



## Antibacterial and Toxicity Evaluation of Stem Bark Extract of *Kigelia Africana* (Lam.) Benth

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**Abstract:** The study was to investigate antibacterial and toxicity evaluation of methanolic stem bark extracts of *Kigelia africana* (SBEKa). The antibacterial activities of the extracts were determined against one Gram-positive (*Staphylococcus aureus*) and three Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella* spp). Distilled water was used as negative control and ciprofloxacin was used as a positive control. The SBEKa showed remarkable activity against various bacterial strains as compared to positive control. *S. aureus* and *Salmonella* spp were proved as highly sensitive strains while *E. coli* and *pseudomonas* were the resistant strain as the extracts formed no inhibition zone against it. These studies determine the phytochemical constituents as well as acute toxicity and sub-chronic toxicity test on plant stem bark. The acute toxicity was evaluated in rats according to the guideline of Organization for Economic and Cultural Development (OECD). The level chosen is the one for which we can expect to see mortality among treated animals. The animals were daily observed for 28 days after treatment and no mortality was observed. The animals were sacrificed. The results showed that the LD<sub>50</sub> by oral route in rats was greater than 5000 mg/kg body weight. The extracts were found to contain alkaloids, Saponins, Flavonoids, Anthraquinones, and steroids were present in high amount while Glycosides in moderate amount. Tannins, Cardiac glycosides and Balsams were present in trace amount, while volatile oils were totally absent. At lower concentration SBEKa exhibited antimicrobial and these may justify the medicinal uses of the plants for treatment of microbial infections.

**Keywords:** *Kigelia africana*; Toxicity; antibacterial activity; phytochemical constituents.

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## 1. INTRODUCTION

Traditional medicines are used globally and have a rapid growing economic importance. In developing countries, traditional medicine is often the only accessible treatment available. In some Asian and African countries, up to 80% of the population relies on traditional medicine for their primary health care needs [1]. However, it is very

easy for bacteria to enter through a cut on the skin and penetrate to the rest of the body. Bacteria colonize wounds within 48 hours after injury such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Streptococcus* spp may all cause infection and this prolong inflammatory phase of wound healing [2].

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*Kigelia* is a genus of flowering plants in the family Bignoniaceae. The genus comprises only one species, *Kigelia africana*, which occurs throughout tropical Africa. It is also known as sausage tree (long sausage – like fruit) or cucumber [3]. In Nigeria, the stem bark is used in the treatment of wound and microbial infection. The stem bark of the plant has wound healing and antibacterial properties [4]. The *kigelia* plant have medicinal properties not only because of its perceived characteristics such as bitterness, astringent taste or smell but also because of forces that it seems to emit in connection with its location, orientation and association with other plants. It has long history of use by rural and African countries particularly for medicinal purposes by certain aboriginal people [3]. In Malwi during famine the seeds are roasted to eat. Baked fruits are used ferment beer and boiled ones yield a red dye.

Microbial infections of various types of wounds are a challenge to the treatment of wounds and wound healing. The study was to investigate antimicrobial and antioxidant properties of methanol leaf and stem bark extracts of *Kigelia africana* and methanol leaf and root extracts of *Strophanthus hispidus* and also to determine wound healing properties of the extracts. The antimicrobial activities of the methanol extracts were determined against two Gram-positive and two Gram-negative bacteria and a fungus using agar diffusion and micro-dilution methods [5].

Most commonly traditional healers used it to treat a wide range of skin ailments like fungal infections, boils, psoriasis and eczema. It also has internal application including the treatment in dysentery, ring worm, tape-worm, post-partum haemorrhage, malaria, diabetes, pneumonia and toothache [6]. *Kigelia africana* of the benth family (Rawiyya in hausa) is abundant in the tropics and widely used in Nigeria as herbal remedies for various ailments such as diarrhoea, malaria, rheumatism, retained placenta and dizziness [6]. Plant parts, especially the stem bark is reputed in traditional medicine and is popularly used for wounds, ulcers and combating infections [7]. The stem bark extract possessed antidiabetic effect and could be used in treatment and management of Diabetes mellitus [8].

## 2. COLLECTION OF PLANT

The stem bark of the plant was collected from villages around Taura Local government, Jigawa state, Nigeria. Botanical identification was done at Botany unit, Bayero University Kano and

voucher specimen was deposited in the herbarium of the same institution for reference.

### 2.1 Extraction

The stem bark was washed with clean water, room dried and pulverized into coarse powder. Two hundred gram (200g) of the powdered was extracted with methanol (1000mL) at room temperature for 72 h. The extract was filtered through whatman filter paper (No 1) and concentrated by removing the solvent completely under reduced pressure. The yield of the extract was 16.7% w/w and was reconstituted in sterile distilled water for Antibacterial, toxicity and phytochemical studies. The extraction was repeated using 200g of the powdered part.

### 2.2 Activity Guided Fractionation

This was done by using methanol and different (hexane, chloroform and ethylacetate) organic solvents as described by [9, 10]. The powdered stem bark of *kigelia africana* (200g) was extracted with methanol (1000mL) at room temperature overnight. The extract was filtered and partition in Hexane (HX) separately (300mL) and clarified by further filtration. Evaporation of HX fraction in an oven at 45°C yielded residues (Table1). The aqueous filtrate (water-methanol) of the extract fraction was further partitioned (to obtain fractions of different polarities) with chloroform (300mL) and ethylacetate (300mL) separately. They were evaporated in oven residues to obtain residues (Table 1). All procedures were repeated to obtain more residues. This procedure enable us remove by erasing the possible contributory effects of the organic solvents.

### 2.3 Toxicity Studies

#### 2.3.1 Acute toxicity studies

This was analyzed by the procedures of Organization for Economic and Cultural Development [11].

#### 2.3.2 Sub-chronic toxicity studies

Twenty four (24) albino rats weighing 60-100g were distributed into three (3) groups. Group 1 received 10mL/kg body weight/day of normal saline which served as control. The group 2 and 3 were orally administered with graded doses of 500 and 1000mg/kg body weight/day for 28days respectively. The body weights of individual animals were evaluated weekly for the duration of the experiment. After 28days, the animals were sacrificed and the blood collected was centrifuged to obtain sera for biochemical assays.

### 3. RESULTS

**Table-1: Amount Of Residue Obtained after Extraction with 200g Stem Bark Extract of *Kigelia africana*.**

Fractions	Amount Recovered (%)
Hexane	0.86
Chloroform	0.15
Ethyl acetate	1.54
Last-remaining water-methanol	16.7

**Table-2: Phytochemical constituents of the stem bark of *kigelia Africana*.**

PHYTOCHEMICALS	METHANOL EXTRACT
FLAVANOIDS	+++
ALKALOIDS (Wagners/ mayer's)	+++
TANNINS	+
CARDIAC GLYCOSIDES	+
ANTHRAQUINONE	+++
SAPONINS	+++
STEROIDS	+++
VOLATILE OILS	-Ve
GLYCOSIDES	++
BALSAMS	+

Key: + = Trace; ++ = Moderate; +++ = High; -Ve = Not present

**Table-3: Antibacterial activity of stem bark extract of *Kigelia africana***

Fractions	Concentration (mg/ml)	Staphylococcus Aureus	Escherichia coli	Pseudomonas Aureus	Salmonella spp
L-R	20	9.00±3.61	5.00±0.58	3.70±0.60	6.00±8.70
	30	9.00±1.73	6.30±2.30	5.70±3.05	5.00±1.00
	40	10.70±2.08	5.30±2.50	6.0±0.00	6.70±2.08
	50	12.30±3.06	5.20±2.08	4.70±1.15	14.70±1.50
Hexane	20	9.7±4.00	3.70±1.50	4.00±1.00	7.30±8.30
	30	10.70±3.20	5.00±1.70	6.00±1.00	6.70±2.90
	40	11.60±1.20	4.60±2.30	6.70±1.50	6.30±2.08
	50	13.00±3.60	3.70±2.08	4.70±2.50	13.60±1.52
Ethylacetate	20	6.70±4.10	4.60±1.50	4.00±2.60	7.30±6.10
	30	7.00±1.00	6.00±4.00	7.20±3.50	7.30±3.00
	40	9.00±1.70	5.50±1.00	7.30±1.50	8.30±4.70
	50	10.70±1.50	5.30±3.20	5.00±1.70	11.00±1.00
Chloroform	20	8.00±2.00	5.71±1.50	9.00±1.00	6.00±2.00
	30	7.30±2.08	7.00±3.00	11.60±4.04	10.60±3.05
	40	10.00±2.00	6.00±3.61	10.30±1.50	10.70±2.08
	50	12±2.00	6.00±3.60	6.30±2.00	10.00±2.00
Ciprofloaxin	40	9.00±1.00	5.70±2.10	10.00±3.00	10.00±2.30
Water	2.0.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00

L-R- last remaining water methanol fraction. Values are mean±SD. Values > 6mm indicate some activity. All values are significantly ( $p < 0.05$ ) from the negative control (water) by using ANOVA.

Hole in Plate Bioassay

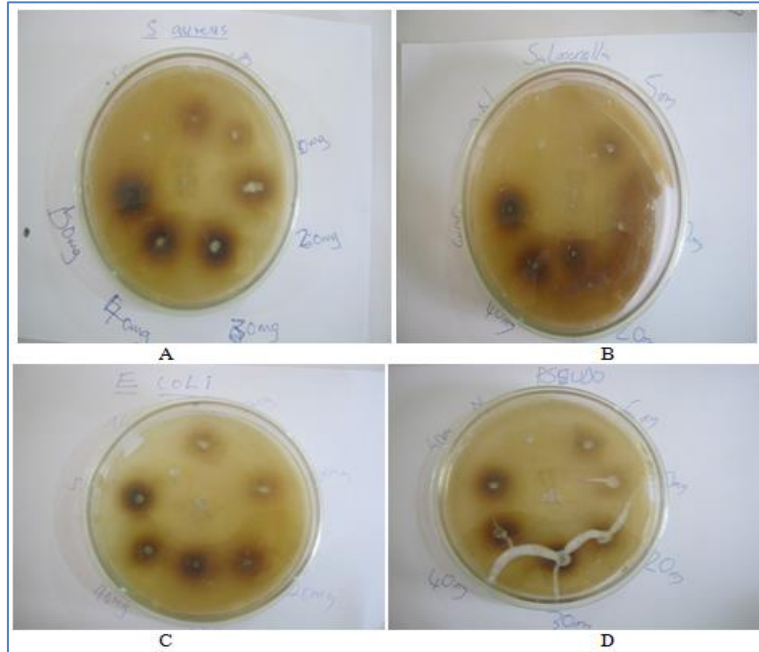


Fig-1.A. Antimicrobial activity of *Kigelia africana* on *Staphylococcus aureus*. B. antibacterial activity of *k.africana* on *salmonella* spp. C. antibacterial activity of *k.africana* on *E. coli*. D. Antibacterial activity of *k.africana* on *Pseudomonas aureus*

**Acute toxicity**

Oral administration of methanolic stem bark extracts of *K. africana* (5000mg/ Kg body weight), did not caused mortality but few clinical sign of toxicity in rats in the first 24 hours and during the

28 days observation period. The animals showed some behavioral changes like excitement, slow movement, weight reduction and loss of appetite. Therefore, the medium lethal dose (LD<sub>50</sub>) is greater than 5000mg/kg.

Table-4: Acute toxicity test of stem bark extract of *Kigelia africana*

METHOD	LIMIT TEST DOSE	OBSERVATION PERIOD	SIGN OF TOXICITY	MORTALITY	LD <sub>50</sub> VALUE
OECD 423 Guidelines (2001)	5000 mg/kg	28-days	Nil	0	> 5000 mg/kg

**Subchronic toxicity**

Effect of methanolic stem bark extract of *k. africana* after 28 days treatment has shown in figure 2. The extract at a dose of 1000 mg/kg produced significant increase (p<0.05) in serum AST, ALT,

total protein, total bilirubin and albumin levels against the control. The result also showed significant decrease (p<0.05) in serum AST at a dose of 500 mg/kg of the test extract.

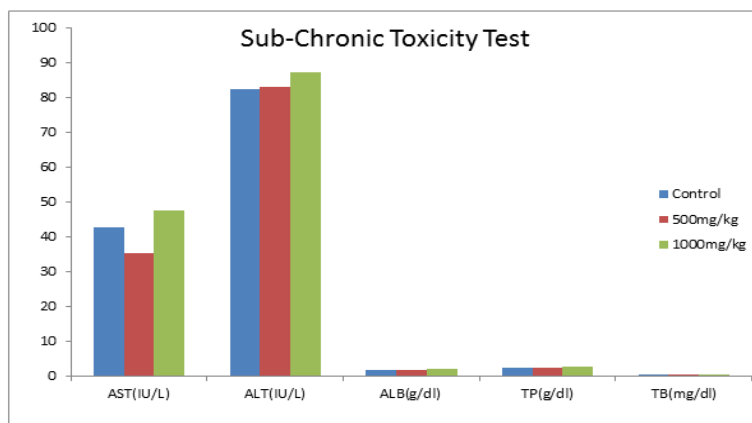


Fig-2: Effect of methanolic stem bark extract of *K. africana* on liver markers.

#### 4. DISCUSSION

The antibacterial activity of *Kigelia africana* bark extract against *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aureus* and *Staphylococcus aureus* by disc diffusion method showed that the methanolic bark extracts were found to exhibit different antibacterial activities. The inhibition against *Escherichia coli* and *Staphylococcus aureus* was moderate and less inhibition was associated with *Pseudomonas aureus*. Chloroform extracts inhibited moderate activity against *Staphylococcus aureus* and the other strains exhibited less activity. The aqueous extracts exhibited less activity against *Staphylococcus aureus* and all remaining strains showed very poor activities. These results were compared with standard antibiotic ciprofloxacin as a standard. In this study, bacterial inhibition was seen maximally in higher concentrations at 40 and 50mg/ml of the extract. The ineffectiveness of the extract in the n-hexane and chloroform solvents might be due to the inability of the solvents to extract the active components responsible for antibacterial activity or these solvents might have denatured the active substances.

Phytochemical screening of the stem bark of (*Kigelia africana*) extract was found to contain some secondary metabolite. From the result, flavonoids, alkaloids, tannins, glycosides, balsams, cardiac glycoside, anthraquinones, saponins and steroids were found to be present in methanol extract. These phytochemicals have been reported to have pharmacological properties [12]. They are used by phytochemists in the production of drugs. They are used as growth regulators and as insect repellents, example alkaloid. They are also used in synthesis of steroid hormones; fire extinguisher etc. The use of secondary plant constituents as drugs by a large number of phytochemists is an indication that medicinal plants are important natural sources of potential new drugs. The residues obtained after extraction and fractionation procedure are presented in table 1, antibacterial activity and phytochemical analysis of the stem bark extract of *K. africana* are presented in table 2 and 3.

According to the OECD (2001) guidelines, substances with an LD<sub>50</sub> value of greater than 5000mg/kg through the oral route are regarded as being safe. Acute toxicity studies of the root extract reveal some behavioral changes such as excitement, slow movement, weight reduction and loss of appetite when 5000mg/kg body weight of *K. africana* bark extracts were administered. No animal died and thus the LD<sub>50</sub> obtained was greater than 5000mg/kg. In subchronic toxicity (figure 2) showed a significant increase in serum AST, ALT, total protein, total bilirubin and albumin levels as

compared to the control group. Transaminases such as AST and ALT are well known good indicators of liver functions and used as biomarkers to conclude the probable toxicity of drugs and xenobiotics. Normally, destruction to the liver parenchymal cells will result in an increase of AST and ALT in the blood. In the present study, the extract produced a significant decrease in serum levels of AST and ALT. This suggests that sub-chronic administration of the extract neither altered hepatocytes of rats nor the normal metabolism of the animals only but also may possess hepatic pharmacological properties. It could also be useful for evaluation of safety and toxicity of plant based chemicals. The significantly ( $p < 0.05$ ) raised activities of ALT and AST may have resulted from possible necrotic injury of the liver and cholestasis [13, 14]. ALP is a marker enzyme for the plasma membrane and endoplasmic reticulum. Alkaline phosphatase (ALP) is typically employed in the assessment of functional integrity of the plasma membrane [15]. High activities of ALP in the serum result from increased synthesis of the enzyme. High ( $P < 0.05$ ) serum TBL and ULBL with decrease of albumin indicate defective liver excretory function and impaired synthetic function of the liver [16].

#### 5. CONCLUSION

It is clear that from the result, the stem bark extract of *K. africana* has antibacterial properties. The plant extracts have great potentials as antimicrobial principles against microorganisms and that can be used in the treatment of infectious diseases caused by resistant microorganisms. Although the LD<sub>50</sub> value of the extract is greater than 5000 mg/kg, the extract is relatively toxic at high dose. The plant at higher dose may be potentially toxic to the liver. Low doses of the stem bark extracts should be cautiously used.

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