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Pharmacological and Toxicological Properties of Leaf Extracts of *Kingelia africana* (Bignoniaceae)

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ABSTRACT

Leaf extracts of *Kingelia africana* were evaluated for wound healing, antibacterial, toxicological and chemical properties. Antibacterial activity was done using hole-in-plate bioassay, wound healing by circular incision, toxicological and chemical properties were evaluated using standard methods. The results show a more rapid wound healing at all the hydromethanolic concentrations employed than 90 mg mL^{-1} of procaine penicillin on the 4, 7, 10, 13, 16 and 19th day. Exudation was more prominent in control and antibiotic treated groups compared to other groups on day 2 of wounding. Clinical features revealed redness, exudation, scab formation and other changes. The aqueous and organic solvent leaf extracts exhibited significant ($p<0.05$) antibacterial activity against *Pseudomonas aeruginosa, Staphylococcus aureus* and *Escherichia coli* at concentrations ranging from 30 to 120 mg mL^{-1}. Most of the hepatorenal indices were significantly ($p<0.05$) increased at doses of 2000 to 4000 mg kg^{-1} indicating compromised functions of these organs. The lethal dose (LD_{50}) of the leaf extract was greater than 3000 mg kg^{-1}. Alkaloids (9.80±0.20), tannins (22.80±0.05), saponins (8.85±0.50), flavonoids (7.80±1.00% w/v), glycosides, saponin glycosides, steroids and anthraquinones were detected. Low values of sodium (6.5±0.01) potassium (3.1±0.01), magnesium (0.126±0.03), phosphorus (2.04±0.04) and calcium (0.108±0.01 mg%) were observed. The results show that leaves extracts of *K. africana* could be cautiously used and also provide support for the traditional use of the plant in treating bacterial diseases and wound healing due to its chemical constituents.

Key words: *Kingelia africana*, antibacterial activity, wound healing, hepatorenal function, chemical composition

INTRODUCTION

The use of traditional medicinal remedies and plants in the treatment of burns and wounds is an important aspect of health treatment and at the same time reduces financial burden. Several plants have been reported to treat skin disorders including burns and infected wounds (Starley *et al*., 1999; Mikhalchik *et al*., 2004; Nayak and Pinto Pereira, 2006; Singh *et al*., 2006; Kumar *et al*., 2007). Wound healing or wound repair is the body's natural process of regenerating

dermal and epidermal tissues (Dnyaneshwar *et al*., 2009). Trace elements are required as antioxidant for proper wound healing, bone formation and cross linking of connective tissues (Hassan *et al*., 2007).

Antibiotics used in the treatment of wound infections are now proved to have adverse effects in the human body and pathogens resistance is emerging. Attention has therefore turned towards extracting biologically active compounds from plant species (Essawi and Srour, 2000; Pattanayak and Sunita, 2008).

Kingelia africana is widely distributed in the south, central and West Africa. It is known as the cucumber or Sausage tree (Burkill, 2000). In Nigeria, the leaves, stem bark and fruits of *K. africana* are used in the treatment of wound infection, burns, boils, syphilis, infectious diseases and rheumatism. The leaves are used for oral and topical applications for wound treatment. The plant has analgesic and anti-inflammatory activities (Picerno *et al*., 2005; Owolabi and Omogbai, 2007) and antibacterial activity (Grace *et al*., 2002). The stem bark of the plant has wound healing and antibacterial properties (Hassan *et al*., 2007) and central nervous system stimulant effect (Owolabi *et al*., 2008). To the best of our knowledge there is no previous report on wound healing, antibacterial and toxicological properties of leaves of *K. africana*. Therefore, this study evaluates the pharmacological and toxicological properties of leaves of *K. africana*.

MATERIALS AND METHODS

Chemicals: All the chemicals used were of analytical grade.

Collection of plant and authentication: The leaves of *K. africana* were collected in August 2009, from villages around Usmanu Danfodiyo University, Sokoto, Nigeria. Botanical identification was done at Botany Unit and voucher specimen was deposited in the herbarium (Herbarium number 022), botany unit, of the same institution for reference.

Extraction: The leaves were washed with clean water, room dried and pulverized into coarse powder. One hundred grams of the powdered (190 g) leaves were extracted with 50% methanol-water (1600 mL of 1:1) at room temperature for 48 h. The extract was filtered through Whatman filter paper (No. 1) and concentrated by removing the solvents completely under reduced pressure. The yield of the extract was 19.20% w/w and was reconstituted in sterile distilled water for wound healing and phytochemical studies.

Activity-guided fractionation procedures of Morris and Aziz (1976) and Springfield and Weitz (2006) were adopted for antibacterial studies with modifications. Twenty grams of powdered leaf were extracted with methanol-water (1:1, 500 mL) at room temperature overnight. The extract was filtered and partitioned in hexane (250 mL) and clarified by further filtration. Evaporation of hexane fraction to dryness in an oven at 45°C yielded residue (0.78% w/w). The aqueous filtrate (methanol-water) of the extract fraction was further partitioned (to obtain fractions of different polarities) with petroleum ether (250 mL) and chloroform (250 mL) separately. Evaporation of petroleum ether and chloroform fractions yielded residues (0.50 and 0.9% w/w), respectively. The procedures were repeated to obtain more residues. Two hundred grams (200 g) of powdered leaves were extracted with 500 mL of distilled water for 24 h and filtered. All the residues obtained were reconstituted in sterilized distilled water and screened for antibacterial activity.

Chemical analysis: Mineral elements were estimated according to procedure of Black *et al.* (1965) and phytochemical analysis was done using the procedures of Persinos and Quimby (1967), Harborne (1973), Trease and Evans (1978) and El-Olemyl *et al.* (1994).

Animals: For wound healing, 25 rabbits of either sex weighing 2.80 to 5.40 kg were purchased from Sokoto Central Market. They were divided into 5 groups of 5 rabbits each and were kept in separate cages. Albino rats were obtained from animal house, Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria and were used for the toxicological studies. All the animals were acclimatized for a week, allowed free access to food and water and maintained on animal feeds *ad libitum* (Bendel feeds and flour mills, Edo state, Nigeria) and fresh vegetables. Animal treatment and handling were done according to the ethical guidelines as reported by Zimmerman (1983) and in accordance with US guidelines as contained in the National Institute of Health guide for the care and use of laboratory animals (NIH Publication No. 18-23).

Preparation of wound: The rabbits were divided into 5 groups of 5 animals each. Wounds were created according to procedures of Dash *et al.* (2001) with some modifications. Rabbits in each group were anaesthesized with lignocaine and the hair on the skin was clipped. The clipped area was disinfected with 70% ethanol. A circular incision 15 mm in diameter (horizontal and vertical) was made on the disinfected area of the skin surface and the skin was carefully dissected out. The wound area was measured immediately by using a disinfected ruler and recorded in cm. Treatment was started immediately after the wound incision by applying a drop (twice daily) of 30, 60 and 90 mg mL^{-1} of the leaf extracts of *K. africana* on the wound to three test groups A, B and C, respectively. Groups D and E (controls) were treated as above with sterile distilled water and 90 mg mL^{-1} of procaine penicillin (a product of Ningbo Second Pharmaceutical Factory, Nigeria), respectively. The wound area of each animal was measured under light lignocaine anesthesia on the 1st, 4, 7, 10, 13, 16 and 19th day post-surgery. The wound healing activity of the extract for these days was measured (vertically and horizontally) in cm. The data obtained were statistically analyzed using one way Analysis of Variance (ANOVA).

Antibacterial activity: The antibacterial activity was done using hole-in-plates bioassay procedures of Hugo and Russel (1983) and Vlietinck *et al.* (1995). Pure cultures of the organisms were inoculated with Muller-Hinton nutrient broth (Oxoid, England), incubated for 24 h at 37°C, diluted with sterile nutrient broth to a density of 9×10^{8} cfu mL^{-1} equivalent to McFarland test tube 3. The suspension was used to streak for confluent growth on the surface of Muller-Hinton agar plates with sterile swab. Using a sterile cork-borer of 6 mm diameter, four holes were made on to the set agar in Petri-dishes containing the bacterial culture. Concentrations of 30 to 120 mg mL^{-1} of the extracts were poured into the wells. Ciprofloxacin (90 mg mL^{-1}), a product Maxheal Pharmaceuticals, India was used as positive control. The plates were placed in the incubator at 37°C overnight. Antibacterial activity was recorded if the zone of inhibitions were greater than 6 mm. Significance ($p<0.05$) of the antibacterial activities was tested by one way analysis of variance (ANOVA).

Acute toxicity studies: A 1 mL leaf extracts of *Kingelia africana* (3000 mg kg^{-1} b.wt.) was administered to 5 groups of 1 rat each (one after the other at a grace observation period of 48 h) in a single oral dose using feeding needle. Another group (control) received distilled-water.

Observation for toxic symptoms was made and recorded systematically 1, 2, 4 and 6 h after administration. Finally the number of survivors was noted after 48 h. The toxicological effect was assessed on the basis of mortality, which was expressed as LD_{50} and was calculated using the limit test dose, up and down procedure of Organization for Economic and Cultural Development (OECD, 2001).

Sub-chronic toxicity: A total of 25 albino rats were divided into 5 groups of 5 rats each. Animals in groups 2, 3, 4 and 5 were orally administered graded doses of the plant extract (1000, 2000, 3000 and 4000 mg kg^{-1} b.wt.; all contained in 1 mL) once daily for 28 days, respectively. Animals in group one served as the control group (i.e., 0.00 mg kg^{-1}) and received distilled water. They were sacrificed on the 29th day and blood samples were collected, allowed to clot and centrifuged to obtain sera. Serum Alanine Amino Transferase (ALT) and Aspartate Amino Transferase (AST) were estimated by Randox assay kit (Reitman and Frankel, 1957). Alkaline phosphatase activity was assayed by the Randox kit (colorimetric) of Rec GSCC (1972). Albumin (Bromocresol green) was assayed by the method of Cheesbrough (1991). Total protein was assayed using Gornall *et al.* (1949). Total bilirubin was estimated using the method of Malloy and Evelyn as reported by Varley *et al.* (1991). Urea was by the method of Wybenga *et al.* (1971) and uric acid by Collins and Diehl (1959) and Morin and Prox (1973). Electrolytes were estimated by the method of Uriyo and Singh (1974) and creatinine by Jaffe (1986).

Statistical analysis: Data were subjected to one way Analysis of Variance (ANOVA), Bonferroni compare all columns using Graph pad Instat Software (San Diego, USA). A p value less than 0.05 was taken as an indication of statistically significant difference.

RESULTS AND DISCUSSION

Result of wound healing activity of the extract is presented in Table 1 and antibacterial activity in Table 2. Rapid wound healing was observed at 30-90 mg mL^{-1} when compared with 90 mg mL^{-1} of the positive control (procaine penicillin), from the 4th to 19th day (Table 1). Redness, scab formation and exudation were the clinical features seen, but exudation was more prominent in positive and negative controls.

The wound healing activity of the plant leaf extracts may be due to its angiogenic and mitogenic potential leading to increased cellular proliferation and increased collagen synthesis (Pattanayak and Sunita, 2008). Collagen gives strength and integrity to the tissue matrix and plays a role in homeostasis and epitheliazation at latter phase of healing (Clark, 1996).

The observed antibacterial activity by 30-120 mg mL^{-1} of the aqueous and organic solvent fractions of *K. africana* against the organisms employed (Table 2) has confirmed the presence of antibacterial agent(s) in the plant. Wound can be affected by microbes and delay natural wound healing process. Healing process takes place naturally, but various factors such as infection and poor nutrition cause delay in healing necessitating the promotion of the process. The topical application of the extract of *K. africana* on the wound may have prevented the microbes to invade the wound and thus, has resulted in rapid wound healing. The inhibition of growth of microbial contaminants of wounds allows normal tissue repair process to occur. From the results, the leaves of *K. africana* are more effective in wound healing when compared with procaine penicillin. Thus, our data reinforce earlier reports on the pharmacological basis for the use of *K. africana* in the treatment of bacterial diseases (Grace *et al.*, 2002; Hassan *et al.*, 2007) and wound healing

Table 1: Wound healing activity of leaves extracts of *Kingelia africana*

	1st day		4th day		7th day		10th day	
Conc. (mg mL^{-1})	Vertical	Horizontal	Vertical	Horizontal	Vertical	Horizontal	Vertical	Horizontal
30	1.50±0.00	1.50±0.00	1.37±0.15	1.45±0.10	0.78±0.19*	1.04±0.23[a]	0.56±0.38*	0.66±0.36[a]
60	1.50±0.00	1.50±0.00	1.28±0.23	1.52±0.28	1.02±0.19*	1.22±0.10	0.66±0.13*	0.86±0.24[a]
90	1.50±0.00	1.50±0.00	0.94±0.13*	1.24±0.11	1.20±0.18*	1.25±0.24	1.03±0.13*	0.9.±0.16[a]
Procaine (90)	1.50±0.00	1.50±0.00	0.90±0.19*	1.18±0.32[a]	0.70±0.25*	0.72±0.29[a]	0.28±0.19*	0.28±0.25[a]
Distilled water	1.50±0.00	1.50±0.00	1.14±0.22	1.34±0.11	0.92±0.13*	0.98±0.18[a]	0.50±0.35*	0.62±0.41[a]

	13th day		16th day		19th day	
Conc. (mg mL^{-1})	Vertical	Horizontal	Vertical	Horizontal	Vertical	Horizontal
30	0.22±0.19*	0.26±0.23[a]	0.00±0.00*	0.00±0.00[a]	0.00±0.00*	0.00±0.00[a]
60	0.40±0.10*	0.40±0.23[a]	0.16±0.11*	0.14±0.15[a]	0.02±0.04*	0.04±0.10[a]
90	0.28±0.17*	0.28±0.10[a]	0.00±0.00*	0.00±0.00[a]	0.00±0.00*	0.00±0.00[a]
Procaine (90)	0.06±0.09*	0.08±0.13[a]	0.00±0.00*	0.00±0.00[a]	0.00±0.00*	0.00±0.00[a]
Distilled water	0.00±0.00*	0.00±0.00[a]	0.00±0.00*	0.00±0.00[a]	0.00±0.00*	0.00±0.00[a]

Values are Mean±SD, *Significantly different from the first day vertical while a- means significantly different from the first day horizontal

Table 2: Antibacterial activity of aqueous and organic solvent leaf extracts of *Kingelia africana*

		Zone of inhibition (mm)		
Fractions	Conc. (mg mL^{-1})	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*
LR	30.00	14.00±1.00	13.00±1.00	13.33±1.53
	60.00	16.00±2.00	17.67±3.00	16.00±4.36
	90.00	19.00±1.00	19.00±3.00	17.00±1.00
	120.00	19.33±0.58	20.67±2.52	18.33±2.08
Hexane	30.00	15.67±3.21	12.33±2.08	12.33±2.08
	60.00	18.67±1.53	15.00±1.00	16.33±1.53
	90.00	19.33±0.58	17.67±1.15	18.00±1.00
	120.00	22.67±2.08	20.33±0.58	20.00±1.00
Petroleum ether	30.00	13.33±1.53	12.67±2.52	14.00±1.00
	60.00	14.33±0.58	15.33±3.21	14.33±0.58
	90.00	15.67±1.53	15.67±2.31	14.33±2.08
	120.00	11.33±1.15	16.67±3.06	17.00±0.00
Chloroform	30.00	14.00±1.00	11.67±1.53	12.67±2.52
	60.00	15.00±1.00	15.00±1.00	15.00±0.00
	90.00	16.67±1.53	14.67±2.52	15.33±2.89
	120.00	20.00±1.00	20.00±1.00	18.33±0.58
Ciprofloxacin	90.00	16.00±1.00	17.00±1.73	16.00±1.73
Distilled Water		6.00±0.00	6.00±0.00	6.00±0.00

LR: Last Remaining Water Methanol Fraction. Values are Mean±SD. Values greater than 6 mm indicate some activity. All values are significantly ($p<0.05$) different from the negative control (water) by using analysis of variance Graph pad Instat Software San Diego, USA

(Hassan *et al*., 2007). It also supports the traditional usage of the plant for the treatment of wounds and infectious diseases as previously reported (Owolabi *et al*., 2008).

The results of toxicological effects are summarized in Table 3-5. The results obtained have confirmed that *K. africana* is non toxic at low concentrations. It is also in agreement with the findings of Hassan *et al.* (2007) that the plant could be toxic at high concentration. A significant ($p<0.05$) change in body weights (Table 3) of the rats that received 4000 mg kg^{-1} of the extract was observed. This may possibly be due to presence of antinutrients that may cause poor feed utilization expressed as weight loss (Muyibi *et al.*, 2000).

In this study, administration of 3000 and 4000 mg kg^{-1} of the extract has indicated significant changes ($p<0.05$) in the renal (Table 4) and hepatic (Table 5) indices. Thus, the plant is not safe at these doses. The observed increases ($p<0.05$) of ALT and AST (Table 5) at higher doses of the plant extract observed may probably be a necrotic injury of the liver or cholestasis (Lott and Wolf, 1986). Increase of Alkaline phosphatase activity suggests obstructive jaundice and intrahepatic cholestasis (Van Hoof and De Broe, 1994).

Decrease of serum albumin with increase of total bilirubin observed (Table 5) may be indicative of impaired liver excretory and synthetic functions. Also the increased serum bilirubin may arise from excessive haemolysis and obstruction of the bile duct. The observed increase ($p<0.05$) of serum urea, creatinine and uric acid and decrease of sodium (Table 4) seen in animals administered with 4000 mg kg^{-1} of the leaves extracts of *K. africana* suggest renal malfunction (Cheesbrough, 1991). Enzymes and non-enzyme indices in tissues and body fluids are important

Table 3: The effect of four weeks administration of aqueous leaf extracts of *Kingelia africana* on total body weights of albino rats

Dose (mg kg^{-1})	Initial weight (g)	1st week	2nd week	3rd week	4th week
1000	112.17±2.08	114.27±6.10	116.00±7.10	118.33±17.85	121.5±24.25
2000	120.50±1.73	127.00±1.32	121.00±3..52	123.30±5.30	125.20±3.76
3000	125.50±1.00	130.67±2.70	133.80±21.92	144.00±3.12	130.17±12.50
4000	138.83±1.76	146.00±2.29*	206.30±3.79*	194.33±16.50*	219.33±10.50*
000	119.83±1.53	123.17±2.52	124.50±2.65	127.83±3.79	130.33±5.80

n = 5, values are Mean±SD. *Significantly different ($p<0.05$) from the initial weight using analysis of variance (ANOVA). Bonferroni compare all columns Instat Software (SAN Diego USA)

Table 4: The effect of four weeks administration of aqueous leaf extracts of *Kingelia africana* on kidney function indices in albino rats

Dose (mg kg^{-1})	Creatinine (μmol L^{-1})	Urea (Mmol L^{-1})	Uric acid (μmol L^{-1})	Sodium (ppm)	Potassium (ppm)
1000	59.73±4.43	8.047±0.62	3.23±0.042	49.33±1.15	20.67±1.15
2000	74.48±5.57	9.63±0.79*	4.07±0.061*	50.67±2.31	20.00±2.00
3000	92.92±5.85*	12.42±0.04*	4.24±0.06*	52.00±2.00	12.67±1.15*
4000	117.26±5.86*	19.01±0.33*	6.06±0.072*	42.00±4.00*	8.133±0.11*
000 (control)	58.99±10.91	7.457±0.12	2.95±0.042	54.67±1.15	21.33±1.15

n = 5, values are Mean±SD. *Significantly different from the control using Analysis of Variance (ANOVA) Bonferroni compare all pairs of columns Instat Software (San Diego USA)

Table 5: Effect of leaf extracts of *Kingelia africana* on some serum liver function indices in albino rats

Dose (mg kg^{-1})	ALT (U L^{-1})	AST (U L^{-1})	ALP (U L^{-1})	Albumin	T. protein	T. Bilirubin
1000	18.07±0.81	53.03±0.91	85.56±2.76	3.77±0.070	5.45±0.107	0.34±0.025
2000	20.07±0.95	72.96±0.91*	103.04±6.95	3.65±0.534	5.76±0.090	0.57±0.02
3000	20.27±0.57*	75.60±0.36*	115.92±5.52*	4.19±0.055	6.10±0.147*	0.73±0.0026*
4000	26.50±0.36*	83.60±1.06*	133.40±8.43*	4.15±0.020	6.43±0.123*	0.96±0.011*
0000	17.97±0.80	52.47±0.38	85.56±5.52	4.33±0.538	4.56±0.065	0.32±0.0152

n = 5, T. = Total, values are Mean±SD. *Significantly ($p<0.05$) different when compared to the control, using Analysis of Variance (ANOVA), Bonferroni compare all columns Instat Software (San Diego, USA)

in aiding disease investigation and diagnosis (Malomo, 2000). Enzymes are released in to circulation from affected organ or tissue due to damage.

The phytochemicals (alkaloids, tannins, saponins, flavonoids, saponin glycosides, steroids and anthraquinones) may be responsible (table not shown) for the wound healing and antibacterial properties observed. These phytochemicals have been documented to have antimicrobial and wound healing properties (Sodipo *et al*., 1991; Okwute and Mann, 1999; Hassan *et al*., 2007).

The low values of sodium (6.5±0.01), potassium (3.1±0.01), calcium (0.108±0.01), phosphorus (2.04±0.04) and magnesium (0.126±0.03 mg%) in the plant extract (table not shown) may contribute to cross linking of connective tissue, epithelial collagen, bone formation, immune function and as antioxidant for proper wound healing (Hassan *et al*., 2007).

CONCLUSIONS

It is clear from the result that the leaf extracts of *K. africana* has wound healing and antibacterial properties. The plant at higher dose may be potentially toxic to liver and kidney. Low doses (1000-2000 mg kg^{-1}) of the leaf extracts should be cautiously used. Structural elucidation of the active agent(s) is recommended.

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