International Journal of Sciences: Basic and Applied Research (IJSBAR)

ISSN 2307-4531

(Print & Online)

http://gssrr.org/index.php?journal=JournalOfBasicAndApplied

Wound Healing, Antioxidants and Toxicological Properties of Root Extracts of *Kigelia africana* (Lam.) Benth

Hassan S. W. [a]*, Mshelia P. Y. [b], Abubakar M. G. [c], Adamu Y. A. [d], Yakubu A. S. [e]

[a,b,c]*Department of Biochemistry,* [d]*Department of Veterinary Medicine,* [e]*Department of Veterinary Surgery and Radiology, Usmanu Danfodiyo University, P.M.B. 2346, Sokoto, Nigeria*

[a]*hassanwara@gmail.com*

[b]*patmshel@gmail.com*

[c]*magusau@hotmail.com*

[d]*yunusaadamu2000@gmail.com*

[e]*rahmsad@yahoo.com*

Abstract

The root extracts of *Kigelia africana* were screened for antibacterial and wound healing properties, using hole-plate bioassay and excision wound model on rats, respectively. Catalase activity, glutathione level and lipid peroxidation were assayed in the granulated tissues and liver homogenates. Chemical compositions of the root were determined using standard methods. Complete wound healing was observed on day 16 in group administered with 120mg/ml and on the 19th day in groups administered 90 and 60mg/ml of the extract. Clinical features indicate redness, scab formation, exudations and some other typical changes. The control and antibiotic treated groups show more redness compared to third day.

---

* Corresponding author. Tel.: +2348036355866

E-mail address: hassanwara@gmail.com.

The hydrolytic and organic solvent fractions show significant ($p< 0.05$) inhibitory activities on wound contaminants *Escherichia coli, Streptococcus pneumoniae, Pseudomonas aeruginosa* and *Staphylococcus aureus* at concentrations of 30 to 120 mg/ml and the inhibitory activities were dose dependent. The anti-oxidant activities of catalase and glutathione were increased significantly ($p<0.05$) depending on the dose and reduction in lipid peroxidation was observed. Phytochemicals detected included alkaloids, saponins, tannins, glycosides, steroids, cardiac glycosides, triterpenoids and saponin glycosides. The LD_{50} of the root extract was greater than 5000mg/kg. Higher (3000 mg/kg) dose of the extract was toxic at sub-chronic administration and lower doses could be used in the treatment of wound and bacterial diseases.

Keywords: *Kigelia africana*; wound healing; antioxidants; antibacterial activity; toxicity

1. Introduction

Wound healing involves in array of processes namely induction of acute inflammatory response, parenchymal and connective tissue regeneration and extracellular matrix protein synthesis [1]. Others are connective tissue cell remodeling and wound strength acquisition. Elements such as silicon, zinc, manganese and copper were reported to be involved in wound healing [2]. *Staphylococcus aureus, Streptococcus pyrogens, S. pneumoniae, Klebsiella pneumonia* and *E. coli* are some of the microorganisms infecting wounds **[3].** Several classes of antibiotics are used in the treatment of wound infection both their side effects are becoming apparent. The situation is compounded by resistance to the antibiotics used against these pathogens. Consequently, research attention has now been focused on extraction of biologically active compounds from plants [4].

Several medicinal plants and their phytochemical constituent(s) were used traditionally without toxicological assessments including the roots of *Kigelia africana*. Herbal plants have great beneficial applications in wound healing [5]. Among them, are roots of *Mimosa pudica*, roots of *Paulinia pinnata*, leaves of *Memecylon edule* Roxb, latex of *Carica papaya* and root bark of *Calotropis gigantea* [6,7,8,9].

Reactive oxygen species interfere in the process of healing wound as they have deleterious effects on tissues and cells and they degrade absorbable synthetic biomaterials [10]. **S**cavenging of free radical exert important functions of diminishing, inactivating and elimination of reactive oxygen species. Using such agents topically improves wound healing significantly and also have protective effect on tissues [11]. *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 [12]. Plant parts, especially the stem bark is reputed in traditional medicine and is popularly used for wounds, ulcers and combating infections [13]**.** The plant is associated with analgesic, anticancer, antidiarrhoeal and anti-inflammatory properties [14,15,16,17,]. The stem bark and leaves are employed for wound healing [2,18]. As far as we know, there is no publication on wound healing, antibacterial, antioxidant and toxicological properties of the root extracts of *Kigelia africana*. Therefore, this study reports the evaluation of the wound healing activity, antioxidant and toxicological properties of the roots of *K. africana*.

2. Materials and methods

2.1. Reagents, drugs and equipment

Analytical grade reagents and chemicals were used.

2.2. Experimental albino rats

Albino rats (males and females) weighing 100-150g were obtained from the Department of Biological Sciences (animal farm), Usmanu Danfodiyo University, Sokoto, Nigeria. The albino rats were given water and food *ad libitum*. They were kept under laboratory conditions and acclimatized for 7 days before the research commenced.

2.3. Plant collection

K. africana was identified at Department of Biological sciences, botany unit, Usmanu Danfodiyo University, Sokoto, Nigeria and a specimen voucher (025) was prepared and deposited in the herbarium of the same department.

2.4. Plant processing

The roots were washed and dried in the sun to a constant weight and pulverized with laboratory pestle and mortar and sieved with a $1mm^2$ sieve. The powdered product was preserved in an air tight container until required.

2.5. Preparation of Root Extract

Two hundred grams (200g) of the powdered root was dissolved in1600ml of 50% methanol at room temperature for 48hrs and stirred at intervals of 30mins. The extract was later filtered using a clean, sterile, white muslin cloth and was re-filtered again using a Whatman filter paper No1. The filtrate obtained was evaporated using rotary evaporator at a temperature of 45^0C. The residue (60.9g) obtained was kept at 4^0C until use. Activity-guided fractionation procedures of Morris and Aziz [1976] and Springfield and Weitz [20] were adopted for antibacterial studies with modifications. Twenty grams (20g) of powdered leaf were extracted with methanol water (1:1, 500ml) at room temperature overnight. Filtration and partitioning of the extract in n-hexane and further clarification using filtration was conducted. The solvent was evaporated to dryness completely (45^oC) producing a residue (4g). All the other residues obtained were also dissolved in water (sterilized) and tested for activity against the bacteria employed. The aqueous methanolic filtrate was also partitioned (to obtain fractions of different polarities) with ethylacetate (250ml) produced a residue of 4g. The hydromethanolic extract wash re-constituted by dissolving the dried evaporated samples of the extract in distilled water for phytochemical, wound healing and antioxidant properties (40% was for the toxicological studies). All the other residues obtained were also reconstituted in sterilized distilled water and screened for antibacterial activity.

2.6. Phytochemical screening of extracts

Phytochemical screening was conducted according to procedures of Harborne [21], Trease and Evans [22] Sofowara [23] and El-Olemyl *et al*. [24].

2.7. Evaluation of wound healing

The wound healing activity was evaluated using the excision wound model [25] with some modifications. Thirty six (36) rats were grouped into six (6) groups of six (6) rats each. Each animal was anesthesized using diethylether. The skin's hair at the back was clipped and the area disinfected (using 70% ethanol). An incision (1.5cm in diameter, horizontal and vertical) was made and dissected out carefully. The area of the wound was measured immediately and recorded in cm. Treatment with the extract commenced immediately after the wound incision by applying a drop (twice daily) of 30, 60, 90 and 120mg/ml of the hydromethanolic root extract of *K. africana* on the wound to four test groups. Controls were also treated as above with sterile water (negative control) and 40mg/ml of procaine penicillin (positive control). The area of the wound was measured on the following days 1, 4, 7, 10, 13, 16 and 19th day after the excision. The wound healing activity of the extract was measured (vertically and horizontally) in cm.

2.8. Antibacterial activity

The antibacterial activity was assessed using hole in plate bioassay procedures of Hugo and Russell [26] and Vlietinck *et al*. [27]. Cultures of pure organisms were transfered onto Muller-Hinton nutrient broth (product of Oxoid, England). Incubation was done for 24 hours at 37^0C. Dilution was done using nutrient broth (sterile) to a density of 9×10^8 cfu /ml which is Mcfarland test tube 3. On the surface of Muller-Hinton agar plates, a suspension was streaked for confluent growth using sterile swab. Holes (four, 6mm) were drilled into the agar at 30 to 120mg/ml concentrations. The root extracts were then poured into these wells. Ciprofloxacin, 90mg/ml (a product of Maxheal pharmaceutical India) was used as a reference drug. The agar plates were incubated at 37^0C overnight. The activity was measured and recorded for inhibition zones beyond 6mm. The data (antibacterial), was analyzed for significance using one way analysis of variance.

2.9. Antioxidant activity

Evaluation of Antioxidant activity in Granulated Tissue

The antioxidant activity was assayed out using the dead space wound healing model by Udupa et al. [28] with some modifications. The granulated tissue formed on the wound surface was collected at the point of epithelization into a test tube containing phosphate-buffered saline for assay of Superoxide Dismutase, Reduced Glutathione (GSH), catalase and free radical scavenging activities.

2.9.1. Evaluation of In Vivo antioxidant activity

Eighteen (18) rats were divided into three (3) groups of six (6) animals each. The animals were administered with 200, 400 and 600mg/kg body weight (b.w.) of the extract once daily. After 14days, the rats were sacrificed and the liver of the rats were removed. The organ (liver) was dipped in 0.86% chilled saline to remove red blood cells and suspended in 10% 0.1M phosphate buffer (chilled, pH 7.4) and macerated into small pieces. Homogenization and centrifugation were done. The resultant post mitochondrial supernatants were used to assay for Superoxide Dismutase (SOD), Reduced Glutathione, catalase and lipid peroxidase.

2.9.2. Determination of reduced glutathione

This was determined using the method of Patterson and Lazarow [29].

Measurement of Tissue Lipid Peroxidation (Thiobarbituric Acid Reactive Substances (TBARS- Method)

Fifty microlitres (50µl) of the serum was diluted to 500µl with double deionized water; 250µl of thiobarbituric acid (1.34%) was transferred, then trichloacetic acid (40% equal volume). Shaking and incubation were done (at 90°C, hot boiling water, 30 minutes). After the tubes were cooled at room temperature, the absorbances were read (at 532nm). The content of malondialdehyde was estimated by extrapolation [30].

2.9.3. Catalase assay

Catalase was assayed by the method described by Beers and Sizer [31].

2.10. Toxicity studies

2.10.1 Acute toxicity studies

This was analyzed by the procedures of Organization for Economic and Cultural Development [32].

2.10.2. Sub-chronic toxicity studies

Thirty-six (36) albino rats weighing 60-180g were distributed into six (6) groups. The rats (groups 2, 3, 4, 5 and 6) were orally administered with graded doses of the LD_{50} of the extract (10, 20 40, 60 and 80% of the LD_{50} equivalent to 400, 800, 1600, 2400, and 3200 mg/kg b.w. once daily for 28days respectively). Animals in group one served as control (0.00mg/kg) and received distilled water. The body weights of all the animals were taken before and every week for the duration of the experiment. After 28days, the animals were sacrificed and the blood collected was centrifuged to obtain sera for biochemical assays. The kidneys and liver of the animals were removed and preserved in 10% formalsaline. Histopathological specimens of the organs were prepared as described by Rivera *et al.* [33].

2.10.3 Clinical chemistry

Aspartate Amino Transferase (AST) and Alanine Amino Transferase (ALT) were assayed[34]. The assay of Alkaline Phosphatase (ALP) employed the procedure of Rec [35]. Total Bilirubin (TBL), Conjugated Bilirubin

(CBL) and unconjugated Bilirubin (UCBL) were estimated by the methods of Jendrassik and Grof [36] and Sherlock [37]. Albumin was assessed using Cheesbrough [38]. Urea was estimated using Wybenga *et al*. [39]. Electrolytes and creatinine were done by the methods of Uriyo and Singh [40] and Henry [41] respectively. Uric acid was by the method of Collins and Diehl [42] and Morin and Prox [43].

2.10.4 Histopathological assessment

Liver and kidney of the rats were fixed in formalsaline, dehydrated in ethanol (50-100%). The tissues were cleared and embedded in xylene and paraffin respectively. A 4-6µM thick were cut. Staining was done with Hematoxylin and Eosin (H-E) and photomicroscopic examination conducted according to Roy *et al*. [44].

2.11. Statistical analysis

The results were presented (mean ± standard error of mean). Data from the groups were subjected to one way Analysis of Variance, Graph Pad Instat (Benferoni compare all columns). Values of $p<0.05$ were considered as statistically significant.

3. Results

3.1 Percentage yield and phytochemistry

The percentage yield of hydromethanolic root fraction of *K. africana* was 14.07% w/w, while n-hexane and ethylacetate yielded 0.69% w/w and 0.62% w/w respectively. Phytochemicals of the hydromethanolic root fractions revealed tannins, steroids, alkaloids, saponin glycosides, cardiac glycosides, triterpenoids, glycosides, anthraquinones but flavonoids were not detected.

3.2 Wound healing

The wound healing activity results of the hydromethanolic fractions of the root of *Kigelia africana* are shown in table 1. The roots of *K. africana* fractions have significant ($p<0.05$) effect on wound healing. The extract was able to effectively reduce the wound healing time when compared to distilled water and Procaine penicillin. All the different doses of the extract 30, 60, 90 and 120mg/ml had a significant wound healing activity. Their effect on the wound was dose dependent with 120mg/ml having the highest effect on 4th day of administration. At 90mg/ml, the effect was already visible on the 7th day of administration. Thus at 60, 90, and 120mg/ml, complete healing of the wounds was observed on 16 to 19th day. The 30mg/ml dose was least effective and the healing was not completed on the 19th day.

Characteristic clinical features of different stages of wound healing such as exudation, reddening, dryness, pigmentation and cicatrisation were observed in animals treated with the roots extracts (table 3). Exudation was only seen in those treated with procaine penicillin or distiller water while faster reddening, dryness, pigmentation and cicatrisation was observed in the groups treated with the root extract.

3.3 Antibacterial activity

The hydromethanolic root extracts of *Kigelia africana* had the highest inhibition against the microorganisms employed. The effect of the n-hexane extract was minimal when compared to positive control and the hydromethanolic extract. However, it was more effective than negative control and the ethylacetate extract. The ethylacetate extract did not show any activity against all the microorganisms employed (Table 2).

3.4. Toxicity studies

3.4.1 Acute toxicity

Oral administration of hydromethanolic root extracts of *K. africana* (5000mg/ Kg body weight), the animals showed some behavioral changes like excitement, respiratory distress, slow movement, weight reduction and loss of appetite. There was also death of (1) one animal. Therefore, the medium lethal dose (LD_{50}) is greater than 5000mg/kg.

3.4.2. Subchronic toxicity

The effect of the hydromethanolic root extract after 28 days treatment is presented in table 4. Total protein, total bilirubin, alkaline phosphatase and the activities of transaminases increased significantly at 3200 mg/kg against the control (table 4). From the renal function indices, an increased ($p<0.05$) in urea was observed in groups administered with dosages of 2400 and 3200mg/kg. Uric acid was raised ($p<0.05$) at the highest dose level (Table 5). The sodium levels decreased ($p>0.05$) generally while potassium levels were raised non significantly ($p>0.05$).

3.4.3. Histopathological

From histopathological analysis, the kidney of rats administered with 400, 800, 1600 and 2400mg/kg of the extract showed normal glomeruli and tubules. In rat administered with 3200 mg/Kg, a mild nephritic distortion was observed. In the liver of the rats administered 400, 800, 1600 mg/Kg, normal hepatocytes were observed with no sign of any lesion while those administered 2400 and 3200 mg/Kg showed some degree of distortion of the hepatic structure (Table 8).

3.5. Antioxidants

The levels of antioxidants are shown in tables 6 and 7. Catalase and reduced glutathione levels were raised in all the groups administered with the extract against the control group. Lipid peroxidation decreased in a significant ($p<0.05$) manner across all groups when compared to control group. In the liver homogenate, increase ($p<0.05$) of catalase, reduced glutathione activities and a decrease in levels of the lipid peroxidation were observed.

Table 1: Wound healing activities of hydromethanolic root extracts of *Kigelia Africana*

	0 day	4th day	7th day	10th day
Conc (mg/ml)	Vertical horizontal	Vertical horizontal	Vertical horizontal	Vertical horizontal
Water	1.50±0.00 1.50±0.00	1.41±0.02 1.48±0.01	1.28±0.03 1.36±0.02	0.95±0.10* 1.11±0.07^y
Procaine 40	1.50±0.00 1.50±0.00	1.35±0.02 1.41±0.02	1.24±0.04 1.37±0.03	1.13±0.07 1.23±0.06
30	1.50±0.00 1.50±0.00	1.36±0.02 1.41±0.02	1.15±0.05 1.34±0.03	0.82±0.04 * 0.92±0.05 ^y
60	1.50±0.00 1.50±0.00	1.21±0.02 1.34±0.04	1.02±0.05* 1.11±0.09	0.70±0.05* 0.81±0.04 ^y
90	1.50±0.00 1.50±0.00	1.13±0.03 1.31±0.03	0.96±0.04* 1.04±0.06	0.58±0.05* 0.67±0.05 ^y
120	1.50±0.00 1.50±0.00	1.03±0.04* 1.20±0.02	0.87±0.07* 0.96±0.07 ^y	0.51±0.04* 0.61±0.04 ^y

Table 1: Continuation

	13th day	16th day	19th day
Conc. (mg/ml)	Vertical horizontal	Vertical horizontal	Vertical horizontal
Water	0.58±0.02* 0.67±0.01^y	0.31±0.05* 0.43±0.04v	0.13±0.04* 0.19±0.05 ^y
Procaine 40	0.82±0.01* 0.91±0.01^y	0.42±0.04* 0.48±0.05 ^y	0.11±0.02* 0.15±0.03 ^y
30	0.41±0.02* 0.51±0.01^y	0.21±0.04* 0.30± 0.05 ^y	0.03±0.01* 0.07±0.01 ^y
60	0.37±0.01* 0.48±0.03 ^y	0.15±0.03* 0.22±0.04 ^y	0.00±0.00* 0.00±0.00 ^y
90	0.24±0.01* 0.31±0.01 ^y	0.02±0.01* 0.05±0.01 ^y	0.00±0.00* 0.00±0.00 ^y
120	0.21±0.20* 0.27±0.02 ^y	0.00±0.00* 0.00±0.00 ^y	0.00±0.00* 0.00±0.00 ^y

Values are mean ± standard error of mean.*=significantly different (P<0.05) from vertical of the zero day. ^y significantly different (P<0.05) from Horizontal of the zero day by using analysis of variance. Benferoni Multiple Comparism, using Graph Pad Instat software.

Table 2: Antibacterial activities of root extracts of *Kigelia Africana*

Extracts	Conc. (mg)	*Escherichia Coli*	*Streptococcus pneumoniae*	*Pseudomonas aeruginosa*	*Staphylococcus Aureus*
W.E	30	6.00 ± 0.00	6.00 ± 0.00	10.25 ± 0.34	10.22 ± 0.41
	60	10.27 ± 0.49	9.23 ± 0.35	10.20 ± 0.40	13.00 ± 0.47
	90	10.32 ± 0.30	11.43 ± 0.51	12.20 ± 0.50	18.40 ± 0.62
	120	12.33 ± 0.60	16.30 ± 0.58	14.32 ± 0.49	20.35 0.34
H.ME	30	9.20 ± 0.23	11.33 ± 0.37	8.25 ± 0.39	10.33 ± 0.37
	60	10.22 ±0.30	14.41 ± 0.63	10.10 ± 0.33	12.40 ± 0.33
	90	12.35 ± 0.28	16.23 ± 0.70	11.23 ± 0.48	14.54 ± 0.43
	120	18.43 ± 0.33	18.43 ± 0.53	16.00 ± 0.51	16.33 0.68

N.HE	30	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00
	60	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	9.00 ± 0.35
	90	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	10.23 ± 0.35
	120	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	12.35 0.54
E.AE	30	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00
	60	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00
	90	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00
	120	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.0 0.00
N.C	30	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00
	60	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00
	90	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00
	120	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.0 0.00
P.C	30	16.20 ± 0.60	16.35 ± 0.55	8.33 ± 0.34	10.10 ± 0.28
	60	20.34 ± 0.72	18.27 ± 0.77	10.20 ± 0.41	14.30 ± 0.53
	90	20.33 ± 0.68	20.43 ± 0.56	10.28 ± 0.47	19.00 ± 0.67
	120	24.45 ± 0.80	22.00 ± 0.75	12.25 ± 0.49	22.00 ± 0.78

Values more than 6mm indicate activity. NHE=N-Hexane extract, N.C= negative control (distilled water), P.C=Positive control (Procaine penicillin) EAE= Ethylacetate extract, HME= Hydromethanolic extract, W.E= Water extract

Table 3: Characteristic clinical features of different stages of wound healing in rats administered with root extracts of *Kigelia africana*

DAYS	CHANGES	PP40	WT	30	60	90	120mg/ml
1st	Exudation	-	-	-	-	-	-
2nd	Exudation	+	+	-	-	-	-
	Reddening	+++	+++	+	+	+	+
5th	Reddening	++	++	+	+	+	-
	Dryness	-	-	+	++	++	+++
8th	Dryness	+	-	++	+++	+++	+++
	Pigmentation	-	-	+	+	++	+++
11th	Dryness	++	+	++	+++	+++	+++
	Pigmentation	+	-	+++	+++	+++	+++
	Cicatrisation	+	-	+	+	+	+++
14th	Dryness	++	+	+++	+++	+++	+++
	Pigmentation	++	+	++	+++	+++	+++
	Cicatrisation	++	+	++	+++	+++	+++
16th	Pigmentation	++	+	+++	+++	-	--
	Cicatrisation	++	+	+++	+++	-	-

+ =mild, - = absence, ++ Moderate, +++ Massive, pp = Procaine penicillin, WT =Sterile distilled water

Table 4: Liver function indices of rats administered with hydromethanolic root extracts of *Kigelia Africana*

Dose ($mgKg^{-1}$)	ALT (UL^{-1})	AST (UL^{-1})	ALP (UL^{-1})	ALB (gdL^{-1})	TP (gdL^{-1})	TB ($mgdL^{-1}$)	CB ($mgdL^{-1}$)
Control	15.83±1.14	33.00±2.35	95.46±2.81	2.93±0.17	6.27±0.29	0.39±0.06	0.14±0.01
400	17.17±1.30	38.33±2.42	97.57±3.01	3.11±0.16	6.34±0.19	0.49±0.07	0.15±0.01
800	18.83±1.40	41.50±1.63	93.81±2.51	2.96±0.11	6.10±0.19	0.45±0.07	0.14±0.01
1600	20.81±1.49	42.50±3.25	94.77±3.92	3.09±0.14	6.21±0.25	0.51±0.06	0.15±0.01
2400	21.83±2.59	43.16±4.36	95.31±3.11	3.01±0.24	6.61±0.14	0.55±0.04	0.15±0.01
3200	26.00±1.24*	51.17±5.30*	110.37±4.14*	3.67±0.20	7.70±0.48*	0.69±0.05*	0.19±0.03*

Values are mean ± SEM, (n=6).*= Significantly different ($p<0.05$) from control group by using analysis of variance (ANOVA). ALT= Alanine amino transferase AST= Aspartate amino transferase ALP= Alkaline phosphatase ALB= Albumin TP= Total protein TB= Total Bilirubin CB= Conjugated bilirubin.

Table 5: Renal function indices of rats administered hydromethanolic root extracts of *Kigelia africana*

Dose ($mgKg^{-1}$)	Urea (mgL^{-1})	Creatinine ($mgdL^{-1}$)	Uric acid ($mgdL^{-1}$)	Na+ ($mmolL^{-1}$)	K+ ($mmolL^{-1}$)	HCO_3^- ($mmolL^{-1}$)
Control	32.56 ±1.14	1.05±0.12	2.55±0.14	132.33±1.16	3.82±0.24	23.83±3.18
600	33.44±1.14	1.10±0.11	2.81±0.29	133.33±1.16	3.68±0.59	24.33±3.02
1200	36.11±1.04	1.24±0.24	3.48±0.22	131.50±0.56	3.62±0.75	23.68±3.01
1800	32.64±2.21	1.18±0.22	3.50±0.21	132.17±0.98	3.58±0.68	23.50±3.01
2400	37.38±2.15	2.40±0.29*	3.54±0.37	133.00±0.97	3.35±0.74	24.83±3.11
3000	43.14±3.22*	2.60±0.59*	4.05±0.53*	135.50±0.92	3.60±0.64	27.67±4.05

Values are mean ± SEM, (n=6).*= Significantly ($p<0.05$) different from control group by using analysis of variance (ANOVA).

Table 6: Effect of root extracts of *Kigelia africana* on antioxidant levels in granulated tissue in rats

(Mg/ml)	Catalase (µ/mg tissue)	Reduced glutathione (mg/100ml)	Lipid peroxidation (nmole of MDA/g tissue) $\times 10^{-5}$
Control	0.76±0.04	104.55±3.52	0.52±0.09
PP 40	1.28±0.09*	116.41±8.19	0.12±0.12*
30	1.56±0.09*	152.35±8.58*	0.27±0.01*
60	2.00±0.02*	185.93±7.58*	0.17±0.01*
90	2.61±0.13*	193.46±7.20*	0.13±0.01*
120	3.04±0.09*	246.40±9.64*	0.06±0.01*

PP=Procaine penicillin, Values are mean ± SEM, (n=6).*= Significantly ($p<0.05$) different from control group by using analysis of variance (ANOVA).

Table 7: Effects of root extracts of *Kigelia africana* on antioxidant levels in liver homogenate tissue in Rats

(mg/ml)	Catalase (μ/mg tissue)	Reduced glutathione (mg/100ml)	Lipid peroxidation (nmole of MDA/g tissue) × 10^{-5}
Water	2.64±0.28	178.16±7.11	0.27±0.02
200	3.60±0.22*	211.35±6.51*	0.20±0.02*
400	3.67±0.16*	218.80±5.56*	0.15±0.01*
600	4.17±0.06*	288.53±8.14*	0.15±0.01*

Values are mean ± SEM, (n=6).*= significantly (p<0.05) different from control group by using analysis of variance (ANOVA

Table 8: Histopathological properties of Kidney and Liver of Rats Administered with doses of *Kigelia Africana*

Dose ($mgkg^{-1}$)	Kidney	Liver
Control	Normal glomeruli and tubules seen	Normal hepatocytes seen
400	Normal glomeruli and tubules seen	Normal hepatocytes seen
800	Normal glomeruli and tubules seen	Normal hepatocytes seen
1600	Normal glomeruli and tubules seen	Normal hepatocytes seen
2400	Normal glomeruli and tubules seen	Slight distortion of hepatic architecture seen
3200	Mild nephrosis/ distortion of glomeruli Was seen	Mild distortion of hepatic architecture seen

4. Discussion

The phytoconstituents of *K. africana* as indicated in the results may be responsible for its wound healing potentials. Researches on some plants have revealed that triterpenoids are involved in the promotion of wound healing due to their antimicrobial action [45,46]. Glycosides are known to serve protective functions of detoxifying many substances and defense against invasion of tissues by microorganisms. Since many aglycones are antisceptic and bacteriacidal in character, these actions might have contributed to the increased healing activity of the root extract of *K. africana*. Therapeutic and toxic effects of Cardiac glycosides are well known and their toxic nature might have contributed to the root extract being toxic on the microbes. Phenolic, tannins and saponins were reported to prevent lipid peroxidation and bleeding through a number of mechanisms [47]. Tissue formation, homeostasis, re-epithelization, granulation, tissue remodeling are hallmark of wound healing [47]. The process of healing follows a natural course not dependent upon much external imputes and some certain conditions interfere with the process. Hence their effect demands attention to gain fuller understanding

of how the process can be promoted. Use of *K. africana* topically results in significant wound healing, probably due to angiogenic and mitogenic properties. It was observed that the prohealing properties of the extract was significant at all parameters were markedly affected.

The constituents present in *K. africana* fraction may promote collagen formation at cell proliferation stage. The result shows significant ($p<0.05$) daily decrease in wound area in all treatment groups especially at 90 and 120mg/ml of the extract and the impact was dose dependant. Wound healing was slower in groups treated with distilled water, 40mg/ml procaine penicillin and 30mg/ml of the extract. It is evident in these findings that all the treatment groups had higher activity than procaine penicillin. The exudation, reddening, dryness of wound and pigmentation were observed in the treated groups. Exudation occurred on the second day of wounding in positive (procaine penicillin) and negative (sterile distilled water) controls and 30mg/ml treated groups. The healing process started from the 2nd day with thin scab formation to drying of the exudates on the wound surface. On the 2nd to 6th days, reddening were prominent in control and antibiotic treated groups but moderate in all the extract treated groups. The wound had dried in all the extract treated groups but moist in sterile distilled water and antibiotic treated groups at day 5 of wounding. On the 8th day, moderate reddening was observed in positive and negative controls but absent in all plant- extract treated groups. Cicatrisation and pigmentation were observed in all extract treated groups but was more prominent at concentrations of 90 and 120mg/ml from day 8 to day 11 of wounding. Mild cicatrisation and pigmentation were observed in positive control group from day 11 and these changes were observed from the 14th day of wounding.

In the present study, the rate of healing of wound varied from 2nd to the 19th day. The highest rate of healing was observed in groups treated with 90 and 120mg/ml of the plant extracts. These findings corroborate that of Udupa et al. [28] who reported that pigmentation and cicatrizaiton were found in all treatment groups from 9th day onwards. This observation may be due to rapid wound healing. The extract of *K. africana* probably promotes and accelerates wound healing through coagulation (which prevent blood loss), inflammation and debridement of wound repair including cellular proliferation, tissues remodeling and collagen formation as these are some of the many wound healing stages which are concurrent, yet not independent. A combination of these properties is also possible in some of the medicinal plants used in wound care. Wound provide environment suitable for microbial growth. Typically bacterial implicated in wounds cover a wide spectrum with *E. coli, P. aeruginosa, S. aureus,* being most prominant [48].

From the results, evaluation of the effectiveness of the extracts on the bacterial wounds contaminants exhibited different degrees of inhibitory activities against these organisms. Wounds infected by bacteria take longer time to heal and promotes enhanced acute inflammatory response often leading to further tissue injury [49,50,51]. Hence, antibacterial action of the fraction may be enforced by disinfection providing room for the wound healing process to take its natural course. The result also suggests the root fraction promoting wound healing by knocking out established infections. Antibacterial action of *K. africana* is underlined hinging on its utility as alternative therapy for wounds. In this study, bacterial inhibition was seen maximally in higher concentrations at 90 and 120mg/ml of the extract. The ineffectiveness of the extract in the n-hexane and chloroform solvents might be due to the in ability of the solvents to extract the active components responsible for antibacterial activity or these solvents might have denatured the active substances.

Results (Tables 7 and 8), show reduced glutathione and catalase in both the granulated tissue and liver homogenate were found to increase which were dose dependent. A decrease also was observed in lipid peroxidation level decrease in group treated with the extract. Antioxidant activities were more evident in the liver homogenate because these enzymes are an intricate part of detoxification [52]. According to Rice-Evans *et al.* [53], radical scavenging activities of phenolics are responsible for their antioxidant activities. The phenolics serve as reducing agent, hydrogen donors in addition to role in singlet oxygen quenching. It can therefore be assumed that antimicrobial and antioxidant actions working in concert may promote the process of wound healing. The assay of organ based enzymes is critical and helpful in disease diagnosis and prognosis. It could also be useful for evaluation of safety and toxicity of plant based chemicals. Acute toxicity studies of the root extract reveal some behavioral changes such as excitement, respiratory distress, slow movement, weight reduction and loss of appetite when 5000mg/Kg body weight of *K. africana* roots extracts were administered. One (1) animal died and thus the LD_{50} obtained was greater than 5000mg/kg. Significant changes of the hepatorenal indices at concentration of 3200mg/kg body weight were observed (Table 10). Thus, this did not provide evidence of clinical safety of the root extract at higher concentration. The significantly ($p<0.05$) raised activities of ALT and AST may have resulted from possible necrotic injury of the liver and cholestasis [54,55]. The increased serum enzyme levels indicate that the extract at higher concentration (3200mg/kg) may cause cytotoxic effect on the liver. The aqueous root extract of *K. africana* may affect the permeability of the cell membrane making it leaky. These will cause their leakage into the circulation in event of necrosis or hepatocellular injury leading to their subsequent elevation in the serum. 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 [56]. 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 [38] and impaired synthetic function of the liver [38]. High levels ($P<0.05$) of serum urea, creatinine and uric acid observed at highest dose of the extracts is an indication of damaged renal function [38]. The levels of sodium, potassium and bicarbonate did not significantly change. The results obtained are in conformity with findings made by Hassan *et al*. [18] that the stem and leaves of the plant could be toxic at higher doses.

The histopathological anomalies observed in hepatocytes of the rats administered with 3200mg/kg body weight have justified the hepatorenal indices. Histopathologicial examination of the liver and kidneys indicated presence of lesions in the hepatocytes and kidneys of experimental rats administered with the highest dose of the extract (3200mg/kg body weight). Thus, the liver and kidneys might have been exposed to certain toxic principle present in the extract at higher concentration. In conclusion the results of this study have justified the incorporation of the Roots of *K. africana* in the management of wounds. Treatment with the plant extract may have beneficial influence on wound healing by increasing tensile strength due to increase in collagen synthesis and the antibacterial properties of the plant might have contributed in faster healing. Further work is required to test the plant in the management of chronic wounds and to isolate the active component(s) of *K. africana* fractions and their mechanisms of action(s).

References

[1]. S.C. Ramzi, V. Kumar and S.L. Robbins. *Robbin's Pathologic Basis of Disease.* 5th Edn. W.B. Saunders company, Philadelphia, pp. 86-88, 1994.

[2]. S.W. Hassan, R.A. Umar, A.S. Yakubu, Y.U. Dabai and A.Y. Abbas. Wound healing, and chemical properties of Stem bark extracts of *Kigelia africana* (Bignoniaceae). *Nig. J. Biochem. Mol. Biol*., Vol. 22, pp. 5-12, 2007.

[3]. P.M. Mertz and L.G. Ovington. Wound healing microbiology. *Dermatol. Clin.*, Vol. 11, pp. 739-747, 1993.

[4]. T. Essawi and M. Srour. Screening of some Palestinian medicinal plants for antibacterial activity. *J. of Ethnopharmacol.*, Vol. 70, pp. 343-349, 2010.

[5]. D.J. Mackay and A.I. Miller Nutritional support for wound healing. *Altern. Med.* Rev., Vol. 8(4), pp. 359-377, 2003.

[6]. D.K. Dnyaneshwar, R.Y. More, M.B. Kale, M.N. Nehele, P.C. Mehendele and C.H. Gadgoli Evaluation of wound healing activity of root of *Mimosa pudica. J. Ethnopharmacol.*, Vol. 124, pp. 311-315, 2009.

[7]. A. Kofi, G. Raghavan and K. Emelia. Wound healing and cytoprotective actions of *Paullinia pinnata* L. *Pharmacogn*., J.,Vol. 2, pp. 345-350, 2010.

[8]. G. Shila and N. Skalko-Basnet. Wound healing properties of Carica papaya latex: *In* evaluation in mice burn model. J. *Ethnopharmacol.*, Vol. 121, pp. 338-341, 2009.

[9]. P.T. Deshmukh, J. Fernandes, A. Akarte, T. Emmanuel. Wound healing activity of *Calotropis gigantea* root bark in rats. *J. Ethnopharmacol.*, Vol. 125, pp. 178-181, 2009.

[10]. E. Aliyev, U. Sakallioglu, Z. Eren and G. Acikgoz. The effect of polylactide membranes on the levels of reactive oxygen species in periodontal flaps during wound healing. *Biomaterials*, Vol. 25, pp. 4633-4637, 2004.

[11]. M. Singh, R. Govindarajan, V. Nath, A.K.S. Rawat and S. Mehrotra. Antimicrobial, wound healing and antioxidant activity of Plagiochasma appendiculatum Lehm. et Lind. *J. Ethnopharmacol*., Vol. 107 (1), pp. 67-72, 2006.

[12]. LS. Gill. *Ethnobotanical Uses of Plants in Nigeria*. Uniben press, Benin City, p. 143, 1992.

[13]. H.M. Burkill. The Useful Plants of West Tropical Africa. Vol. 1. *Royal Botanic Gardens,* Kew, London. Pp. 254-257, 1985.

[14]. P.J. Houghton, A. Photiou, S. Uddin, P. Shah, M. Browning, S.J. Jackson and S. Retsas. Activity of extracts of *Kingelia pinnata* against melanoma and renal carcinoma cell lines. *Planta medica*, Vol. 60(**5**), pp. 430-433, 1994.

[15]. A.P. Akah. Antidiarrheal activity of *Kigelia africana* in experimental animals. *Journal of Herbs Spices. Med. Plants*, Vol. 4, pp. 31-38, 1996.

[16]. P. Picerno, G. Autore, S. Marzocco, M. Meloni, S. Rokia and R.P. Aquino. Anti-inflammatory activity of *vernimoside* from *Kigelia africana* and evaluation of cutaneous irritation in cell cultures and reconstituted human epidermis. *J. Nat. Prod.*, Vol. 68, pp. 1610-1614, 2005.

[17]. J. Omonkhilin, O.J. Owolabi and K.. Erick. Analgesic and Anti-inflammatory activities of the ethanolic stem bark extract of *Kigelia africana S*(Bignoniaceae). *Afr. J. Biotech*., Vol. 6(**5**) pp. 582-585, 2007.

[18]. S.W. Hassan, M.G. Abubakar, R.A. Umar, A.S Yakubu., H.M. Maishanu and G. Ayeni Pharmacological and toxicological Properties of leaf extracts of *Kingelia africana. Journal of Pharmacol. Toxicol.*, Vol. 6, pp. 124-132, 2011.

[19]. K.S. Morris and K. Aziz. Tumour inhibitors 114 Aloe Emodin: Antileukemic principleisolated from *Rhamnus frangular* L. *Liloydia*, Vol. 39, pp. 223-224, 1976

[20]. E.P. Springfield and F. Weitz. The scienctific merit of *Capobrotus melei* L. based on antimicrobial activity and chemistry profiling. *Afr. J. Biotechnol*., Vol. 5, pp. 1289-1293, 2006.

[21]. J.B. Harborne. Phytochemical Methods: *A Guide to Modern Techniques of Plant Analysis*. 3rd Edn., Chapman and Hall, London, pp.7-13, 89, pp. 131-135, 1973.

[22]. G.E. Trease and W.C. Evans. *Pharmacognosy.* 11th edn. Braillar Titidel Can. MacmillianPublishers., 1989.

[23]. A. Sofowora. *Medicinal Plants and Traditional Medicine in Africa*. Spectrum Books Ltd., Ibadan, Nigeria,pp. 289, 1993.

[24]. M.M. El-Olemyl, F.J. Al-Muhtadi and A.A. Afifi. *Experimental Phytochemistry*, A Laboratory Manual. King Saud University Press, UK., pp. 1-134, 1994.

[25]. G.K. Dash, P. Suresh and S. Gomathy. Studies on hypoglycemic and wound healing activities of *Lantana comara Linn. J. Nat. Remedies*, Vol. 1, pp 105-110, 2001.

[26]. W.B. Hugo and A.D. *Russell Pharmaceutical Microbiology.* 3nd Edn., Blackwell, UK., pp. 33-35, 51, 1983.

[27]. J. Vlietinck, L. Van Hoof, J. Totte, A. Lasure, D.V. Berghe, P.C. Rwangabo and J. Mvukiyumwami. Screening of hundred Rwandese medicinal plants for antimicrobial and antiviral properties. *J. Ethnopharmacol.,* 46 pp. 31-47, 1995.

[28]. S.L. Udupa, S. Shetty, A.L . Udupa and S.N. Somayaji. Effect of *Ocinum sanctum* on normal and dexamethasone suppressed wound healing. *Indian J. Exp. Biol*., Vol. 44 pp. 49-54, 2006.

[29]. J.W. Patterson and A. Lazarow. *Determination of glutathione. Meth. Biochem. Anal.*, Vol.2, pp. 259-278, 1955.

[30]. P.E. Hartman. Putative mutagens and carcinogens in foods. IV. Malondialdehyde (Malondialdehyde). *Environ. Mutagen.,* Vol. 5, pp. 603-607, 1983.

[31]. R.F. Beers and I.W. Sizer. A spectrophotometric method of for measuring the breakdown of hydrogen peroxide by catalase. *J. Biol. Chem.*, Vol. 195, pp. 133-140, 1952.

[32]. OECD. Guidelines for testing of chemicals. Acute oral toxicities-up and down procedure, *OECD Guideline* 425, pp. 1-26, 2001.

[33]. E.S. Rivera, N. Andrade, G. Martin, G. Melito and G. Cricco. Induction of mammary tumours in rat by intraperitoneal injection of NMU: Histopathology and estralcycle influence. *Cancer Lett*., Vol. 86(2), pp 223-228, 1994.

[34]. S. Reitman and S. Frankel. A colourimetric method for the determination of serum glutamate-oxaloacetate and pyruvate transaminases. *Am. J. Clin. Pathol*., Vol. 28, pp. 56-63, 1957.

[35]. G.S.C.C. Rec (DGKC). Optimized standard colorimetric methods. *J. Clin. Chem. Clin. Biochem.*, Vol. 10, pp. 182-182, 1972.

[36]. L. Jendrassik and P. Grof. Simplified photometric methods for the determination of bilirubin. *Biochem Zschr.,* Vol., 297, pp. 81-89, 1938.

[37]. S. Sherlock. *Liver disease* (Determination of total and direct bilirubin, colorimetric method. Churchill, London, p. 204, 1951.

[38]. M. Cheesbrough. *Medical Laboratory Manual for Tropical Countries.* Vol. II., Microbiology Tropical Health Technology/Buterworths Scientific Publications, Boston, pp. 167-214, 1991.

[39]. D.R.D. Wybenga, J. Glorgio and V.J. Pileggi. Determination of Serum Urea by Diacetyl Monoxime Method. *J. Clin. Chem.,* Vol. 17, pp. 891-895, 1971.

[40]. A.P. Uriyo and B.R. Singh. *Practical Soil Chemistry Manual*. Branch, Morogoro University, Daressalaam, pp.12-14, 1974.

[41]. R.J. Henry. *Clinical Chemistry: Principles and Techniques*. 2nd Edn., Harper and Row, Hagerstown, MD, USA., P. 525, 1974.

[42]. P.F. Collins and H. Diehl. *Determination of uric acid*. J. Ann. Chem., Vol. 31, pp. 1862-1867, 1959.

[43]. L.G. Morin and J. Prox. Reduction of ferric phenanthroline-a procedure for determining serum uric acid. *Am. J. Clin. Pathol.*, Vol. 60, pp. 691-694, 1973.

[44]. S. Roy, S. Khanna, K. Nallu, T.K. Hunt and C.K. Sen. Dermal wound healing is subject to redox control. *Mol. Ther.*, Vol. 13, pp.: 211-220, 2006.

[45]. M. Scorchitini and P. Pia. Preliminary *in vitro* evaluation of antimicrobial activity of triterpenes and terpenoids towards Erwinia amylovera (burrill). *J. Bactriol*., Vol. 71, pp. 109-112, 1991.

[46]. B.S. Nayak, S. Sandiford and A. Maxwell. Evaluation of the wound-healing activity of ethanolic extract of Morinda citrifolia L. leaf. Evid.-Based Comp. *Alternat. Med.*, Vol. 6(3), pp.n351-356, 2009.

[47]. T. Sawa, M. Nakao, T. Akaike, K. Ono and H. Maeda. Alkyperoxy radical scavenging activity of various flavonoids and other phenolic compounds; implications for the anti-tumor- promoter effect of vegetables. *J. Agric. Food Chem*.**, Vol. (47**), pp.397-402, 1999.

[48]. F.E. Emele, M.I. Izomoh and E. Alufohai. Microorganisms associated with wound infection in Ekpoma, Nigeria. *West Afr. J. Med.*, Vol. 18, pp. 97-100, 1999.

[49]. J.T. Johnson and V.L. Yu. Role of anaerobic bacteria in postoperative wound infections following oncologic surgery of the head and neck. Ann. Otol. *Rhinol. Laryngol. Supl*.,154, pp. 46-48, 1991.

[50]. K . Whaley and A. Burt. *Inflammation, Healing and Repair*. In: Muir's Textbook of Pathology, MacSween, R.M.N. and K. Whaley (Eds.). 13th Edn., Arnold, London, pp. 112- 165, 1996.

[51]. P.G. Bowler, B.I. Duerden and Armstrong DG. Wound Microbiology and Associated Approaches to wound management. *Clin. Microbiol. Rev.*, Vol. 14, pp. 244-269, 2001.

[52]. I.C. Tayes. Antioxidant defenses and lipid peroxidation in human blood plasma. *Carcinogenesis,* Vol. 10, pp. 921-925, 1989.

[53]. C.A. Rice-Evans, N. Miller and G. Paganga. Antioxidant properties of phenolic compounds. *Trends Plant Sci.*, Vol. 2(4), pp. 152-159, 1997.

[54]. H.J. Speech and H. Liehr. Of what value are SGOT/SGPT, GGT/AP and IgA ratios in the differential diagnosis of advanced liver diseases? *Z. Gastrol Enterol.,* Vol. 21, pp. 89-96, 1983.

[55]. J.A. Lott and P.L. Wolf. *Alanine and Aspartate* Animo *Transferase*: Clinical Enzymology. Field Rich and Associates, New York, pp. 111-138, 1986.

[56]. M.A. Akanji and S.E. Nlumanze. Alkaline phosphatase activities following repeated suramin administration in some rat tissues in relation to their functions. *Pharm. Toxicol.*, Vol.1, pp. 182-183, 1987.