

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

ABSTRACT

Objective: To evaluate the antibacterial activity and toxicity effect in rats of the leaves extract of *Guiera senegalensis*

Methods: plant material was extracted with methanol and aqueous solvents using sohxlet extractor and assayed for qualitative/quantitative phytochemicals, the extracts were bioassayed against clinical isolates of some selected gram negative bacterial (*Echerichia coli*, *Salmonella typhi*, *Klebsiella pneumoniae* and *Pseudomona aeruginosa*). Tetracycline and chloramphenicol were used as positive control. Acute toxicity studies were conducted by administering different doses (100, 200, 400, 600, 800, 1000, 1200 mg/kgbw) of the extract to eight groups (4 per group). The selected safe dose was used for the repeated dose in the sub-acute study.

Results: Analysis showed that the extracts contain alkaloids, phenols, tannins, steroids and saponins. Estimation of phytochemicals revealed high concentration 87.3 ± 0.50 and 123.3 ± 0.34 of phenols, while saponins, flavonoids, tannins and alkaloids were less and at varying concentrations. The aqueous extract shows appreciable antibacterial activity at 40 mg/ml concentration against all isolates with diameters zone of inhibitions ranging 14.40 ± 1.60 to 15.60 ± 0.51 mm. Tetracycline and Chloramphenicol used as positive control demonstrated higher zones of inhibition than the extract. Low MIC and MBC values were observed for *E.coli*, *K. pneumoniae*, *P.aeruginosa* except *S. typhi*. The selected safe dose was 800 mg/kgbw and LD_{50} was 894.43 mg/kgbw. Haematological, and biochemical analysis revealed no toxic effects after weeks 1 and 5. However, cumulative toxic effects manifested after week 10. The result of histopathology of liver and kidney revealed mild necrosis of hepatocytes and nephrotoxicity.

Conclusion: The results suggest that although the use of the leaf extracts of *G. senegalensis* is relatively safe, hepatic and renal toxicity may occur with prolonged usage.

Keywords: *G.senegalensis*, Haematology, Biochemical, Phytochemical, Antibacterial

INTRODUCTION

Plants are natural reservoir of medicinal agents almost free from the side effects increasingly gaining acceptance even among the literates in urban settlements, probably due to the increasing inefficacy of many modern drugs used for the control of many

infections such as typhoid fever, gonorrhea and tuberculosis as well as increase in resistance by several bacterial to various antibiotics and the increasing cost of prescription drugs, for maintenance of personal health (Smolinski *et al.* 2003).

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.

Acute toxicity tests measure the adverse effects that occur within fourteen days after administration of a single dose of a test substance. This is performed principally on rodents (mice or rats) and is usually done early in the development of a new chemical or product to provide information on its potential toxicity. Traditionally, acute oral toxicity testing has focused on the immediate determination of the dose that kills half of the animals (i.e., the median lethal dose or LD₅₀), the timing of lethality following acute chemical exposure, as well as observing the onset, nature, severity and reversibility of toxicity. But in recent times, after the immediate observation is done, toxicological parameters (biochemical,

haematological and histopathological) to assess potential adverse effects are carefully chosen and measured (Dekant and Vamvakas, 2005).

Repeated-dose toxicity studies assess the toxic effects resulting from the accumulation of a compound or its metabolites in an organism. Unlike acute studies, they are measures of toxic effects that appear with repeated doses of the toxicant; hence, continuous dosing is essential to assess the long-term toxicity effect of substances. Chronic toxicity is usually performed on mice, rats, rabbit and guinea pigs for at least six months and on dogs for 12 months (Ramaroa and Lercelus, 2006). The dose levels are usually selected on the basis of the results from acute and sub-acute toxicity studies. (Dekant and Vamvakas, 2005).

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 (Minna ,Niger State Nigeria 2015 NIPRD/6732), 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 for 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 and at different wavelenght flavonoid (415nm), phenols (765nm), alkaloid (565nm), saponins (490nm) and tannins (760nm) using spectrophotometer.

Partioning of Extracts

The methanol and aqueous extracts were soblimised in 100ml of water and partitioned with 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 using rotary evaporator and 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 sealed the bottom of the bored wells so as to prevent the extract from sipping beneath the bottom of the agar. Sterile cotton swab stick was used to streak onto the surface of the agar plate with the standardized test organism a. Zero point two gram of the extract each was reconstituted in 5ml of DMSO (200 mg/5ml $\approx$ 40 mg/ml), and 100 μ l of the reconstituted extract (40 mg/ml) was 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 37⁰C for 18 h. The zones of inhibition were then measured in millimeter.

The above procedure was repeated for the fraction ethyl acetate/methanol (100 mg/ml). All tests were carried out in triplicates and the mean of the triplicate results were taken. Test with ten millimeter (10mm) zones of inhibition were considered sensitive to the plant extract.

Ethical approval for the animal studies was obtained from Biochemistry department of Federal university of Technology Minna Nigeria.

Acute oral Toxicity Study

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

Repeated Dose Toxicity Test

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

Collection of Blood Samples

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

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 aminotransferase (AST), alkaline phosphatase, urea, uric acid, sodium, potassium, chloride ion 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 h and afterwards dehydrated with methanol in increasing concentration ranging from 70, 90 and 100% at ascending concentration and different time in order to remove water from the tissues. Then, clearing with xylene was done for 2 h to remove alcohol and prepare the tissue for waxing. Embedding was done using parafin 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(70%, 90% and 100%) of alcohol respectively.

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 was later cleared with xylene. A mounting medium, dibutylphthalate xylene (DPX) was dropped on the tissue section and they were viewed through the microscope.

3.10 Statistical Analysis

All data generated from the study were subjected to analysis of variance (ANOVA) to test for the significant difference between the plant crude extracts, fractions and toxicological parameters tested. SPSS version 19 package was used.

The analysis was performed using Microsoft excel 2007 The results were expressed at $P < 0.05$ level of significance.

Results and Discussion

Results

Table 1 shows the result of qualitative phytochemical constituents present in the leaf extracts of the plant *G. senegalensis*. Tannins, alkaloids, saponins, flavonoids, phenols, steroids were present in the plant while volatile oil and terpenoids were not detected (Table 1).

Table 2 shows the results of the quantitative estimation of phytochemicals. Phenols were found to be present in high concentration 87.3 ± 0.50 and 123.3 ± 0.34 in the crude extract of *G. senegalensis* while saponins flavonoids and tannins alkaloids were present at varying concentrations (Table 3).

The results of the antibacterial screening of the methanol and aqueous extracts on the test strains are shown in Table 3. The aqueous extract showed more activity on the test isolates than the methanol extract (14.40 ± 1.60 to 15.60 ± 0.51 mm) The standard drugs (Chloramphenicol and Tetracycline) used inhibited the growth of all tested organisms with varying and yet higher diameters zone of inhibitions than that of the extracts.

The results in table 4 showed varied yields of fraction obtained after partitioning. The yield was dependent on the polarity and the solubility of the material in the different solvents. Residual aqueous fraction was the highest, followed n-hexane and chloroform fraction.

Fig. 1&2 shows the result of the antibacterial activity of chloroform aqueous and methanol 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	+	+	+	-	+	-	+	+

Key: + =present and - =Absent

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	0.40±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	Aqueous fraction
Methanol	3.95	4.03	1.66	16.01
Aqueous	7.00	1.83	1.77	13.10

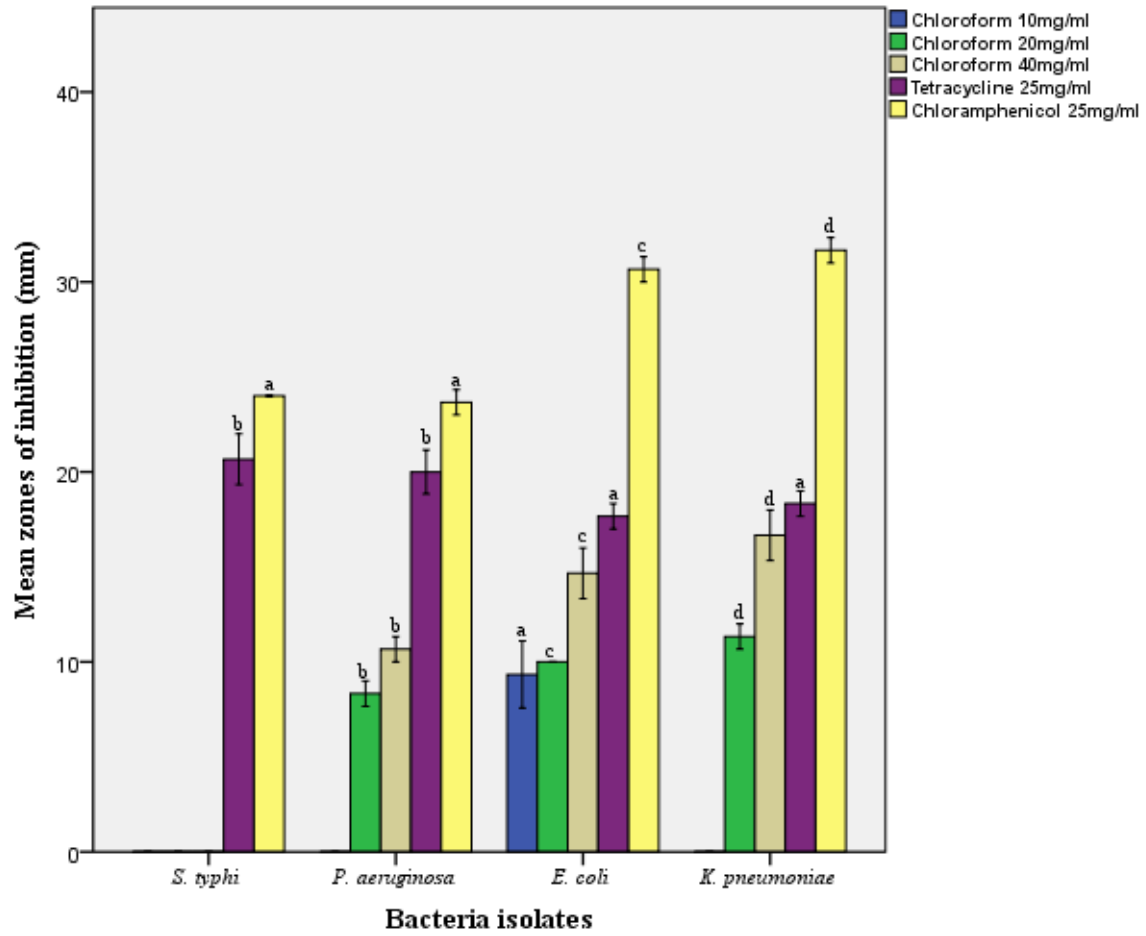


Fig 1: Mean zones of inhibition of chloroform fraction of aqueous *G. senegalensis* extract

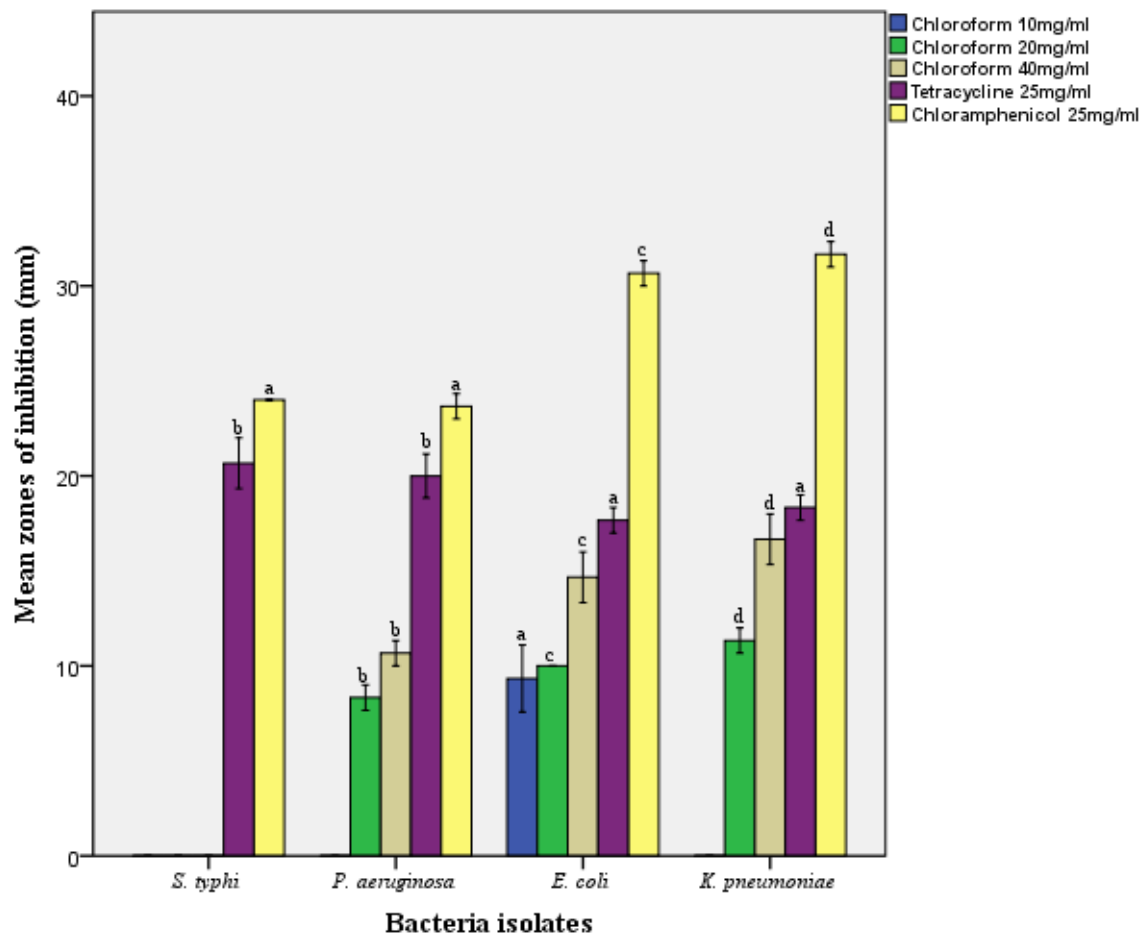


Fig 2: Mean zones of inhibition of chloroform fraction of methanol *G. senegalensis* extract

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 varied concentrations (100, 200, 400, 600, and 800 mg/kgbw) did not show alterations in behaviour after 72 hours of the experiments. Abrupt cessation of physical activity, depressed respiratory and cardiac activity as well as mortality were observed at dose concentrations of 1000 and 1200 mg/kgbw. Thus the selected safe dose was 800mg/kgbw and the LD₅₀ dose was 894.43mg/kgbw.

Haematological study: Table 5 below shows the results of haematological study. There was a significant increase ($p < 0.05$) in the of heamoglobin (Hb), parcked cell volume (PCV) mean corpuslar volume (MCV), mean corpuslar hemoglobin (MCH)and mean corpuslar hemoglobin concentration(MCHC) values after 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 platelates in all groups were not affected significantly when compare to the control groups. The weight of the rats significantly reduces after week 5 and 10 in treatment groups (Table 5).

Liver function parameters : Results 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, after 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 (after week 1, 5 and 10). While serum glucose was slightly affected (Table 6).

Kidney function parameters : The results in table 6 did not shows much difference after treatment with the extract. However, slight increased in serum leves of creatinine urea, HCO_3^- and K^+ was observed after week 5 and 1. While Cl^- was not affected (Table 7).

Histopathology of Liver and Kidney: There were no treatment related changes in the organs observed in the study after week 1 & 5 from the biochemical parameters evaluated (Table 6&7).after week 10, mild morphorlogical inflammation of the portal tract, thrombosis and congestion of hepatic vessels with prominent nucleic and feathery degeneration and moderate hepatocyte necrosis were observed on the liver. While the kidney shows mild intercellular infileration of the glomerulus when compared to the control (Plate 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 Corpuslar Volume; WBC – White Blood Corpusles.

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 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 ion; K⁺ - Pottasiun ion; Cl⁻ - Chloride ion; HCO₃⁻ - Hydrogen bicarbonate iron.

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.

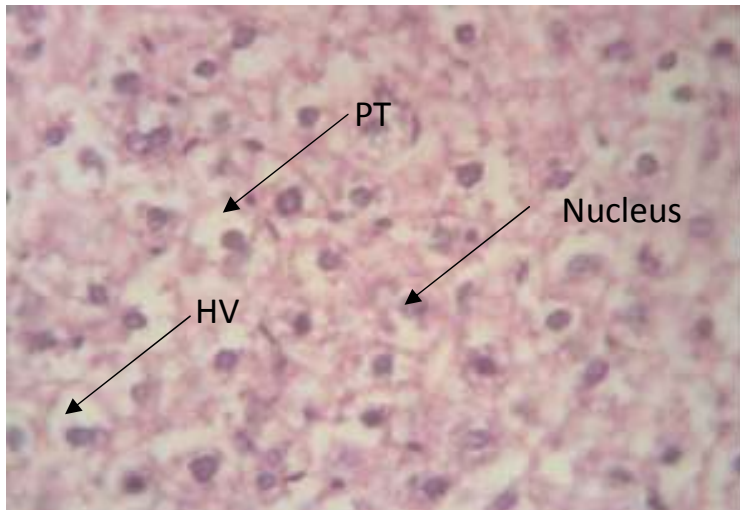


Plate I : Liver section of control rat showing moderate portal tract (PT) inflammation and interphase hepatitis, moderate congestion and thrombosis of hepatic vessels (HV), moderate pleomorphic hyperchromatic to vesicular nuclei with prominent nucleoli and feathery degeneration with mild hepatocyte necrosis.

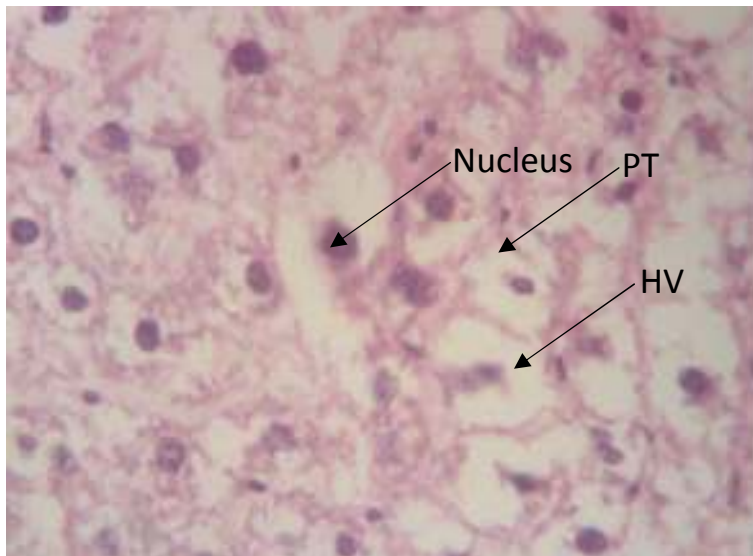


Plate II : Liver section of rat tested with 800 mg/kgbw crude extract of *G. senegalensis* showing mild portal tract (PT) inflammation and interphase hepatitis, mild congestion and thrombosis of

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

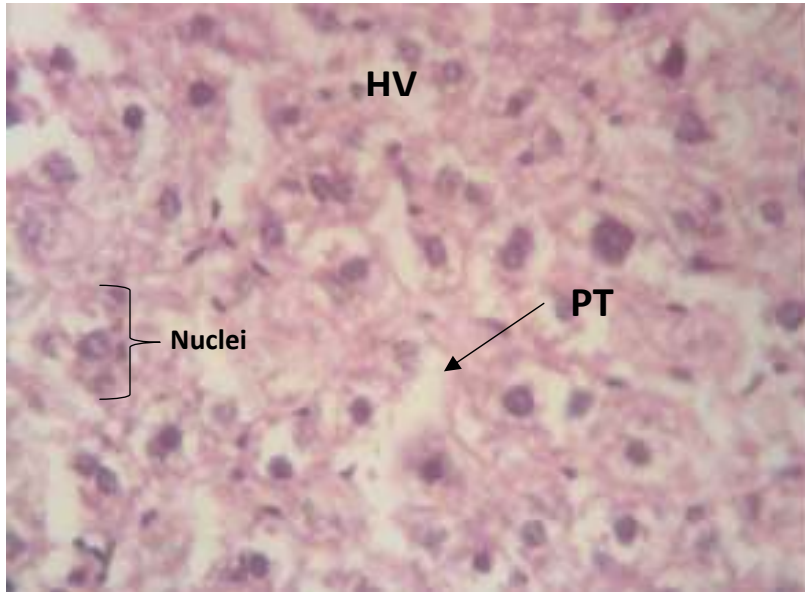
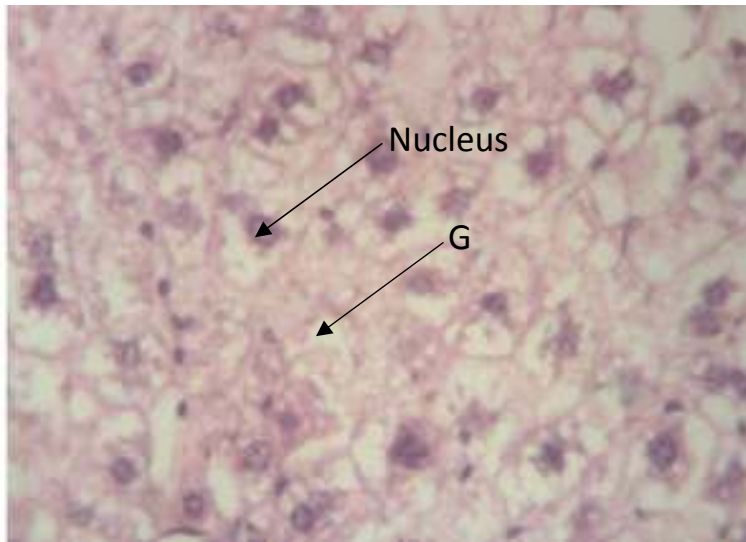
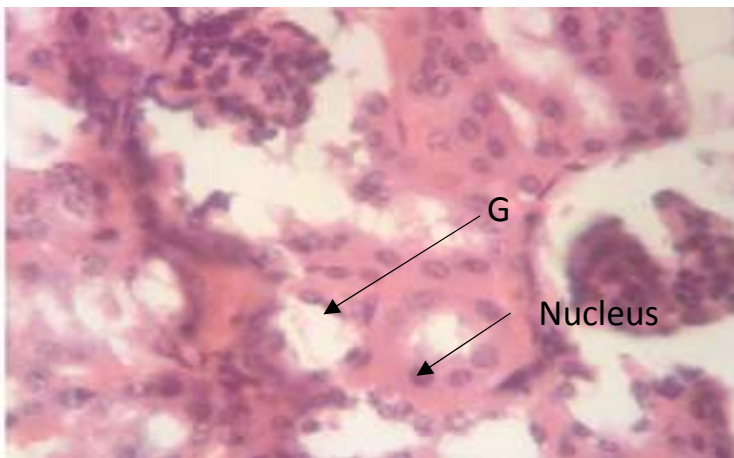


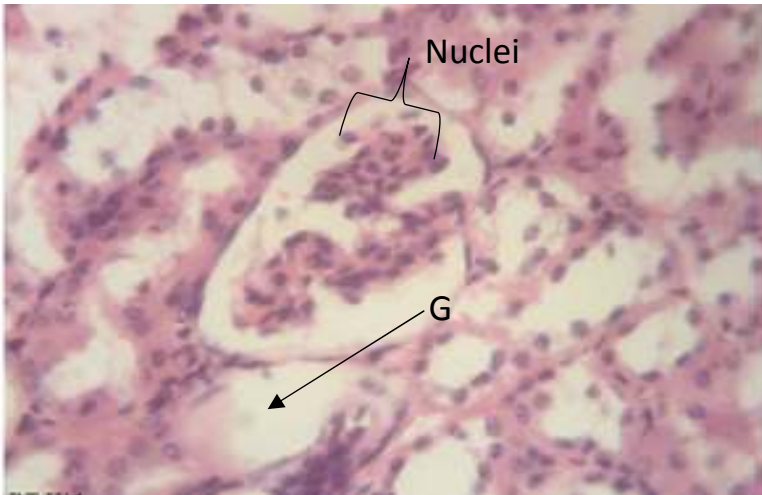
Plate III : Liver section of rats tested with 800 mg/kgbw crude extract of *G. senegalensis* showing moderate portal tract (PT) inflammation with interphase hepatitis. Mild congestion and thrombosis of hepatic vessels (HV). Mild pleomorphic hyperchromatic nuclei with irregular nuclear membrane. Feathery 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 800 mg/kgbw of crude *G. senegalensis* extract showing mild tubulointerstitial lymphocyte infiltration, haemorrhage/fibrin and mild thrombosis.



PlateVI Kidney section of rat tested with 800 mg/kgbw of *G. senegalensis* extract showing moderate cellular 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 monitoring 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 the following phytochemicals; alkaloids, flavonoids, tannins saponins, steroids and phenols. Volatile oils and terpenoids were not detected (Table 1). These findings were in accordance with the results Bello *et al.*, (2013), Uchechukwu *et al.*, (2014), and Ogbaba 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 at 40mg/ml concentration 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 observed against the test organism means that the plant has the potential to be used to treat bacterial infection effectively. However, the high MIC and MBC values were 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 extracts revealed high concentration of phenols (87.3 ± 0.50 and 123.2 ± 0.4) in table 2. Alkaloid and saponin were also detected in 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 (*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 therefore 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 1400 mg/kgbw did not show alteration in behavioral signals during 72 h but abrupted cessation 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 800 mg/kgbw . The selected safe dose concentration 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, Forty(40) rats control and forty(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 weeks 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 5) 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 (Table5).

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 comparism with the control which could be attributed to the prolonged used of the extract and its cumulative effects . The group treated at week1 did not shows significant ($p<0.05$) variations in the serum level of the enzymes with the control group, we can infer that prolonged used of extracts of *G. senegalensis* has lead to hepatic and renal injury.

Similarly, the extracts has 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 creatinine is also suggestive of kidney malfunctioning as observed after week 5. Significant increased 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 (Table 7). 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 leisions. 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 (plates I-VI).

CONCLUSION : The plant *G. senegalensis* demonstrated appreciable spectrum of acivity on gram negative bacteria (*S. typhi*, *P.aeruginosa*, *E.coli*, and *K. pneumoniae*).

2. The toxicological evaluation of 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.

REFERENCES

- Adeshina, G. O., Onaolapo, J. A., Ehinmidu, J. O. and Odama, L. E., (2012). Antimicrobial activity of the aqueous ethyl acetate sub-fractions of *Alchornea cordifolia*. *European Journal of Medicinal Plants*. **2(1)**, 31-41.
- Almeida, R. N., Navarro, D. S., and Barbosa-Filho, J. M., (2001). Plants ith central analgesic activity. *Phytomedicine*. **4(6)**,382-388.
- Baragob, A.E.A., Abdulwahab, H., Marem, M., Algili, I., Samia, H.A., Khojali, S.M.E., Zakia, A.O.A., Almalki, W.H. and Faragalla, H.E.H.(2013). Study of antimicrobial activities of *Guira senegalensis* leaves. *Research Journal of Pharmacology*, **7:31**, 29-36.
- Bello, M. I., Raji, L. M. and Modibbo, A. A. (2013). Phytochemical screening and antimicrobial activity of stem bark extract of *Deterium microcarpum*. *Asian Journal of Biomedical and pharmaceutical Research*. 3(3), CODEN (USA): AJB PAD.
- Cheesebrough, M. (2006). District Laboratory Practices in Tropical Countries. Cambridge University Press, Edinburgh, United Kingdom. 382 – 407.
- Cowan, M.M. (1999) Plant products as antimicrobial agents. *Clinical Microbiology Reviews*. 12(4), 564 – 582.
- Dekant, W. and Vamvakas, S. (2005). Toxicology. In: *Toxicology in Occupational and Environmental Setting*. WILEY-VCHVerlag GmbH& Co. KGaA, Weinheim, 87-89.
- Dubey, S., Maity, S., singh, M., Saraf, S. A., and Saha, S. (2013). Phytochemistry, Pharmacology and Toxicology of *Spilanthes acmella*:A Review. *Advance in Pharmacological Science*. 423750.
- Dutta, K. N., Chetia, P., Lahkar, S., and Das, S. (2014). Herbal Plants Used as Diuretics: A comprehensive Review. *Journal of Pharmacological, Chemical and Biological Science*. **2(1)**,27-32.
- El-Mahmood, A. and Ameh J. M (2009). Antibacterial Activity of Crude Extracts of *Euphorbia hirta* Against Some Bacteria Associated With Enteric Infections. *Journal of Medicinal Plants Research*. **3**, 498-505.
- Gonzales, G. F., and Valeiro, L. G (2006). Medicinal plants from Peru: A review of plants as potential Agents Against Cancer. *Anticancer Agents. Medicinal Chemistry*. **6(5)**, 429-44.
- Havagiray, R. C., Ramash, G., Mehid, A. D., and Sadhara K. (2004). Studies on anti-diarrhoea activity of *Calotropis gigantea* in experimental animals. *Journal of Pharmaceutical Science*. **7(1)**, 70-73.
- Hemalta, M.,Thirumalai, T., Saranya, R., Elumadai, E. K., and David, E (2013). a review on Antimicrobial Efficacy of Some Traditional Medicinal Plants in Tamilnadu. *Journal of Acute Diseases*. **2(2)**, 99-105

- OECD(1998). Guideline for testing of chemicals (TG 408). Repeated dose 90-day oral Toxicity study in Rodents. OECD/OEDC.
- Ogbeba J., and Iruolaji F.O.(2015) Antimicrobial Efficacy of *Guiera senegalensis* and *Prosopis africana* leave extract on some bacterial pathogens. Proceeding of the Academic Conference of African Scholar Publications and Research International on African Sustainable Development 2, No.2.
- Ogbuewu, I. P., Unamba-Oparah, I. C., Odoemenam, V. U., Etuk, I. F., and Okoli, I. C (2011). The potentiality of medicinal plants as the source of new contraceptive principles in males. *North American Journal of Medical Sciences*. **3(6)**, 255-63.
- Oloyed, O.I.(2005). Chemical profile of unripe pulp of *Carica papaya*. *Pakistan Journal of Nutrition*, 4, 379-381.
- Pierre, A.E.D., Ahamed Y.C., Muhammad I.C., Kiessoum K., Estelle Y., Nacoulma O.G.(2013) GC-MS analysis and antifungal activity from gall of *Guiera senegalensis* J.F Gmel (*Combretaceae*) *J. of Applied Phar. Sci.* **3(12)**, 006-012.
- Prakash, O., Kumar, A., and Kumar, P (2013). A jeet Anticancer Potential of Plants and Natural Products: a review. *American Journal of Pharmacological Sciences*. **1(6)**, 104-115.
- Ramara, N., Lereclus, D., 2006. Adhesion and cytotoxicity of *Bacillus cereus* and *Bacillus thuringiensis* to epithelial cells are FlhA and PlcR dependent, respectively. *Microbes and infection*, **8**, 1483-1491.
- Rates, S. M. K (2001). Plants as source of drugs. *Toxicon* **39(5)**, 603-613.
- Sarker, D. S., Latif, Z., and Gray, I. A. (2006). *Natural Products Isolation*. Second Edition. p. 4-70
- Shilpi, J. A., Islam, M. E., Billah, M., Islam K. M. D., Sabrin, F., Uddin, S. J., Nahar, L., and Saker, S.D (2012). Antinociceptive Anti-inflammatory and Antipyretic Activity of Mangrove Plants: A mini Review. *Advance in Pharmacological Science*. 576086.
- Silva, N. C. C., and Fernandes, J (2010). A Biological properties of Medicinal Plants: a review of their antimicrobial activity. *J. Venom. Anim. Toxins Incl. Trop. Dis.* **16(3)**, 402-413.
- Smolinski, M.S., Hamburg, M.A and Lederberg, J.(2003). *Microbial Threats to Health: Emergency, Detection, and Response*. Washington, DC: Institute of Medicine, National Academies Press. pp 203-210.
- Sofowora, E .A. (1982). *Medicinal Plants and Traditional Medicine in Africa*. John Wiley and Sons Ltd., New York, pp. 256-257.
- Stefano, B., Enrico, C., Vivian, O., Paola, B., Serena, C., Paolo, V., Pierangelo, C. and Luca, C.(2014). Antibacterial Activity of Leaf Extract From *Combretum micranthum* and *Guiera senegalensis*. *Research Journal of Microbiology*, **9**, 66-81.

- Singleton, V.L., Orthofer, R. and Lamuela-Raventos, R.M.(1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in Enzymology*, 299, 152-178.
- Trease, G. E. and Evans, W. C. (1978) Pharmacognosy. 11th ed. Bailliere Tindall; London, Phytochemistry : Introduction and General Methods; 2, 227-247.
- Uchechukwu, U. N., Grace, E. O. and Anthony, I. Okoli (2014). Assemblies of *T. indica* extract for antibacterial activity. *International Journal of Molecular Science*. **12(10)**, 6385-6396
- Yadav, U., Hugo, A., Boon, E. K., Saroj, Y., and Krishna, K. S (2010). Indigenous Use and Bio-Efficiency of Medicinal Plants in the Rasuwa District Central Nepal. *Journal of Ethnomedicine*. **6(3)**, 2.

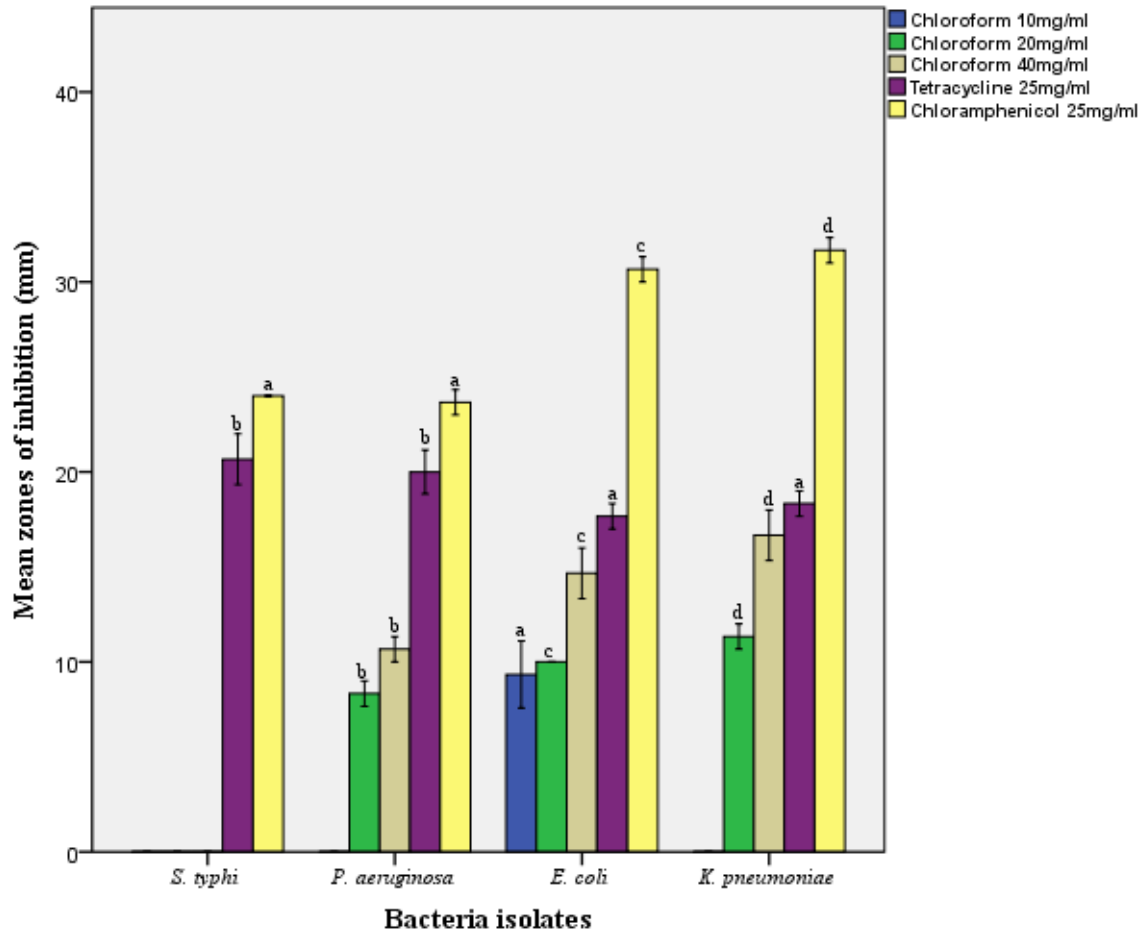


Fig 2: Mean zones of inhibition of chloroform fraction of methanol *G. senegalensis* extract

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.

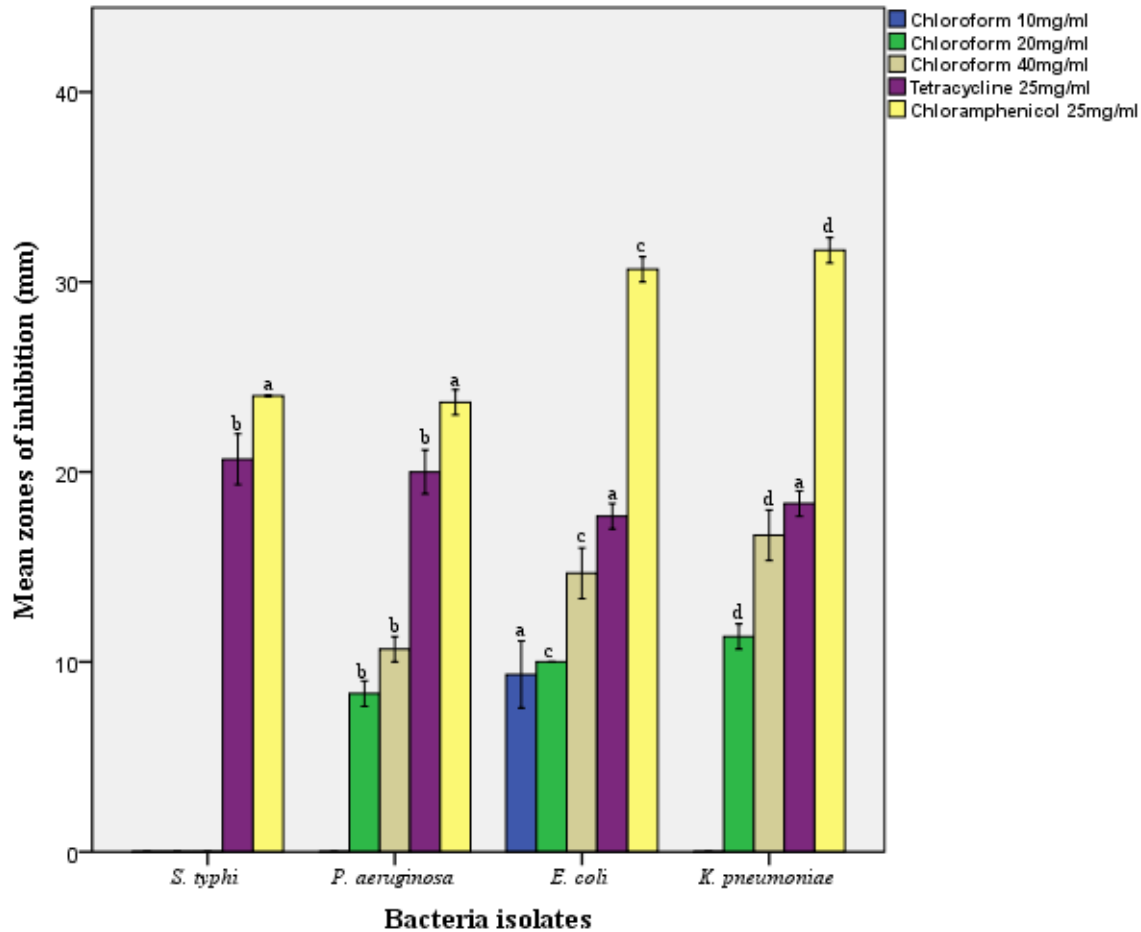


Fig 3 : Mean zones of inhibition of chloroform fraction of aqueous *G. senegalensis* extract

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.