

1 **ANTIBACTERIAL AND TOXICOLOGICAL EVALUATION OF LEAF EXTRACTS**
2 **OF *GUIERA SENEGALENSIS* AGAINST GRAM NEGATIVE BACTERIA.**

3 **ABSTRACTS**

4 This study evaluated the antibacterial activity of the leaves extract of *Guiera senegalensis* by
5 extracting with methanol and aqueous using sohxlet extractor. Qualitative phytochemical
6 analysis showed that the extracts contain alkaloids, phenols, tannins, steroids, saponins and
7 glycosides. Quantitative estimations of phytochemicals revealed 123.2 ± 0.04 , 13.95 ± 0.91 and
8 9.20 ± 0.01 of phenols, saponin and alkaloids respectively. The various extracts were
9 investigated for antibacterial activity using agar diffusion methods of susceptibility testing
10 and only the aqueous extract and chloroform fraction were found to be active against clinical
11 isolates of *Echerichia coli*, *Salmonella typhi*, *Klebsiela pneumoniae* and *Pseudomona*
12 *aeruginosa* (14.40 ± 1.60 to 15.60 ± 0.51 mm). However, the standard drug(tetracycline and
13 choramphenicol) used as positive control demonstrated higher zones of inhibition. Low MIC
14 and MBC values were observed for *E.coli*, *K. pneumonia*, *P.aeruginosa* except *S. typhi*. The
15 toxicity profile of the crude was evaluated on haematological, biochemical and
16 histopathology of the organs. The result revealed that prolonged used of the extracts could led
17 to liver and kidney damage. The morphological changes in the liver and the kidney were
18 randomly distributed between the treated animals and the control animals, and the incidences
19 were within the range of normal background leisions. It can therefore be inferred that all
20 observed changes were mild. This result suggests that although the use of the leaf extracts of
21 *G.senegalensis* is relatively safe, hepatic and renal toxicity may occur with prolonged
22 usage.

23 **Keywords:** *G.senegalensis*, Haematology, Biochemical, phytochemical, Antibacterial

24 **INTRODUCTION**

25 Plants are natural reservoir of medicinal agents almost free from the side effects increasingly
26 gaining acceptance even among the literates in urban settlements, probably due to the
27 increasing inefficacy of many modern drugs used for the control of many infections such as
28 typhoid fever, gonorrhea and tuberculosis as well as increase in resistance by several bacterial
29 to various antibiotics and the increasing cost of prescription drugs, for maintenance of
30 personal health(Smolinski *et al.*,2003) .Unfortunately, rapid explosion in human population
31 has made it almost impossible for modern health facilities to meet health demands all over
32 the world, thus putting more demands on the use of natural herbal health remedies. Current
33 problem associated with the use of antibiotics, increased prevalence of multiple-drug resistant

(MDR) strains of a number of pathogenic bacteria such as methicillin resistant *Staphylococcus aureus*, *Helicobacter pylori*, and MDR *Klebsiella pneumonia* has revived the interest in plant with antimicrobial properties. In addition, the increase in cases of opportunistic infections and the advent of Acquired Immune Deficiency Syndrome (AIDS) patients and individuals on immunosuppressive chemotherapy, toxicity of many antifungal and antiviral drugs has imposed pressure on the scientific community and pharmaceutical companies to search alternative and novel drug sources.

The importance of plants in medicine remains of great relevance with the current global shift to obtain drugs from plant sources. This is because attention has been drawn to the safety, efficacy and economic considerations of herbal remedies (Abubakar *et al.*, 2009). Numerous studies have shown that medicinal plants are the oldest source of bioactive products with proven efficacy (Yadav *et al.*, 2010; Hemalatha *et al.*, 2013) serving as the basic components of several drugs (Rates 2001, analgesics (Almeida *et al.*, 2001), anesthetics (Dubey *et al.*, 2013) antibiotics (Cowan, 1999), anticancer (Gonzales and Valerio, 2006., Prakash *et al.*, 2013), anti-parasitic, anti-inflammatory (Shilpi *et al.*, 2012), oral contraceptives (Ogbuewu *et al.*, 2011; Umadevi *et al.*, 2013) and diuretic drugs (Dutta *et al.*, 2014) among others.

Several ailments including fever, asthma, constipation, diarrhoea, esophageal cancer and hypertension have been treated with traditional medicinal plant. Different plant parts and components (root, leaves, stem bark, flowers or their combinations, essential oils) have been employed in the treatment of infectious diseases

Guiera senegalensis commonly known as Sabara in Hausa is a shrub of the savannah region of west and central Africa. It is widely being used in traditional medicine for the remedy of

many ailments/diseases. Its leaves extract is being used against dysentery, diarrhoea, gastrointestinal pain and disorder, rheumatism and fever (Piere *et al.*, 2013).

The antibacterial activities of plants are due to the presence of biologically active compounds such as alkaloid, tannins, flavonoid, phenols and phenolic compounds (Havagiay *et al.*, 2004). Unfortunately along with these bioactive agents, plants also produce potentially toxic compounds.

The rapid increase in the antibiotic resistance of microorganism to available synthetic drug and the discomforting side effects of synthetic antibiotic necessitate the search for alternative source of antibiotics. This study was there for design to access the antibacterial potential and toxic level of the plant.

Matreials and Methods

Plant Processing and Extraction

Fresh leaves of the plant *Guiera senegalensis* was collected, air dried and processed into fine powder using mortar and pestle. Six hundred grams (600 g) of the plant powder was used for extraction with methanol and aqueous using Soxhlet extractor apparatus.

Qualitative Phytochemical Screening of Extracts

Preliminary qualitative phytochemical screening which involves performing simple chemical tests to detect the presence of secondary metabolites such as tannins, flavonoids, phenols, phenolic compounds, saponins, glycosides, and volatile oil, was carried out according to the methods of Sofowora, (1982), and Trease and Evans, (2002).

Quantitative Determination of Phytochemicals

Quantitative estimation of phytochemicals such as alkaloids and saponins was carried out according to Oloyed (2005), total phenolic content using Singleton *et al.* (1999) and

flavanoids using Aluminum Chloride colorimetric method. The absorbance of the reaction mixture was taken at different wavelength flavonoid (415nm), phenols (765nm), alkaloid (565nm), saponins (490nm) and tannins (760nm) using spectrophotometer.

Partitioning of Extracts

The methanol and aqueous extracts were solubilised in 100ml of water and partitioned between n-hexane, chloroform, ethyl acetate and aqueous residue. The partition was done starting with the non-polar solvent to the polar solvent. The resultant fraction was evaporated to dryness at room temperature so as not to destroy some of the thermo labile materials that may be present in the fractions and the weight of each of the fraction was taken (Sarker *et al.*, 2006).

Susceptibility Testing of Plant Crude Extracts and Fractions from Partitioning.

Sterile molten Mueller Hinton agar (20 ml) was poured into sterile Petri dish and allowed to set. A sterile cork borer of diameter 6 mm was used to bore equidistant wells into the agar plate. One drop of the molten agar was used to seal the bottom of the bored wells so as to prevent the extract from sipping beneath the bottom of the agar. Sterile cotton swab sticks were used to streak on the surface of the agar plates with the standardized test organisms and 100 µl of the extract (40 mg/ml) of methanol and aqueous extract and 100 µl of ethyl acetate/methanol (100 mg/ml) of the sub-fractions were added to each of the bored wells and 25 mg/ml of the standard drug (Tetracycline and chloramphenicol) were used as a positive control while dimethylsulfoxide (DMSO) served as the negative control. One hour pre-diffusion time was allowed after which the plates were incubated at 37°C for 18 h. The zones of inhibition were then measured in millimeter. The above method was carried out in triplicates and the mean of the triplicate result were taken. Test with ten millimeter (10mm) zones of inhibition was considered sensitive to the plant extract.

104 **Acute Toxicity Study**

105 Acute toxicity of *Guiera senegalensis* extract was determined in rats according to the OECD
106 guide line 2008. Rats fasted for 16 h were divided into groups of four(4) per group. Graded
107 doses of the extract (200,400,800,1000,1200 and 1400) were separately administered to the
108 rats in each group by means of canular needle. All rats were allowed free access to food and
109 water and observed for period of 72 h for signs of acute toxicity. The number of deaths within
110 this period was recorded.

111 **Repeated Dose Toxicity Test**

112 The study applied the OECD guidelines on repeated dose. Forty (40) rats for the test and
113 another fourty (40) rats for the control group. The selected safe dose (800ml/kgbw). from
114 acute toxicity study was used. The treatment lasted for ten weeksand the administration was
115 done using metal oropharyngeal cannula. Observation were made twice daily for mortality
116 and changes in general appearance or behavior. The body weight were recorded every week.
117 In addition measurement of food and water comsumption was performed weekly.

118 **Collection of Blood Samples**

119 Four(4) rats were randomly selected at the end of each week and aliquot of the blood
120 collected
121 into sample bottles containing EDTA for haematological analysis while the remaining blood
122 was kept in plain bottles from which serum was collected for and stored for biochemical
123 analysis. liver and kidney function parameters.

124

125

Biochemical and Haematological Analysis

At the end of the experiment, the blood of the animal was collected and haemologic analysis performed using the method described by Ochei and Kolhatkar (2000) for packed cell volume(PCV), white blood count(WBC), differential white blood count(Diff.WBC). neutrophil, mean corpuscular volume(MCV), mean corpuscular haemoglobin(MCH), mean corpuscular haemoglobin concentration(MCHC) and platelets. For biochemical parameters, total protein, albumin, alanine aminotransferase(ALT), aspartate aminotranferase (AST), alkaline phosphatase ,urea, uric acid, sodium, potassium, chloride iron concentrations and creatinine (Ochei and Kolhatkar, 2000).

Histopathological Analysis

The histopathological analysis of the kidney and the liver, excised from the experimental animals were carried out in the histopathology laboratory of the Teaching Hospital, Gwagwalada, Abuja Nigeria. The various organs were cut and placed in embedded cassettes. Thereafter, they were fixed with 10% formalin for 1 hour and afterwards dehydrated with methanol (70, 90 and 100%) at different concentration in ascending concentration and different time in order to remove water from the tissues. Thereafter, clearing with xylene was done for 2 to remove alcohol and prepare the tissue for waxing. Embedding was done using paraplast wax by impregnating cassettes with molten wax at 60°C for 3 h. Slicing was done at 5 microns using a microtome. The slide was dyed for 20 min on hot plate. Afterwards, dewaxing and hydration were done using xylene and various percentage of alcohol respectively.

Thereafter, staining was done with cole's hematoxylin for 10 min to stain the nucleus while eosin was used to stain the cytoplasm for 3 min. Dehydration was once again carried out in alcohol and alcohol cleared with xylene. A mounting medium, dibutylphthalate xylene (DPX) was dropped on the tissue section and they were viewed through the microscope.

151 **Results and Discussion**

152 **Results**

153 Table 1 is the result of qualitative phytochemical constituents present in the leaf extracts of
 154 the plants. Tannins, alkaloids, saponins and flavonoids were present in majority of the plants
 155 while phenols, terpenoids, steroids were present in the plant (Table 1).

156 The results of the antibacterial screening of the methanol and aqueous extracts on the test
 157 strains are shown in Table 2. The aqueous extract showed more activity on the test isolates
 158 than the methanol extract (14.40 ± 1.60 to 15.60 ± 0.51 mm) . The standard drug used inhibited
 159 the growth of all tested organisms with varying diameter zones of inhibition higher than that
 160 of the extracts.

161 The result in table 3 showed varied yields of fraction obtained after partitioning. The yield
 162 was dependent on the polarity of the material, and the solubility of the material in the
 163 solvent. Residual aqueous fraction was the highest, followed by n-hexane and chloroform
 164 fraction.

165 Table 4 below shows the result of the quantitative estimation of phytochemicals, phenol was
 166 found to be present in high in concentration in the aqueous and methanol extract of *G.*
 167 *senegalensis* 87.3 ± 0.50 and 123.3 ± 0.034 while saponin and alkaloid were present at
 168 varying concentrations (table 3).

169 Fig. 1&2 shows the result of the antibacterial activity of chloroform aqueous and methanol
 170 of *G. senegalensis*. The fractions inhibited all the test organisms except *S. typhi* .

Table1 Phytochemical Constituents of leaf extract from *G. senegalensis*

Extract	Phenols	Alkaloids	Tannins	Volatile oils	Steroids	Terpenoids	Saponins	Flavonoids
Methanol	+	-	+	-	+	-	+	-
Aqueous	-	+	+	-	+	-	+	-

Table 2: Quantitative estimation (mg/g) of leaf extract of *G. senegalensis* plants

Plants	Flavonoids	Phenols	Alkaloids	Tannins	Saponins
Methanol	1.67±0.02	87.3±0.50	7.41±0.03	0.07±0.18	10.88±0.53
Aqueous	-14.40±0.01	123.2±0.4	9.20±0.01	0.07±0.25	13.95±0.91

Table 3: Mean zones of Inhibition of Aqueous and Ethanol Extracts.

Extracts	<i>S. typhi</i>	<i>P. aeruginosa</i>	<i>E. coli</i>	<i>K. pneumonia</i>
(40mg/ml)				
Methanol	2.87±1.74 ^a	4.00±2.47 ^c	8.40±4.00 ^b	3.67±2.08 ^d
Aqueous	14.40±2.48 ^a	14.40±1.60 ^c	15.60±0.51 ^b	14.80±0.86 ^{bc}
Tetracycline	20.67±0.67 ^b	20.00±0.58 ^b	17.67±0.33 ^a	18.33±0.33 ^a
(25mg/ml)				
Chloramphenicol	24.00±0.00 ^a	23.67±0.33 ^a	30.67±0.33 ^b	31.67±0.33 ^c
(25mg/ml)				

Values are expressed as mean ± standard error of mean

Values with the same superscript in the same column are not significantly different at p>0.05

Table 4: Yields of Partitioned Fractions of *G.senegalensis* Extract

Extract	n-hexane fraction	Chloroform fraction	Ethylacetate fraction	Residual Aqueous fraction
Methanol	3.95	4.03	1.66	16.01
Aqueous	7.00	1.83	1.77	13.10

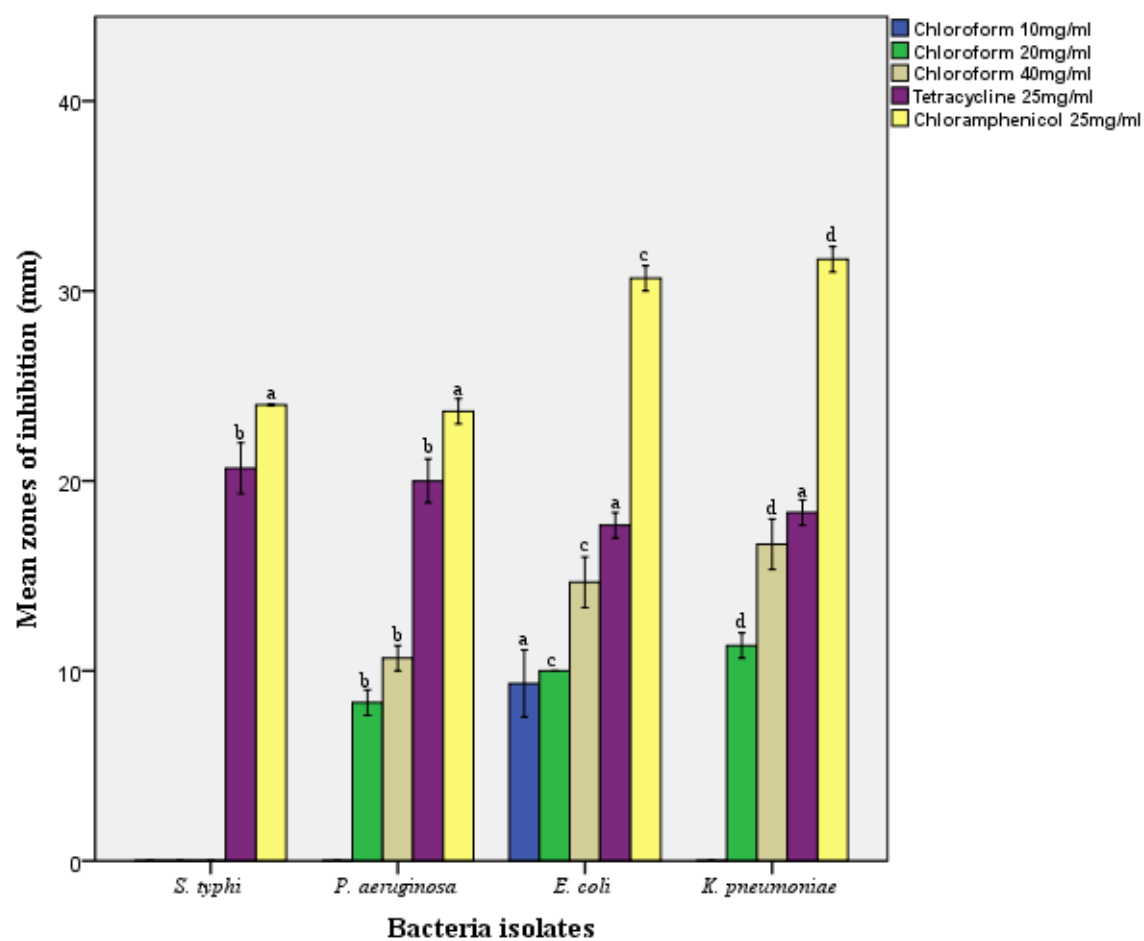


Fig 1: Mean zones of inhibition of chloroform fraction of aqueous *G. senegalensis* extract

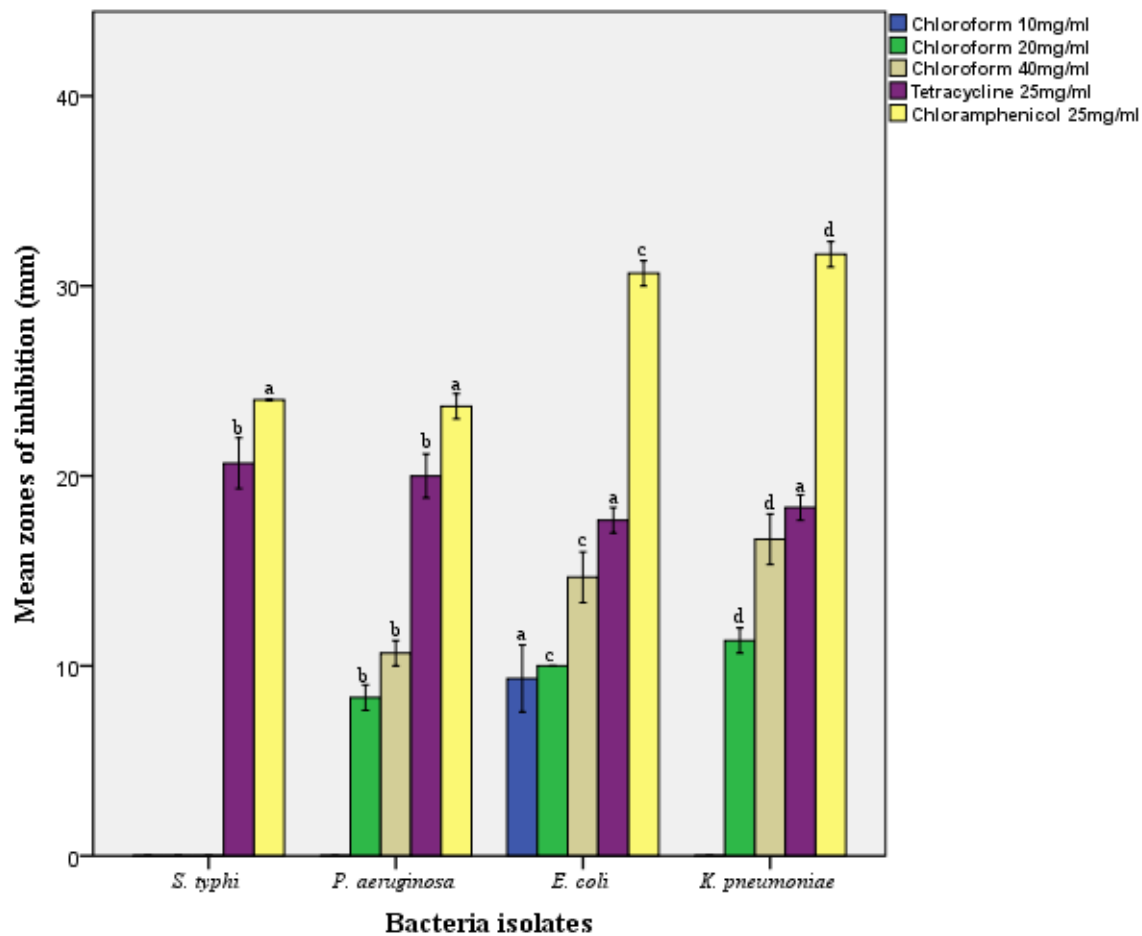


Fig 2: Mean zones of inhibition of chloroform fraction of methanol *G. senegalensis* extract

Toxicity study.

Effects of different doses of crude extracts of *G. senegalensis*: The result of oral toxicity assays showed that the group of rats treated with the crude extract of *G senegalensis* 100, 200, 400, 600, and 800 mg/kg bw did not show alteration in behavioural signal during 72 hours of the experiments. However at 1000 and 1200mg/kgbw there was abrupt ceasation of physical activity, depressed respiratory and cardiac activity. Mortality was observed at 1000 and 1200mg/kgbw. Thus the selected safe dose was 800mg/kgbw and the LD₅₀ dose was 894.43mg/kgbw.

Haematological study:Table 5 below shows the result of haematological study. There was a significant increase ($p < 0.05$) in the of hemoglobin(Hb), packed cell volume(PCV) mean corpuscular volume(MCV), mean corpuscular hemoglobin(MCH)and mean corpuscular hemoglobin concentration(MCHC) values at week 1 in the treated group from the control group but at week 5 and 10, no significant difference at ($p > 0.05$) was observed . Also the serum level of neutrophil lymphocytes, Eosinophils and platelets in all groups were not affected significantly when compared to the control groups. The weight of the rats significantly reduces at week 5 and 10 in treatment groups (Table 5).

Liver function parameters : Result in table 6 revealed no significant difference in the serum level of Alkaline Phosphatase(ALP), Aspartate Aminotransferase(SGOT) and Alanine Aminotransferase enzymes of the treated group and control group at week 1. However, at week 5 and 10 there was significant increase($p > 0.05$) in ALP, SGOT, SGPT serum level in treated groups from the control group (Table 6 below). Serum level of albumin, total bilirubin and conjugated bilirubin not significantly affected. Serum level of Triglycerides decreases in the treated groups (at week 1, 5 and 10). While serum glucose was slightly affected (Table 6 below).

Kidney function parameters : The in table 6 shows no significant differences in the indices after treatment with the extract. However, slight increase was observed in the creatinine at week 5 and 10 (Table 7). The serum level of urea, HCO_3^- , K^+ have increase at week 5 and 10 while Cl^- and creatinin indices not affected (Table 7 below).

Histopathology of Liver and Kidney: There were no treatment related changes in the organs observed in the study at week 1&5 from the serum readings obtained for liver and kidney parameters (Table 6&7) (plates I-VI) .

Table 5: Effects of crude extracts *G. senegalensis* on Haematology Parameters in rats

Parameters	Control	Week 1	Control	Week 5	Control	Week 10
Weight (g)	192.89±5.18 ^b	181.24±3.08 ^a	182.13±1.47 ^b	164.97±2.10 ^a	182.22±0.74 ^b	128.58±3.07 ^a
Hb (g/L)	10.46±0.29 ^a	13.85±0.08 ^b	11.18±0.25 ^a	11.73±0.10 ^a	12.27±0.22 ^a	13.88±0.18 ^b
PCV (%)	30.75±0.33 ^a	44.37±2.44 ^b	34.30±0.59 ^a	33.38±0.24 ^a	36.69±0.19 ^a	40.99±0.43 ^b
MCHC (g/dL)	28.45±0.36 ^a	40.58±0.30 ^b	33.32±0.72 ^a	33.94±0.59 ^a	34.55±0.23 ^a	39.39±0.18 ^b
MCH (pg)	17.50±0.51 ^a	31.27±0.50 ^b	22.78±0.13 ^a	22.60±0.20 ^a	23.87±0.10 ^a	29.82±0.11 ^b
MCV (fL)	58.36±1.04 ^a	87.04±0.81 ^b	66.28±0.07 ^a	66.26±0.18 ^a	68.85±0.39 ^a	85.02±0.28 ^b
WBC (x10 ⁹ /L)	10.65±0.23 ^b	9.25±0.11 ^a	9.51±0.09 ^a	9.99±0.23 ^a	10.12±0.41 ^a	9.55±0.31 ^a
Neutrophil (%)	40.23±0.45 ^b	37.65±0.89 ^a	29.86±0.13 ^a	31.80±0.54 ^a	30.00±0.42 ^a	30.96±0.21 ^a
Lymphocytes (%)	68.62±0.50 ^b	65.54±0.37 ^a	69.61±0.45 ^a	69.94±0.85 ^a	69.84±0.28 ^a	68.25±0.24 ^a
Eosinophils (%)	0.89±0.03 ^a	0.89±0.02 ^a	0.88±0.23 ^a	0.83±0.01 ^a	0.95±0.01 ^b	0.89±0.01 ^a
Platelets (x10 ⁹ /L)	97.46±0.84 ^a	97.84±0.65 ^a	97.75±0.23 ^a	98.33±0.90 ^a	97.91±0.39 ^a	98.22±0.09 ^a

Key: Hb – Haemoglobin; PCV – Packed Cell Volume; MCHC – Mean Corpuslar Haemoglobin Concentration; MCV – Mean Corpsular Volume; WBC – White Blood Corpusles.

Values are expressed in mean ± standard erro of mean

Values with the same superscript in the same column are not significantly different at p>0.05.

Table 6: Effects of crude extracts on liver Biochemical Parameters in rats.

Parameters	Control	Week 1	Control	Week 5	Control	Week 10
GLC (mg/dL)	96.06±0.66 ^a	92.16±1.12 ^a	101.78±2.64 ^a	107.93±7.39 ^a	91.19±1.42 ^a	107.94±2.87 ^b
TAGs (mg/dL)	143.31±4.24 ^b	132.17±2.14 ^a	151.08±4.75 ^b	125.99±2.03 ^a	150.57±0.90 ^b	125.27±1.73 ^a
Total protein (g/L)	7.00±0.24 ^b	5.09±0.08 ^a	7.30±0.19 ^b	4.36±0.23 ^a	8.01±0.08 ^b	3.60±0.26 ^a
Albumin (g/L)	3.87±0.06 ^a	3.30±0.14 ^a	3.81±0.05 ^a	3.59±0.06 ^a	3.80±0.05 ^a	3.69±0.04 ^a
Total bilirubin (mg/L)	3.90±0.06 ^a	5.25±0.27 ^b	3.71±0.08 ^a	5.03±0.15 ^b	3.71±0.10 ^a	5.81±0.12 ^b
Conjugated bilirubin (mg/L)	1.71±0.03 ^a	1.89±0.02 ^a	1.72±0.07 ^a	2.16±0.20 ^b	1.82±0.04 ^a	2.77±0.05 ^b
ALP (U/L)	104.30±1.07 ^a	102.19±0.42 ^a	108.26±0.85 ^a	125.85±0.79 ^b	102.59±1.10 ^a	174.23±0.87 ^b
SGOT (U/L)	34.19±0.92 ^a	38.23±0.89 ^b	41.83±0.86 ^a	83.37±1.45 ^b	37.40±1.12 ^a	90.45±1.42 ^b
SGPT (U/L)	30.25±0.85 ^b	23.67±0.71 ^a	35.68±1.13 ^a	61.44±0.89 ^b	37.11±0.93 ^a	66.44±0.80 ^b

Key: Glu – Glucose; TAG – Triglyceraldehyde; ALP – Alkaline Phosphatase; SGOT – Aspartate Aminotransferase; SGPT – Alanine Aminotransferase.

Values are expressed in mean ± standard error of mean

Values with the same superscript in the same column are not significantly different at p>0.05.

Table 7: Effects of crude extracts *G. senegalensis* on some Kidney Biochemical Parameters in rats.

Parameters	Control	Week 1	Control	Week 5	Control	Week 10
Urea (mg/dL)	35.66±0.64 ^a	39.00±1.25 ^b	32.05±1.02 ^a	50.88±0.51 ^b	36.06±0.43 ^a	53.07±0.76 ^b
Creatinin (mg/dL)	5.73±0.14 ^a	8.74±0.14 ^b	0.46±0.23 ^a	7.80±0.17 ^b	6.08±0.22 ^a	6.41±0.34 ^a
Uric acid (mg/dL)	49.67±0.78 ^b	19.04±0.36 ^a	41.66±0.83 ^a	26.11±0.70 ^b	40.18±0.42 ^b	33.35±0.28 ^a
Na ⁺ (mmol/L)	133.25±1.80 ^a	138.17±0.72 ^b	131.71±0.85 ^b	110.19±35.20 ^a	140.75±0.48 ^a	151.10±0.76 ^b
K ⁺ (mmol/L)	4.64±0.23 ^a	3.82±0.06 ^a	0.20±0.10 ^a	4.72±0.08 ^a	3.59±0.08 ^a	6.07±0.05 ^b
Cl ⁻ (mmol/L)	87.17±0.43 ^a	101.35±0.54 ^b	92.17±1.10 ^a	113.29±0.49 ^b	110.22±0.44 ^a	113.78±1.17 ^a
HCO ₃ ⁻ (mmol/L)	23.49±0.60 ^a	27.56±0.86 ^b	21.46±0.73 ^a	30.29±0.84 ^b	28.22±0.40 ^a	36.35±0.90 ^b

Key: Na⁺- Sodium iron; K⁺- Pottasiun iron; Cl⁻ - Chloride iron; HCO₃⁻ - Hydrogen bicarbonate iron.

Values are expressed in mean ± standard erro of mean

Values with the same superscript in the same column are not significantly different at p>0.05.

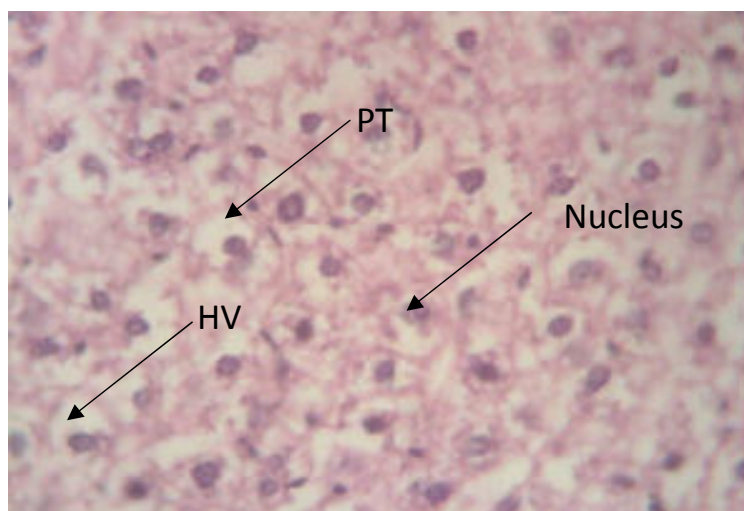
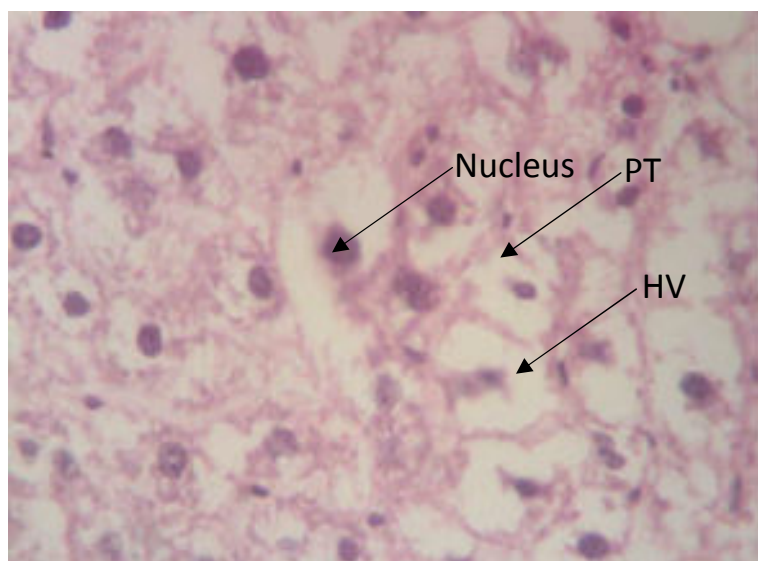
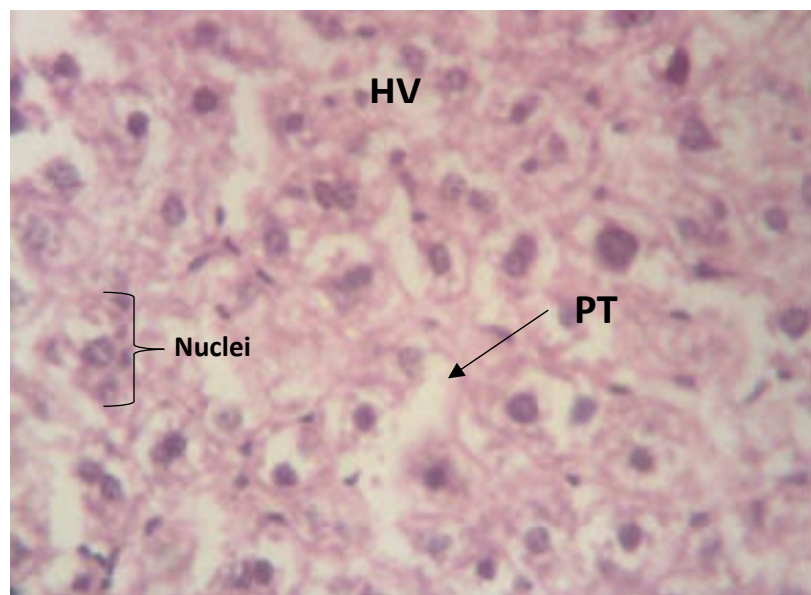


Plate I Liver section of control rat showing moderate portal tract (PT) inflammation and interphase hepatitis, moderate congestion and thrombosis of hepatic vessels (HV), moderate pleomorphic hyperchromatic to vesicular nuclei with prominent nucleoli and feathery degeneration with moderate hepatocyte necrosis.

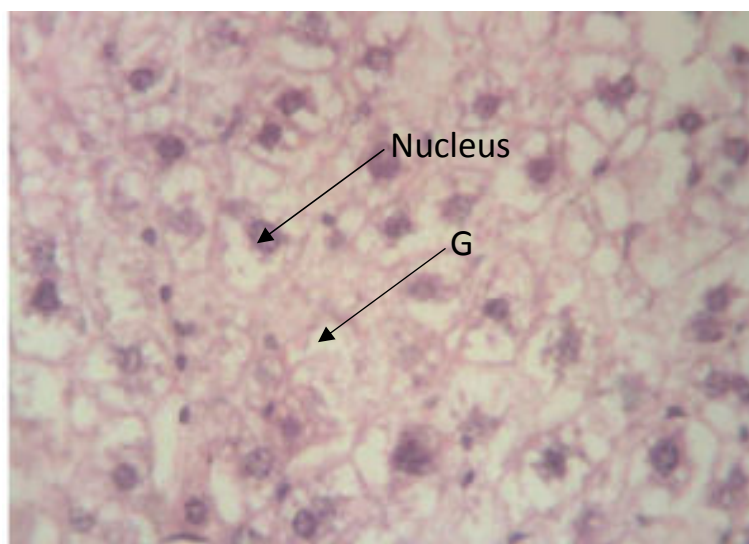


PlateII Liver section of rat tested with 800mg/kgbw crude extract of *G. senegalensis* showing moderate portal tract (PT) inflammation and interphase hepatitis, moderate congestion and

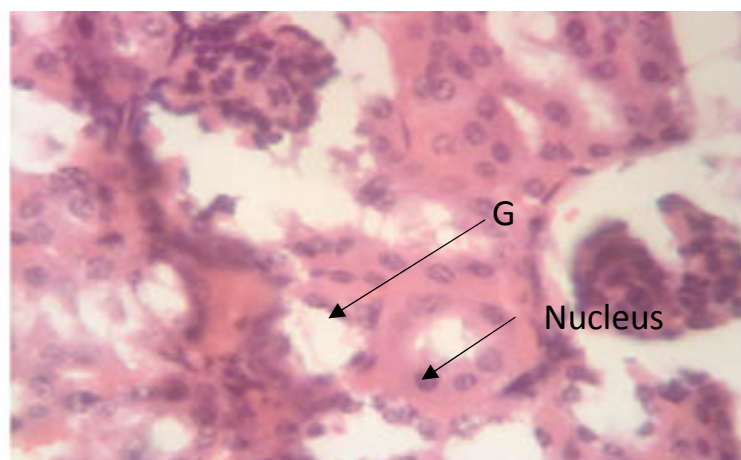
thrombosis of hepatic vessels (HV), moderate pleomorphic hyperchromatic to vesicular nuclei with prominent nucleoli and feathery degeneration with moderate hepatocyte necrosis.



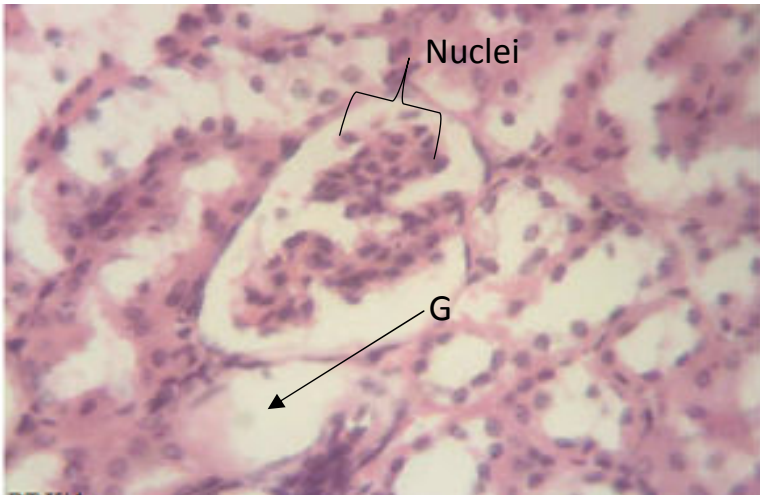
PlateIII Liver section of rats tested with 800mg/kgbw crude extract of *G. senegalensis* showing moderate portal tract (PT) inflammation with interphase hepatitis. Moderate congestion and thrombosis of hepatic vessels (HV). Moderate pleomorphic hyperchromatic nuclei with irregular nuclear membrane. Feathery degeneration with moderate hepatocyte necrosis.



PlateIV Kidney section of control rat showing moderate cellular mesangium of the glomerulus (G), tubulointerstitial inflammation, congestion and thrombosis of renal vessels.



PlateV Kidney section of rat tested with 800mg/kgbw of crude *G.senegalensis* extract showing mild tubulointerstitial lymphocyte infiltration, haemorrhage/fibrin and mild thrombosis.



PlateVI Kidney section of rat tested with 800mg/kgbw of *G. senegalensis* extract showing moderatecellular mesangium of the glomerulus (G), tubulointerstitial inflammation and moderate congestion and thrombosis of the renal vesicles.

DISCUSSION

Plants extraction permits the demonstration of their biologically active compounds. It also facilitates fractionation and monitory of their antimicrobial potential which may lead to isolation of active compound and elucidation of the structure of the active compound(Stefano *et al.*, 2014) The phytochemical screening of the extracts and fractions showed that the plant contains one or more of the following phytochemicals in high amount; alkaloids, flavonoids, tannins saponins, glycosides, steroids and phenols. Other phytoconstituents detected in trace amount include steroids, glycosides and terpenoids (Table 1). These findings were in accordance with the results Bello *et al.*, (2013),Uchechukwu *et al.*, 2014,and Ogbeba and Iruolaje,2015 who identify the same phytochemicals in the plants.

Results recorded by agar diffusion methods indicated that the strongest antibacterial activity was obtained for the aqueous extracts of *Guiera senegalensis* with 100% inhibition because it inhibited the growth of all bacterial isolates with zones of inhibition ranging from 14-15mm (Table3) Stefano *et al.*, 2014 and Baragob *et al.*,2013 reported on the antibacterial efficacy of crude and purified fractions of *G.senegalensis* and *D.microcapum* against gram positive and less effective against gram negative bacterial strains. This result did not agree with their finding, it is worthy of note that antimicrobial activity results of the same plant part tested most of the time varied from researcher to researcher. This is possible because concentration of plant constituents of the same plant organ can vary from one geographical location to another depending on the age of the plant, differences in topographical factors, the nutrient concentrations of the soil, extraction method as well as method used for antimicrobial study. It is therefore important that scientific protocols be clearly identified and adequately followed and reported.. The differences in the observed activity of the various extracts may be caused by the varying solubility of the

active constituents in the primary solvents (methanol and aqueous) and the secondary solvents; Dimethylsulfoxide (DMSO). It is well known that different solvents have diverse solubilization capacity for phytoconstituents. The antibacterial activity of the extract may also be attributed to the presence of the phytochemicals present as reported by Bello *et al.*, (2013).

The standard antibiotic chloramphenicol used showed larger zones of inhibition against all the test organisms compared to the extracts. This may be due to the fact that conventional antibiotics are usually prepared from synthetic materials by means of reproducible manufacturing techniques and procedures while herbal medicinal products prepared from plant and animal origins are prone to contamination and deterioration (El-Mahmood and Ameh, 2007).

The low MIC and MBC values were observed against the test organism means that the plant has the potential to be used to treat bacterial infection effectively. At the same time high MIC and MBC values however, is an indication of lack of efficacy of the plant extracts against the pathogen as observed with respect to *S. typhi*.

Quantitative estimation of phytochemicals in the plant revealed high concentration of phenols in all the four active plant (123.2 ± 0.4) in table 4. Alkaloid and saponin were also detected in all the plant in varied concentration, this may explain why the extracts showed good activity against *S. typhi*, *P. aeruginosa*, *E. coli* and *K. pneumoniae* inspite the reports by researchers (Adesina *et al.*, (2012), Bello *et al.*, (2013) of the multidrug resistance strains of the above species of organisms. This result agrees with the work of Garba *et al.*, 2013 who earlier reported the antibacterial activities of total alkaloid extracted from the same medicinal plant (*P. africana* and *G. sensgalensis*). Phenol is generally a protoplasmic poison and is toxic to all types of cells, precipitation of proteins occurs with high concentrations of phenol while at low concentrations it denatures proteins without coagulating them. It is free to penetrate the tissue because of its

denaturing activity. Saponins are surface active agents which interfere with or alter the permeability of the cell wall thus facilitating the entry of toxic materials or linkages of the vital components from the cell (Sarker *et al.*,2006).

Toxicity study of extracts from *G. senegalensis*

In evaluating the effects of different doses of the crude extracts, the 8 groups of rats(4per group) treated with doses of *G. senegalensis* 100,200, 400,800,1000,1200, and 1400mg/kgbw did not show alteration in behavioral signals during 72h but abrupted ceasation of physical activity and depressed respirational cardiac activity were observed, and mortality recorded at 1000 and 1200 mg/kgbw(for *G.senegalensis*) Thus the selected safe doses was 800mg/kgbw . The selected safe dose concentrations was used for the sub-chronic and chronic test. No significant differences were observed in food and water consumption in all the groups. The results from oral toxicity evaluation indicated that the crude plant extracts from *G.senegalensis* have low toxicity (Table 4.12).

In the repeated dose sub- acute and chronic study, Fourty(40) rats control and fourty(40) rats tes for period of ten weeks and four rats were selected randomly and evaluated for heamatological, liver and kidney indices. The observed lost in body weight recorded in the groups at week 5 & 10) could have been as result of factors like the age, metabolic and physiochemical changes in the animal and effects of prolonged use of the extracts. However, the haematological indices (Table 4.13) demonstrated significant increase ($p<0.05$) in parcked cell volume (PCV) mean corpuslar volume (MCV), mean corpuslar hemoglobin (MCH) and mean corpuslar hemoglobin concentration (MCHC) in animals treated at week1,5 and 10 with repeated dose of 800mg/kgbw of the extract. There was slight increase in haemoglobin (Hb), This

result indicates that the components of *G.senegalensis* extract may have not affected the production of red blood cell negatively (Table 5).

Evaluation of the activities of basic liver function enzymes in serum are used to indirectly access the integrity of the organs after exposure to pharmacological agents (Uboh *et al.*, 2010). Alkaline phosphatase is a marker enzyme of the plasma membrane as well as the endoplasmic reticulum and is present in the cells lining the biliary duct of the liver (Ezeja *et al.*, 2014). Therefore, increase in serum ALP activities may indicate alteration in the permeability of the plasma membrane and cholestatic diseases such as gall stone, while alteration in the activity of serum Aspartate Aminotransferase (SGOT) may produce consequential effects on the metabolism of amino acid and its biochemical regulation. Biochemical analysis for the liver function indices (Table 6) revealed significant ($p > 0.05$) increase in the serum level of ALP (140.30 ± 1.07 to 174 ± 0.23), SGOT (34.19 ± 0.91 to 92.63 ± 0.24) and SGPT (30.25 ± 0.85 to 66.44 ± 0.80) for *G. senegalensis* and ALP (87.55 ± 1.21 - 110.47 ± 0.25), SGOT (17.51 ± 2.63 - 31.76 ± 0.31) and SGPT (12.41 ± 0.14 - 32.90 ± 0.59) in the test animals at week 5 & 10 in comparison with control which could be attributed to the prolonged use of the extract and its cumulative effects. The group treated at week 1 did not show significant ($p < 0.05$) variations in the serum level of the enzymes with the control group, we can infer that prolonged use of extracts of *G. senegalensis* has led to hepatic and renal injury.

Similarly, the extracts have affected the kidney. The level of serum urea increased significantly ($p < 0.05$) in the treated group at week 10 compared to the control. Healthy kidney filterate urea, which is a waste product of protein metabolism out of the blood. High level of urea in the blood is an indication of impaired kidney. Increase in serum level of creatinin is also suggestive of kidney malfunctioning as observed at week 5. Significant increase in serum level of HCO_3

(week 5 and week 10) was also observed. The mild to moderate alterations observed in the liver and kidney appeared to be as a result of prolonged used and cumulative effects of the extracts administered to the rats. The morphological changes in the liver and the kidney were randomly distributed between the treated animals and the control animals, and the incidences were within the range of normal background lesions. It can therefore be inferred that all observed changes were mild This result suggests that although the use of the leaf extracts of *G.senegalensis* is relatively safe, hepatic and renal toxicity may occur with prolonged usage (table7).

CONCLUTION : The plant *G. senegalensis* demonstrated significant spectrum of activity on gram negative bacteria and the toxicological studies on the crude extract suggested that *G. senegalensis*, has the potential to be use in herbal medicine for the treatment of ailments caused by these pathogens due to their low toxicity.

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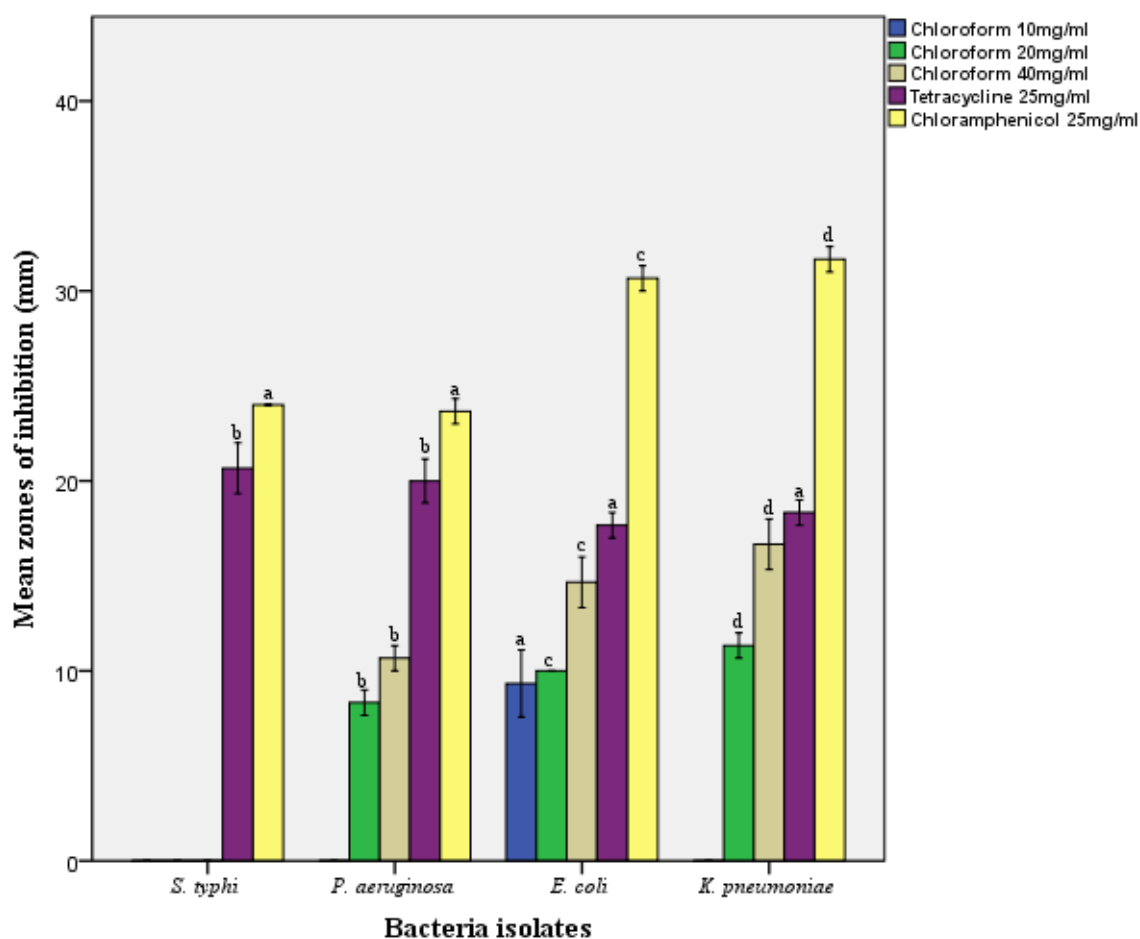


Fig 2: Mean zones of inhibition of chloroform fraction of methanol *G. senegalensis* extract

Chloroform fractions of *G. senegalensis* methanol extract showed varying diameters of mean zones of inhibition at concentrations of 20mg/ml and 40mg/ml of the extract (Fig 2) against all isolates except against *S.typhi* where all concentrations (10,20.and 40mg/ml) did not inhibit growth of the organism. At a concentration of 40mg/ml, the activity was almost measuring up with that of tetracycline antibiotic in respect to *E. coli* and *K. pnemoniae* as shown in fig.2 below.

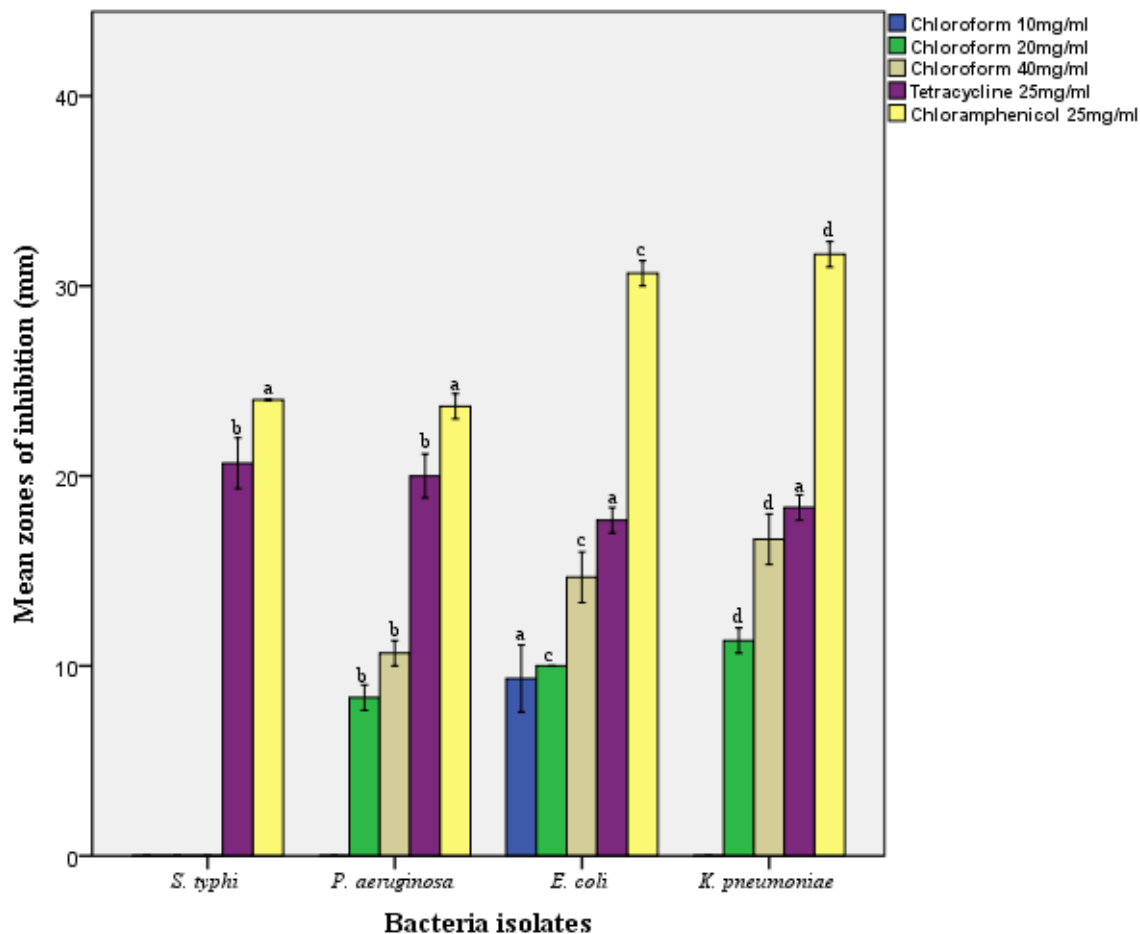


Fig 3 : Mean zones of inhibition of chloroform fraction of aqueous *G. senegalensis* extract

Chloroform fractions of *G. senegalensis* aqueous extract showed varying diameters of mean zones of inhibition at concentrations of 20mg/ml and 40mg/ml of the extract (Fig 4. 8) against all isolates except against *S.typhi* where all concentrations (10,20.and 40mg/ml) did not inhibit growth of the organism. At a concentration of 40mg/ml, the activity was almost measuring up with that of tetracycline antibiotic in respect to *E. coli* and *K. pnemoniae* as shown in fig.3 below. While the standard chloramphenicol was still better in activity than the extracts at all concentrations.