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Full Length Research Paper

Antifungal and antibacterial activities of the ethanolic and aqueous extract of *Kigelia africana* (Bignoniaceae) stem bark

Omonkhelin J. Owolabi[1*], Eric K. I. Omogbai[1] and Osahon Obasuyi[2]

[1]Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Edo State, Nigeria.
[2]Department of Pharmaceutical microbiology, Faculty of Pharmacy, University of Benin. Edo State, Nigeria.

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Studies on the antibacterial and antifungal activities of the stem bark of *Kigelia africana*, (LAM). Benth (Family: Bignoniaceae), a medicinal plant used in South, Central and West Africa for the treatment of various ailments and infection was carried out using agar diffusion technique. The results revealed that the crude ethanolic extract exhibited antibacterial and antifungal activities against *Staphylococccus aureus* and *Candida albicans* with zones of inhibition measuring 15.0±0.95 and 20.75±4.6mm respectively. The aqueous extract exhibited no antibacterial or antifungal activity. The minimum inhibitory concentration for the extract was 6.25 ± 1.07 mg/ml for *S. aureus* and 7.92 ± 1.52 mg/ml for *C. albicans.* The ethanolic extract was also compared with various standards; Ampicillin, Gentamicin, Ceftriaxone and Ciprofloxacin. The ethanolic extract (20mg/ml) produced similar zone of inhibition with 25µg disc of amoxicillin.

Key words: *Kigelia africana*, antimicrobial activity, minimum inhibitory concentration.

INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many of these isolations were based on the uses of the agents in traditional medicine (Cragg and Newman, 2001). This plant-based traditional medicine system continues to play an essential role in health care, with about 80% of the world's inhabitants relying mainly on traditional medicines for their primary health care (Farnsworth et al., 1985). *Kigelia africana* is a plant that is widely distributed in the South, Central and West Africa. Locally known by Europeans as the cucumber or sausage tree because of the huge fruits (average 0.6 m in length and 4 kg in weight), which hangs from long fibrous stalks. The family contains trees, shrubs and climbers. The tree can grow to more than 20 m tall. It is found mostly in riverine areas where distribution is restricted to the wetter areas (Cordell, 2000). Venereal diseases are commonly treated with the extracts usually in palm wine as oral medication. The fruits and barks, grind and boiled in water, are also taken orally or used as enema in treating stomach ailments (Burkill, 1985).

Most commonly, traditional healers have used the sausage tree to treat a wide range of skin ailments from relatively mild complaints such as fungal infections, boils, psoriasis and eczema, through to the more serious disease like leprosy, syphilis and skin cancer (Burkill, 1985). Previous studies of the fruits of *K. africana* showed some antibacterial activity (Grace et al., 2002). However there is no report on the antibacterial and antifungal properties of the stem bark of this plant, this is needful as the organism; *S. aureus* is the most implicated organism in atopic eczema (Burkill, 1985). This report, therefore, presents studies on the antimicrobial properties of the alcoholic and aqueous extracts of the bark using clinical isolates of bacterial and fungal.

*Corresponding author. E-mail: josphineomo@yahoo.com. Tel: 08034120318.

MATERIALS AND METHODS

Plant material

The stem barks were collected based on ethno-pharmacological information. The barks were collected in Okomu forest reserve, Udo in Benin City, Edo State. The botanical identity of the plant and its bark was by Alhaji Alasa Abubakar, of the Department of Pharmaco-gonosy, University of Benin, while it was authenticated at Forestry Research Institute of Nigeria (F.R.I.N., Ibadan) where a specimen (No FHI 107654) was deposited for future reference.

Immediately after collection, the barks were cut into small pieces and dried under sunlight. The dried barks were pulverized into a smooth powder using impact mill, weighed and kept for further analysis.

Drugs and chemicals

Absolute alcohol (Sigma-Aldrich), Mueller Hinton agar medium (Oxiod Limited., Basingstoke, England) Nutrient broth (oxoid cm 31), Sabouraud dextrose broth (oxoid CM 41; Sigma – Aldrich) Amoxicillin, Ciprofloxacin, Gentamicin, Ceftriaxone (silva Hills) and Fluconazole (Drug field).

Extraction of the plant material

The powdered material (500 g) was mixed with absolute alcohol (2.5 l) and left for 72 h. The mixture was stirred at 6 h intervals using a sterile glass rod, while another 500 g was placed into 4 l of distilled water and heated using a hot plate for 30 min. At the end, both extracts were filtered. The filtrates were concentrated in a vacuum (40^{o}C), giving a yield of 3.78% for the ethanolic extract and 10.74% for aqueous extract. They were then stored in universal bottles and refrigerated at 4^{o}C prior to use.

Micro organism

Clinical Isolates of *E. Coli, S. aureus, P. aeruginosa* and the yeast *C. albicans* were all supplied by the Pharmaceutical Microbiology Department of the University of Benin.

Preparation of medium

Mueller–Hinton Agar was supplied by the Department of Pharmaceutical Microbiology, Faculty of Pharmacy University of Benin. Inocula of test organisms obtained from source were prepared by growing each pure isolate in Nutrient broth for 18 hours at 37^{o}C. The overnight broth culture was matched with macfarland turbidity standard to give an approximate 10^{8} cfu/ml. 0.2mls was then used to seed a molten Mueller Hinton agar medium which has been allowed to cool to 45^{o}C to obtain approximately 10^{6} cfu/ml. This was poured into sterile Petri dishes and used for analysis (Ibeh et al 2002).

Antimicrobial susceptibility testing

The agar diffusion method described by Ver-poorte (1988) was used. Inocula of test organisms obtained from source were prepared by growing each pure isolate in Nutrient broth for 18 h at 37^{o}C. The overnight broth culture was matched with macfarland turbidity standard to give an approximate 10^{8} cfu/ml. 0.2 ml was then used to seed a molten Mueller Hinton agar medium which has been allowed to cool to 45^{o}C to obtain approximately 10^{6} cfc/ml. This was poured into sterile petri dishes and used for analysis.

The susceptibility assay was carried out with 20 mg/ml concentration of each of the extracts with bacterial suspensions of 10^{6} organisms/ml. This was delivered into wells (8 mm in diameter) bored unto the surface of the already seeded Mueller Hinton agar plates. Equal volumes of distilled water and ethanol were assayed along to act as negative controls. Commercial discs containing amoxicillin 25 µg, ciprofloxacin 5 µg, gentamicin 10 µg, ceftriaxone 30 µg and 20 mg/ml fluconazole served as positive controls for the antibacterial and anti-fungal activities. *S. aureus* (NCTC 10788) maintained in the pharmaceutical microbiology laboratory was set up along with the test organisms as a check on the effect of the media and inherent sensitivity of isolates on zones of inhibition produced by the anti-bacterial substances. *C. albicans* was first grown on sabouraud dextrose broth and assayed using sabouraud dextrose agar. The plates were incubated at 37^{o}C for 24 h while the plates containing the fungi were incubated at 25^{o}C for 48 h. After incubation, zone of inhibition around the wells and the disc were measured and recorded.

Minimum inhibitory concentration (MIC)

The extracts were incorporated into Mueller Hinton agar at concentrations ranging from 2.5 mg/ml to 20 mg/ml. A control containing the growth medium and each of the test isolates was also set up. A loopful of the organisms previously diluted to 10^{6} cfu/ml was used to inoculate the plates. These were incubated at appropriate temperatures of 37^{o}C for 24 h and 25^{o}C for 48 h for the bacteria and fungi, respectively. The MIC of the extract was regarded as the lowest concentration that did not permit growth of test organism.

Acute toxicity test

24 mice (20 – 25 g) of either sex were obtained from the Animal House of the Department of Pharmacology and Toxicology, University of Benin, Benin city. The animals were randomly divided into six (6) groups of four (4) mice each. The animals were fed with mice pellets and had free access to drinking water but starved for 12 h prior to testing. The first five groups were orally administered with 1, 2, 4, 6 and 8 g/kg of ethanolic extract, respectively. The sixth group was given distilled water (10 ml/kg). General symptoms of toxicity and mortality were observed for 24 h for any sign of delayed toxicity (Lorke, 1983).

RESULTS AND DISCUSSION

The ethanolic extract of *K. africana* possess antibacterial and antifungal activity against *S. aureus* and *C. albicans* but not against the strains of *Escherichia coli* and *P. aeruginosa* tested (Table 1). The water extract showed

Table 1. Mean zone diameter of clinical isolates to the ethanolic extract.

Organisms	Zone of inhibition (mm)	
	Neat	20 mg
Staphylococcus aureus	34.5 ±2.5	15.0 ±0.95
Escherichia coli	-	-
Pseudomonas aeruginosa	-	-
Candida albicans	24.8 ±1.53	0.75 ±4.6

Mean taken was for 10 isolates. Values are mean zone of inhibitions in mm ± SEM.
Zone measuring ≥ 10 mm was accepted as indicating sensitivity of an organism to an antimicrobial agent.

Table 2. Comparison of the antimicrobial activity of commercial antibiotics against the ethanolic extract of *Kigelia africana*.

Organisms	Mean zone of inhibition (mm)					
	Extract (20 mg)	GN (10 µg)	CZ (30 µg)	CIP (5 µg)	AM (25 µg)	F (20 mg)
Staphylococcus aureus	15.0 ± 0.95	19.3 ±1.44	25.3 ±3.01	33.0 ±1.29	15.0 ±1.00	-
Escherichia coli	-	11.0 ±3.00	19.4 ±2.34	23.6 ±2.03	16.5 ±4.50	-
Pseudomonas aeruginosa	-	15.0 ±1.00	16.5 ±2.00	29.6 ±3.28	-	-
Candida albicans	20.75 ±4.60	-	-	-	-	36.0 ±2.28

Values are mean zone of inhibitions in mm ± SEM.
Mean taken was for 10 clinical isolates.
GN: Gentamicin, CZ: Ceftriaxone, CIP: Ciprofloxacin, AM: Amoxicillin, F: Fluconazole.

Table 3. Minimum inhibitory concentration (MIC) of the ethanolic extract of *Kigelia africana*.

Organisms	MIC (mg/ml)
Staphylococcus aureus	6.25 ± 1.07
Escherichia coli	-
Pseudomonas aeruginosa	-
Candida albicans	7.92 ± 1.52

Mean taken was for 10 isolates for *Staphylococcus aureus* and 6 isolates for *Candida albicans*.
-: indicates no zone of inhibition.

Table 4. Acute toxicity effect of the ethanolic extract of *Kigelia africana*.

Treatment (mg/kg)	Log-dose	Percentage mortality (%)	Probit transformation
Control		0	-
1000	3.000	0	-
2000	3.301	0	-
4000	3.602	50	5.000
6000	3.778	75	5.700
8000	3.903	100	-

Values are mean of four per group.

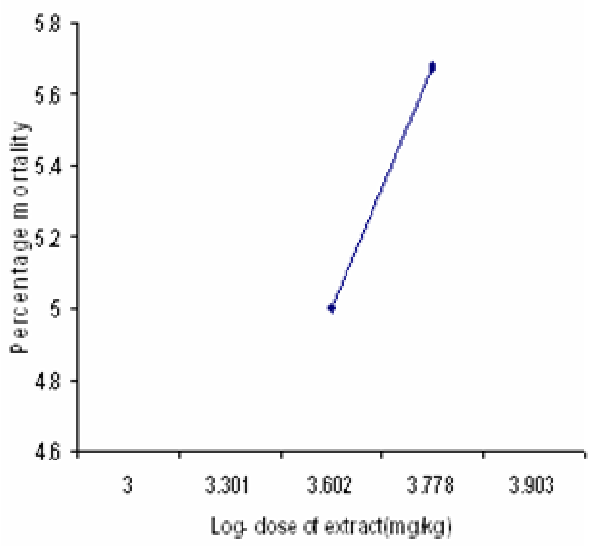

Figure 1. Log dose response curve of the ethanolic extract of *Kigelia africana*.

no activity against all the organisms. The activity of 20 mg/ml of the ethanolic extract of *K. africana* against *S. aureus* was found to be similar to that of 25 µg disc of amoxicillin (Table 2). The MIC was 6.25 ± 1.07 mg/ml for the ethanolic extract against *S. aureus* and 7.92 ± 1.520 mg/ml against *C. albican* (Table 3). Distilled water and ethanol, which served as negative controls, produced no zones of inhibition. Table 4 shows the result of the acute toxicity test done on mice. The LD_{50} was estimated from a log-dose response curve (Figure 1) as 4 g/kg.

The extract was well tolerated by the animals as no signs of acute toxic effects like restlessness, dizziness or seizures were observed after the administration at 1 – 2 g/kg. However at 4 g/kg, the animals showed signs of toxicity like writhes and jerks, with 50% death. At 6 g/kg there was 75% death. While at 8 g/kg, there was 100% death.

The fact that the ethanolic extract of *K. africana* produced zones of inhibition against a Gram positive organism such as *S. aureus* and a fungus *C. albicans* indicate the presence of antimicrobial activity which confirms its use as anti-infection agent. However, it showed no activity against *E. coli* and *P. aeruginosa* thus indicating its narrow spectrum of activity. The aqueous extract showed no activity, indicating that ethanol is a better extracting solvent. *S. aureus* and *C. albicans* have been implicated in the pathology of atopic eczema and psoriasis (Watt and Breyer-Bradwijk, 1962). From the investigation carried out it shows that low doses of 6.25 ±1.07mg/ml and 7.92 ±1.52mg/ml of the ethanolic extract would inhibit the effect of these organisms causing these infections. This thus gives credence to its ethnopharmacological use as a remedy for these skin infections and other infections in which these organisms are implicated.

The ethanolic extract was compared with respect to its antimicrobial activity with some commercially obtained

antibiotics. This is shown in Table 3. Zones of inhibition against Staphylococcus aureus produced by the extract at 20mg were similar to that of 25µg disc of amoxicillin. However its zones of inhibition against Staphylococcus aureus were 78%, 60% and 45% of that produced by gentamicin, ceftriaxone and ciprofloxacin respectively (result not shown). For its antifungal activity the zones of inhibition produced was 58% of that of fluconazole (result not shown).

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