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Antibacterial and Potential Toxicological Evaluation of Leaf Extracts of *Guiera senegalensis* against Gram Negative Bacteria

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Authors' contributions

This work was carried in collaboration among all authors. Author TBS designed the study, wrote the protocol, managed the animals, collected all data, performed the statistical analysis, and wrote the first draft of the manuscript. Authors MG, EMA and AAJ did the literature search and also wrote part of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Objective: To evaluate the antibacterial and potential toxicity effects of leaves extract of *Guira senegalensis.*
Methods: The 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 selected gram negative bacterial (*Echerichia coli, Salmonella typhi, Klebsiela pneumoniae* and *Pseudomona aeruginosa*). Tetracycline and chloramphenicol were used as positive standard drugs. Acute toxicity studies were conducted by administering different doses (100, 200, 400, 600, 800, 1000, 1200 mg/kg) of the extract to eight groups (4 animals per group). A ten weeks sub acute toxicity investigation was also conducted in rats.

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Results: Analysis showed that the extracts contain alkaloids, phenols, tannins, steroids and saponins. Estimation of the phytochemicals revealed high concentration between 87.3±0.5 g/ml and 123.3±0.34 g/ml of phenols, while saponins, flavonids, tannins and alkaloids were present in less amounts and at varied concentrations. The aqueous extract shows appreciable antibacterial activity when tested at 40 mg/ml concentration against all isolates with diameters zone of inhibitions ranging 14.40±1.60 mm to 15.60±0.51 mm. Tetracycline and chloramphenicol demonstrated higher inhibitory zones than the plant extract. Low MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bactericidal Concentration) values were observed for *E. coli, K. pneumonia, P. aeruginosa* except *S. typhi.* The selected safe dose was 800 mg/kg and the LD_{50} value was estimated to be 894.43 mg/kg. Haematological, and biochemical analysis revealed no toxic effects after weeks 1 and 5 of treatment. However, cumulative toxic effects were manifested after week 10 of treatment. The result of liver and kidney histopathology revealed mild necrosis of the hepatocytes and gross nephrotoxicity.
Conclusion: Although the leaf extracts of *G. senegalensis* appeared to be relatively safe, potential hepatic and renal toxicity may occur with prolonged usage.

Keywords: G. senegalensis; haematology; biochemical; phytochemical; antibacterial.

1. INTRODUCTION

Plants are natural reservoir of medicinal agents and are 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 [1].

Plants as a potential source of drugs are of great relevance, especially 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. Numerous studies have shown that medicinal plants are the oldest source of bioactive products with proven efficacy [2]; [3] and are the basic components of several pharmacological classes of drugs [4] such as analgesics [5], anesthetics [6], antibiotics [7], anticancer [8]; [9], anti-parasitic, anti-inflammatory [10], oral contraceptives [11]; [12] and diuretic drugs [13] among others.

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

The rapid increase in the antibiotic resistance of microorganism to available synthetic drugs and their discomforting side effects necessitates the search for alternative source of antibiotics. This study investigated antibacterial and preliminary toxicology of *Guira senegalensis*.

2. MATERIALS AND METHODS

2.1 Plant Processing and Extraction

Fresh leaves of the plant *Guairá 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 the extraction with methanol using Soxhlet extractor apparatus.

2.2 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 conducted according to the methods of [14], and [15].

2.3 Quantitative Determination of Phyto-chemicals

Quantitative estimation of phytochemicals such as alkaloids and saponins was carried out according to [16]; the total phenolic content was measured using [17] method and the flavanoids were measured using Aluminum Chloride colorimetric method. The absorbance of the reaction mixture was performed at different

wavelength for the flavonoid (415 nm), phenols (765 nm), alkaloid (565 nm), saponins (490 nm) and tannins (760 nm) using spectrophotometer.

2.4 Partioning of Extracts

The methanol and aqueous extracts were dissolved in 100 ml of water and partitioned with n-hexane, chloroform, ethyl acetate and aqueous residue. The partition was done starting with non-polar solvent to 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 [18].

2.5 Susceptibility Testing of Plant Crude Extracts and Fractions

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 stick was used to streak onto the surface of the agar plate with the standardized test organism. Two gram of each extract was reconstituted in 5ml of DMSO (200 mg/5ml≈ 40 mg/ml), and 100 µl of the reconstituted extract (40 mg/ml) was added to each of the bored wells and 25 mg/ml of the standard antibiotic drugs (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 37ºC for 18 h. The zones of inhibition were then measured in millimeter.

The above procedure was repeated for the fraction containing 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 (10 mm) 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.

2.6 Acute Oral Toxicity Study

Acute oral toxicity of *Guiera senegalensis* extract was determined in rats according to the OECD (Organization for Economic Community and Development) [19]. 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 in mg/kg) were separately administered to the rats in each group by means of oral dosage cannula. All rats were allowed free access to food and water and while signs of acute toxicity were assessed for a 72 h period. The number of deaths occurring within this period was recorded.

2.7 Repeated Dose Toxicity Test

The study applied the OECD guidelines for the repeated dose toxicity investigation employing forty (40) rats for the test and forty (40) rats for the control group. The selected safe dose (800 mg/kg) was determined from acute toxicity study. The treatment lasted for ten weeks and the administration was done using metal oropharyngeal dosing cannula. Observations were made twice daily for mortality and changes in general appearance or behavior. The body weights were recorded every week. In addition, measurements of food and water consumption were performed weekly.

2.8 Collection of Blood Samples

Four (4) rats were randomly selected at the end of each week and aliquot of the blood collected by cardiac puncture 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).

2.9 Biochemical and Haematological Analysis

At the end of the experiment, animal blood was collected and hematologic analyses were 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, lymphocytes, eosinophil), 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 were assayed [20].

2.10 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 after wards 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 paraffin wax by impregnating cassettes with molten wax at 60ºC 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.

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

3.11 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.

3. 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 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) 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.

Figs. 1 and 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*.

Chloroform fractions of *G. Senegalese* methanol extract showed varying diameters of mean zones of inhibition at concentrations of 20 mg/ml and 40 mg/ml of the extract (Fig. 2) against all isolates except against *S. typhi*

Table 1. 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

where all concentrations (10,20 and 40 mg/ml) did not inhibit growth of the organism. At a concentration of 40 mg/ml, the activity was almost measuring up with that of tetracycline antibiotic in respect to *E. coli* and *K. pneumonia* as shown in Fig. 2.

Chloroform fractions of *G. senegalens* aqueous extract showed varying diameters of mean zones of inhibition at concentrations of 20 mg/ml and 40 mg/ml of the extract (Fig. 1) against all isolates except against *S. typhi* where all concentrations (10,20 and 40 mg/ml) did not inhibit growth of the organism. At a concentration of 40 mg/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. While the standard chloramphenicol was still better in its antibacterial activity than the plant extracts at all concentrations.

Table 3. Mean zones of inhibition of aqueous and ethanol extracts

Extracts (40 mg/ml)	*S. typhi*	*P. aeruginosa*	*E. coli*	*K. pneumonia*
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 (25 mg/ml)	20.67±0.67^{b}	20.00±0.58^{b}	17.67±0.33^{a}	18.33±0.33^{a}
Chloramphenicol (25 mg/ml)	24.00±0.00^{a}	23.67±0.33^{a}	30.67±0.33^{b}	31.67±0.33^{c}

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	Ethyl acetate fraction	Aqueous fraction
Methanol	3.95	4.03	1.66	16.01
Aqueous	7.00	1.83	1.77	13.10

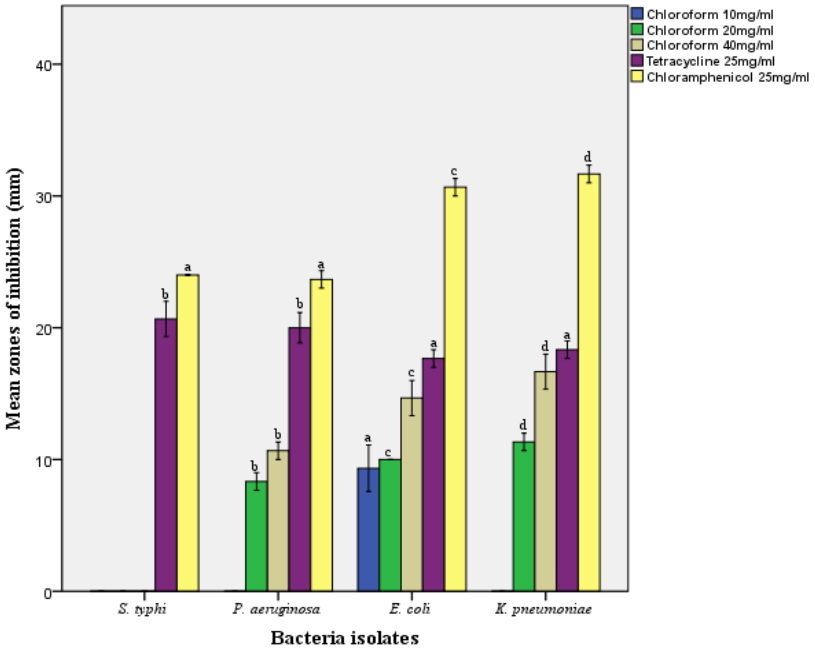

Fig. 1. Mean zones of inhibition of chloroform fraction of aqueous *G. senegalensis* extract

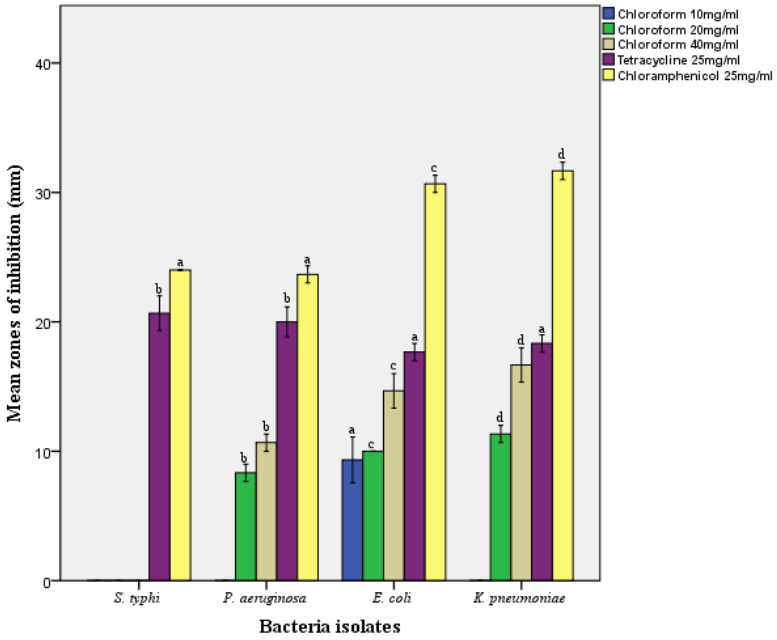

Fig. 2. Mean zones of inhibition of chloroform fraction of methanol *G. senegalensis* extract

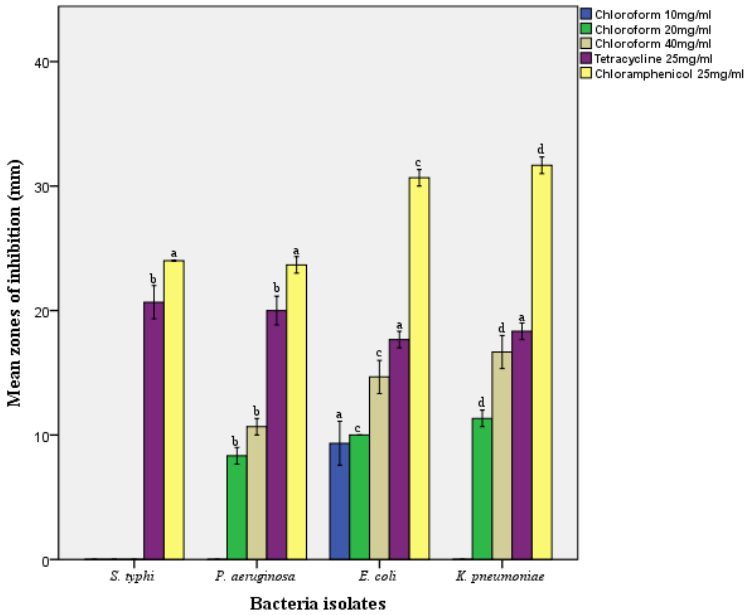

Fig. 3. Mean zones of inhibition of chloroform fraction of aqueous *G. senegalensis* extract

3.1 Toxicity Study

3.1.1 Effects of different doses of crude extracts of *G. senegalensis*

The result of acute oral toxicity assays showed that the group of rats treated with varied concentrations (100, 200, 400, 600, and 800 mg/kg) did not show obvious behavioral alterations after 72 hours of the experiments. Abrupt cessation of physical activity, depressed respiratory and cardiac activity as well as mortality were observed at doses of 1000 and 1200 mg/kg. The LD_{50} dose was calculated to be 894.43 mg/kg, thus, the selected safe dose was established to be 800 mg/kg.

3.1.2 Haematological study

Table 5 shows the results of haematological study. There was a significant increase ($p<0.05$) in the of heamoglobin (Hb), packed 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 plateletes in all groups were not significantly affected when compared with the control groups. The body weights of the rats were significantly reduced after week 5 and 10 in treatment groups (Table 5).

3.1.3 Liver function parameters

Results in Table 6 revealed no significant difference in the serum levels of Alkaline Phosphatase (ALP), Aspartate Aminotransferase (SGOT) and Alanine Aminotransferase (SGPT) enzymes of the treated group and control group at week 1. However, after weeks 5 and 10, there were significant increases ($p>0.05$) in ALP, SGOT, SGPT serum level in treated groups when compared with the control group (Table 6 below). The serum level of albumin, total bilirubin and conjugated bilirubin were not significantly affected. The serum levels of triglycerides decreased in the treated groups (after week 1, 5 and 10), while serum glucose level was slightly affected (Table 6).

3.1.4 Kidney function parameters

The results in Table 6 did not show much difference after treatment with the extract. However, slight increases in serum levels of creatinine, urea, HCO_3^- and K^+ were observed after week 5 and 1, while chlolride level was not affected (Table 7).

3.1.5 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 (Tables 6 and 7). After week 10, mild morphological 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 showed mild intercellular infiltration of the glomerulus when compared to the control (Plates 1-6).

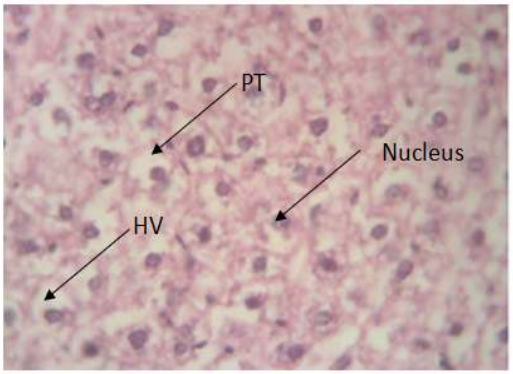

Plate 1. Liver section of control rat showing moderate portal tract (PT) inflammation and interphase hepatitis, moderate congestion and thrombosis of hepatic vessels (HV), moderate pleomorphic hyperchromatic to vessicular nuclei with prominent nucleoli and feathery degeneration with mild hepatocyte necrosis

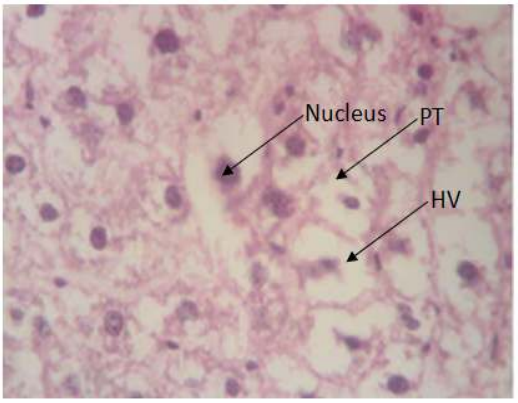

Plate 2. Liver section of rat tested with 800 mg/kg crude extract of *G. senegalensis* showing mild portal tract (PT) inflammation and interphase hepatitis, mild congestion and thrombosis of hepatic vessels (HV), moderate pleomorphic hyperchromatic to vesicular nuclei with prominent nucleoli and feathery degeneration with moderate hepatocytes necrosis

4. 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. [23]) 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 [21], [22], and [23] who identified the same phytochemicals in the plants.

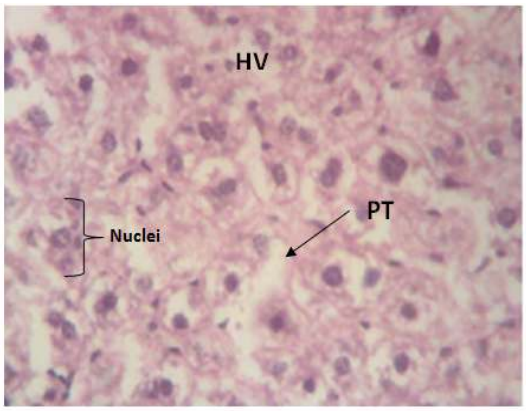

Plate 3. Liver section of rats tested with 800 mg/kg crude extract of *G. senegalensis* showing moderate portal tract (PT) inflammation with interphase hepatitis. Mild congestion and thrombosis of hepatic vessels (HV). Mild pleomorphic hyper chromatic nuclei with irregular nuclear membrane. Feathery degeneration with moderate hepatocytes necrosis

The results recorded by agar diffusion methods indicated that the highest 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 14-15 mm (Table 3) [24] and [25] 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: dimethylsulfuroxide (DMSO). It is well known that different solvents have diverse solubilization capacity for the phytoconstituents. The antibacterial activity of the extract may also be attributed to the presence of the phytochemicals present as reported by [21].

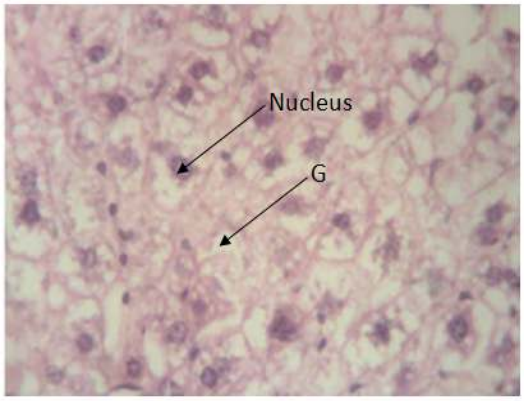

Plate 4. Kidney section of control rat showing moderate cellular mesaugium (define) of the glomerulus (G), tubulointerstitial inflammation, congestion and thrombosis of renal vessels

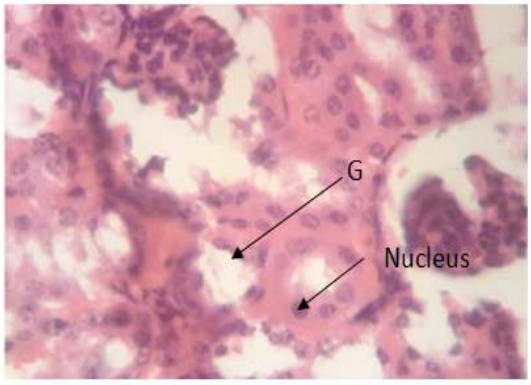

Plate 5. Kidney section of rat tested with 800 mg/kg of crude *G. senegalensis* extract showing mild tubulointerstitial lymphocyte infiltration, haemorrhage/fibrin and mild thrombosis

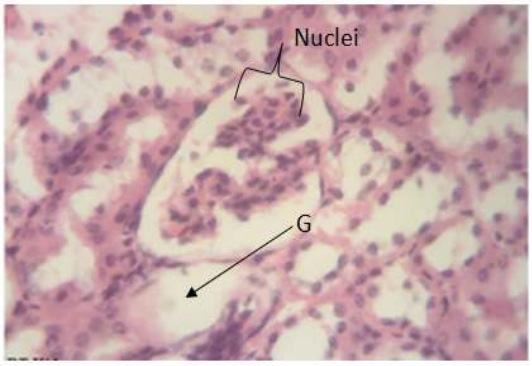

Plate 6. Kidney section of rat tested with 800 mg/kg of *G. senegalensis* extract showing moderatecellular mesangium of the glomerulus (G), tubulointerstitial inflammation and moderate congestion and thrombosis of the renal vesicles

Chloramphenicol 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 [26].

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 some pathogen as observed with respect to *S. typhi*.

The quantitative estimation of phytochemicals in the plant extracts revealed high concetration of

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±o.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}	36.690.19^{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}	29.820.11^{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 error of mean.
Values with the same superscript in the same rows 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 rows 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; HCO_3^- - Hydrogen bicarbonate iron.
Values are expressed in mean ± standard error of mean
Values with the same superscript in the same rows are not significantly different at p>0.05.

phenols (Table 2). Alkaloid and saponin were also detected in the plant in variable concentration, this may explain why the extracts showed good activity against *S. typhi, P. aeruginosa, E. coli* and *K. pneumoniae* despites the reports by researchers [27], [21] of the multidrug resistance strains of the above species of organisms. This result agrees with the work of [28] 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 penetrates 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 [18].

4.1 Toxicity Study of Extracts from *G. senegalensis*

In the acute toxicity assays, LD_{50} values was determined to be 894.43 mg/kgbw, indicating low toxicity. This result conforms with [19] where LD_{50} means that the therapeutic effectiveness of the drug where 50% of the animal died is lethal dose 50 (LD_{50}). The smaller the LD_{50} value, the more toxic is the chemical and. the larger the LD_{50} value, the lower the toxicity. The behavioural profile of all the tested animals revealed that the animals were alert and responded to pain and touch. The animals showed no signs of depression or restlessness. Monitoring the body weight during treatment provides an index of the general health status of the animals; such information may be important for gauging their health [30]. There was significant decreased ($P<0.05$) in the body weight (after week 10) from 182.13±1.47 to164.97±2.10 and 182.22±0.74 to 128.58±3.07 respectively. An effect of the extract in causing drowsiness in all the treated groups was observed for the first 30 minute to 1 h after dosing, compared with control. One mortality was recorded from the treated group throughout the duration of the experiment. Blood is a sensitive index of the physiological changes in an animal in response to any environmental pollutant, and it has been documented that toxicant or potentially toxic substances induce conspicuous and significant changes in the haematological parameters [29].

The haematological parameters demonstrated significant increased ($p<0.05$) in Parcked Cell Volume (PCV) Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) in animals treated after week 1, 5 and 10 with repeated dose of 800mg/kbw. There was slight increase in haemoglobin (Hb), this result indicates that the components in *G.senegalensis* extract did not affected the production of red blood cell negatively (Table 5).

The depletion in the total plasma protein level has also been observed in the present assessment. This may be due to impaired protein synthesis in the damaged liver or to the altered nutritional status of the animals. [30] reported that the synthesis of plasma proteins in the liver was quantitatively and qualitatively affected during liver damage. The decrease in plasma albumin or in the total plasma protein indicated hepatocellular dysfunction or liver disease [28]. Elevated levels of ALT (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) n the test animals after week 5 & 10 observed in this study compared to the control could be attributed to the cumulative effects. It may also be due to pathological changes such as necrosis of hepatocytes, which causes an increase in the permeability of the cell membranes, resulting in the release of aminotransferases into the blood stream [30] and [31]. ALT (SGOT) has been reported to be a marker with a high specificity for acute hepatocellular injury (Table 6) and. ALP as 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. and alteration in the activity of serum Aspartate Aminotransferase (SGOT) may produce consequential effects on the metabolism of amino acid and its biochemical regulation [31] (Table 6).

There was significant increased in plasma urea concentration in rats compared with the control (after weeks 1, 5 and 10). Healthy kidney filterate urea and creatinine waste product of protein metabolism out of the blood. High level of urea in the blood is an indication of impaired kidney. In renal failure, there is an increase in metabolic waste products, especially nitrogenous substances like plasma urea and uric acid. An elevated plasma urea level has been linked to elevated non-protein nitrogen in diseases associated with nephrotoxicity [31].

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. 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 (Table 7).

The mild changes in the histoarchtecture of the liver and kidney may be as a result of accumulation of the toxic effects of the extracts. Also high content of alkaloid present in *G. senegalensis* could be responsible for the liver injury. [31] reported on the effect of alkaloid from the galls of *G .senegalensis* on the liver function in rats Toxicity of herbal preparations could be attributed to several factors including contamination with pesticides, microbes, heavy metals, toxins or adulteration with orthodox drugs (Plates 1-6).

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 administration.

5. CONCLUSIONS

1. Extracts of *G*. *senegalensis* demonstrated appreciable spectrum of antibacterial 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 used in herbal medicine for the treatment of ailments caused by these pathogens due to their low toxicity.

CONSENT

It is not applicable.

ETHICAL APPROVAL

We hereby declare that the “Principle of laboratory animal care” (NIH publication no. 85-23, revised 1985) were followed, as well as specific national laws were applicable. All experiments were examined and approved by the Federal University of Technology Minna ethical committee for the use of laboratory animals.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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