Antimicrobial Activity and Cytotoxicity of Fresh and Dry Fruits of *Kigelia africana*

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ABSTRACT

Objective: The present study seeks to determine the antibacterial and antifungal activities in addition to the cytotoxicity of dry and fresh fruits of *Kigelia africana*.

Methods: Fresh and dried fruits of *Kigelia africana* were collected and extracted separately by maceration, infusion and soxhlet using different solvents. The extracts were screened for the antibacterial and antifungal activities against *Staphylococcus aureus*, *Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa* and *Candida albicans* using disc diffusion and cup plate agar diffusion methods. The minimum inhibitory concentration (MIC) was then determined and the most effective extract was examined for the presence of various phytochemicals. The toxicity of the active extract was also evaluated *in vitro* via the brine shrimp lethality assay to determine the LC50.

Results: Extracts of fresh *Kigelia africana* fruits were more active than dry fruits extracts. Moreover, the aquatic ethanol extract of the fresh fruit exhibited the highest effectiveness among all tested extracts against all mentioned bacteria by both methods. It was more effective against gram positive than the gram negatives. Nevertheless, all extracts were ineffective against *C. albicans*. The phytochemical screening of the aquatic ethanol extract showed the presence of alkaloids, coumarins, tannins, flavonoids, triterpenes, sterols and anthraquinones glycosides. This extract imparted an LC50 value of 1.11 mg/ml which categorized it as non-toxic extract.

Conclusion: The aquatic ethanol extract of fresh fruits of *Kigelia africana* has significant antibacterial activity and safety which enable it treatment option for bacterial resistance.

Key words: Antibacterial, antifungal, phytochemical, cytotoxicity, brine shrimp**,** *Kigelia africana.*

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INTRODUCTION

Medicinal plants have been a major source of drugs till the last century, but with the rapid advance in organic chemistry they were put aside by the designed synthetic compounds [1]. Nonetheless, they still occupy an important place in medicine and approximately 50% of individuals from developed countries especially Africans depend on traditional medicines in form of herbs or herbal products for the treatment of human diseases. This is because of their rich heritage in the field of herbal medicine[2].

Despite of the success of the used plants in treating the targeted conditions, they should be investigatedto understand their properties, safety and efficacy [3]. That what opened a wide door into research in medicinal plants and related fields as they have limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen-substituted derivatives [4]. In many cases, these substances serve as plant defense mechanisms against predation by microorganisms, insects, and herbivores [5].

Acquired antimicrobial resistance is a growing worldwide problem that emerged from the increasing use of antimicrobials in humans, animals and agriculture [6]. This issue necessitated the search for new efficient antimicrobials that lacks side-effects on humans. Thus, several medicinal plants were screened for their potential antimicrobial activities such as Black pepper, Buchu, Caraway, Eucalyptus, Gamboge, Harmel, Hemp, Licorice, Oak,Poppy, Thyme and Turmeric [7,3].

The antimicrobial activity of medicinal plants was attributed to the presence of various phytochemical groups such as flavonoids, tannins, alkaloids, coumarins, saponins, triterpenes, phytosterols, volatile oils, anthraquinones and glycosides [8].

The Sudan Atlas of Medicinal Plants records the scientific name of more than 2000 medicinal herbs collected from different parts of the country, many native to Sudan. All of these herbs are in current use in traditional medicine [2]. *Kigelia africana* Lam. (Benth) belongs to family *Bignoniaceae*, its common name sausage tree has a wide distribution in Africa, America, India and Australia. It has a long history of use by rural and African countries particularly for medicinal properties. Several parts of the plant are employed for medicinal purposes by certain

aboriginal people [9].In Sudan, the dry and fresh fruits of this plant are largely used in the southern and south western parts for treating many local infectious diseases [10,11]. The present study highlights the contribution of*K. africana*fruits in modern system of herbal medicine for new drug development. This study aims at determining the antibacterial and antifungal activities as well as to the cytotoxicity of dry and fresh fruits of *Kigelia africana*.

MATERIALS AND MOETHODS

Materials

Chloroform (MRS, UK), methanol (Applichem, Germany), ethanol (Applichem, Germany), n-hexane (UNI-CHEM, India), ethyl acetate (UNI-CHEM, India), Muller Hinton Agar (Scharlu, Spain), Sabouraud Dextrose Agar (Scharlu, Spain), ciprofloxacin (Hovid, Malaysia), amoxicillin (AurobindosPharmaLtd., India), clotrimazole (Shin Poong, Korea), *Artemia salina* eggs (INVE AQUACULTURE, Thailand), dimethyl sulfoxide (DMSO) (S d FINE CHEM, India) and potassium dichromate (BDH, England).

Methods

Collection and identification of plant material

The mature fresh and dried fruits of *Kigelia africana* from the family *Bignoniaceae* were collected from Khartoum – Sudan, identified and authenticated by botanists in the Medicinal and Aromatic Plants Research Institute (MAPRI), Sudan. The fruits were washed thoroughly with running tap water, cut into slides, then air-dried for one week at room temperature by spreading evenly in an open drying area with a fan. They were then powdered by a grinding mill, packaged and stored in an air tight container with appropriate label.

Preparation of extracts

50 grams of the ground plant material of both fresh and dried fruits were prepared separately for extraction by maceration for 7 days with periodic shaking using chloroform, ethanol, ethanol (70%), methanol and methanol (70%). The aquatic ethanol and methanolic extract were fractionated successively using n-hexane, ethyl acetate and chloroform, while other crude powdered fruits were extracted by infusion using water for 4 hours followed by Soxhlet extraction (ISOLAB-Germany) for 48 hours using chloroform, ethanol and methanol. Next, the extracts were filtered through Whatman No. 1 filter paper and the solvents were removed using a rotary evaporator for organic solvents from BIBBY Sterilin-UK and freeze drying (via a dryer from TELSTAR-Spain) for the aqueous part of solvents. The final extracts were stored in sterile airtight containers at 4 °C in readiness for bioassay tests [12]. The yield % of each extract was

estimated via the following equation:

$$\text{Yield\%} = \frac{\text{Weight of extract}}{\text{Weight of crude drug}} \times 100$$

Antimicrobial assay

Antibacterial and antifungal susceptibility test

The antimicrobial activity of the extracts was determined using the disc diffusion and the cup plate methods against *Staphylococcus aureus*(ATCC 25923), *Bacillus subtilis* (NCTC 8236), *Escherichia coli (*ATCC 25922) and *Pseudomonas aeruginosa (*ATCC 27853)as bacterial strains and *Candida albicans (*ATCC 7596) as tested fungi. Mueller Hinton Agar was used as media for bacterial culture while Sabouraud Dextrose Agar was used as fungal culture media.

Disc diffusion method

6 mm sterile filter paper disk was impregnated into the extract solution (100 mg/ml) and then air dried. After that, the disk was placed aseptically onto inoculated plates with 10^8 CFU/ml (Colony Forming Unit/ml) suspension of each microorganism,allowed to diffuse at room temperature for two hours and incubated for 18 hours at 37 °C for the bacteria whereas for the fungal cultures, incubation was at 25 ºC for 48 hours [13].

Cup plate method

Cup plate method was conducted by inoculation of the agar media was inoculated with 10^8 CFU/ml suspension of each microorganism and left to set and in each of the plates, cups (6 mm in diameter) was made using a sterile cork borer. Alternate cups were filled with 0.1 ml of each extract solution using automatic microtitre pipette, and allowed to diffuse at room temperature for two hours. The plates were next incubated in the upright position at 37 ºCfor 18 hours for the bacteria and at 28 ºC for 48 hours for the fungi [14].

DMSO and distilled water were used as negative controls while amoxicillin and ciprofloxacin hydrochloride were chosen to be the positive counterparts. The inhibition zone in duplicate was determined for test and controls.

Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) of the aquatic ethanol extract was determined via the agar plate dilution method. For that, plates were prepared in series of increasing concentrations of the plant extract in the following order: 3.125, 6.25, 12.5, 25, 50 and 100 mg/ml. The organisms to be tested were grown in broth over-night, and diluted in broth to contain about 10^8 CFU/ ml. Then, a loopful of the diluted culture was spotted with standard-loop that delivered 0.01 ml onto the surface of each segment and incubated at 37°C for 18 hours. The end point (MIC) which is the least concentration of antimicrobial agent that completely inhibits

growth- was recorded in mg/ml of crude extract for each bacteria.

Phytochemical screening

The aquatic ethanol extract was screened for the presence of tannins, triterpenes, sterols, alkaloids,saponins,flavonoids, coumarins,anthraquinones glycosides, cyanogenic glycosides and cardiac glycosides [15, 16, 17, 18].

Cytotoxicity Assay

Brine shrimp lethality test was used for determining the acute cytotoxicity of aquatic ethanol extract. Natural sea water was filtered using filter paper and diluted with purified water to adjust its salinity. The pH of this mixture was adjusted to be neutral which placed in a separating funnel with a turnover and shrimp eggs added to the separating funnel which is darkened by covering it with aluminum foil with continuous aeration by air pump. The illuminated part of funnel was attracted *Artemia salina* larvae (nauplii) to easily separate the larvae from eggs thorough the turnover. 48 hours were allowed at room temperature (22–29°C) for the shrimps to hatch and mature.

For each fraction, 2, 1, 0.1 and 0.01 mg/ml concentrations were prepared and 3 replicates for each concentration were prepared making a total of 12 vials for each extract. After 48 hours and when the brine shrimp larvae matured, 4.5 ml “sea water” was added to each vial with 20 shrimps (larvae) added per vial with the help of Pasteur pipette. The vials were maintained under illumination. The test was also carried out on potassium dichromate ($K_2Cr_2O_7$) as positive control in the concentrations of 0.01 mg/ml, 0.1mg/ml and 1 mg/ml and on natural sea water alone and with DMSO as negative control. After 24 hours period, the number of surviving shrimps were counted with the aid of magnifying glass and recorded. The nauplii were considered moribund if no movement of the appendages was observed within 10 seconds. The mortality in each vial was estimated via the following equation:

$$\text{Mortality (\%)} = Sc - St$$

Where:

Sc = % nauplii that survived for control

St = % nauplii that survived the treatment.

Additionally, the lethality was calculated from the mean survival of larvae in both extract and control samples. The mean percentage mortality was plotted against the concentrations and the lethal concentration killing 50% of the larvae (LC50) was calculated from the linear regression equation using SPSS version 24, IBM Corp [19,20].

RESULTS AND DISCUSSION

Antibacterial susceptibility test

The highest antibacterial activity against all tested bacteria was demonstrated by the aquatic ethanol extract of the fresh *Kigelia africana* fruit that was prepared using maceration. The inhibition zone diameters of this extract, ranged between 21.5 mm and 28 mm for the cup plate method and 18 mm to 25mm for those of the disc diffusion method as shown in Table 1. The antibacterial activity of dry fruits is illustrated in table 2.

The cup plate method results demonstrated better antimicrobial activity than the disc diffusion approach, which may be attributed to retention of some phytoconstituents in paper discs during the diffusion of phytoconstituents into agar in incubation stage.

Extracts of the fresh *K. africana* fruits was found to have greater antibacterial activity than the dry fruits, this may be due to destruction of some phytoconstituents during the storage of dry fruits because of poor storage conditions of whole dry fruits i.e. without warinessto physicochemical factors as temperature and light and biological factors as microbial contaminants and insects that may decompose the active phytoconstituents during the storage of crude drugs [12,21]. This activityagainst theselected bacteria which are common opportunistic pathogens that causes infections agreed with the ethnobotanical uses of the fruit among the African countries for the treatment of infectious diseases such as abscess, wound infection which usually caused by *S. aureus* and *P. aeruginosa* and bacillary dysentery [10,11,22].

It was noticed that the tested plant extracts were more active against gram positive bacteria than the gram negatives, which was explained by the presence of the outer membrane in gram negative bacteria. That membrane is known to be rich in a molecule called lipopolysaccharide which exclude certain drugs and antibiotics from penetrating the cell, thus partially accounting for why gram-negative bacteria are generally more resistant to antibiotics than the gram-positive bacteria [23].

However, the fractionated aquatic ethanol extracts were less active than the crude aquatic ethanol extract. This ensures the synergistic effect of crude extract constituents which is supported by the study stated that that all phytochemical groups of extract usually contribute to the antibacterial activity of most medicinal plants [3,12,24,25].

Table 1: Yield % and mean diameter of inhibition zone of extracts of *Kigelia africana* fresh fruits prepared with various methods and different solvents against *Staphylococcus aureus*, *Bacillus subtilis, Pseudomonas aeruginosa* and *Escherichia coli.*

Extract/ STD	Method	Solvent	Yield% w/w	MDIZ (mm)							
				S.a		*B.s*		*P.a*		*E.coli*	
				C.P	*D.D*	*C.P*	*D.D*	*C.P*	*D.D*	*C.P*	*D.D*
EF1	Maceration	Chloroform	2.3	-	-	-	-	-	-	-	-
EF2	Maceration	Ethanol	16.0	21	20	20.5	18	18	15	15	13
EF3	Maceration	Ethanol 70%	20.3	28	25	25	22.5	21.5	18	23	20
EF4	Maceration	Methanol	17.8	24	21	21	18	18	13.5	18.5	16
EF5	Maceration	Methanol70%	19.1	19	16	17	14.5	16	14	18	15
EF6	Fractionation	n-hexane	2.0	11	-	10	-	-	-	-	-
EF7	Fractionation	Ethyl acetate	1.6	12.5	11	11	-	10	-	-	-
EF8	Fractionation	Chloroform	1.5	-	-	-	-	-	-	-	-
EF9	Fractionation	Ethanol 70%	15.2	13	10	12	10	10	-	11	-
EF10	Fractionation	n-hexane	2.2	10	-	-	-	-	-	-	-
EF11	Fractionation	Ethyl acetate	1.9	11	-	13	10	-	-	-	-
EF12	Fractionation	Chloroform	1.8	13.5	10	12	10	10	-	11	-
EF13	Fractionation	Methanol	11.9	12	10	11	-	-	-	-	-
EF14	Infusion	Water	5.0	13	11	16	13.5	13	11	16	13.5
EF15	Soxhlet	Chloroform	2.9	-	-	-	-	-	-	-	-
EF16	Soxhlet	Ethanol	27.3	18	17	19.5	17	19	16	20	17.5
EF17	Soxhlet	Methanol	25.4	20	18	17	15	16	14.5	15	12
STD1 Amox	NA	NA	NA	30.5	32	25	24	-	-	24	23
STD2 Cip	NA	NA	NA	23	22	20	21	28.5	29	31	33

* C.P: cup plate, D.D: disc diffusion, NA: not applied, MDIZ: mean diameter of inhibition zone, *S.a*: *Staphylococcus aureus, B.s*: *Bacillus subtilis, P.a*: *Pseudomonas aeruginosa* and*E.coli: Escherichia coli*

Table 2: Yield % and mean diameter of inhibition zone of extracts of dry fruits of *Kigelia africana*prepared with various methods and different solvents against*Staphylococcus aureus*, *Bacillus subtilis, Pseudomonas aeruginosa* and *Escherichia coli.*

Extract	Method	Solvent	Yield% w/w	MDIZ (mm)							
				S.a		*B.s*		*P.a*		*E.coli*	
				C.P	*D.D*	*C.P*	*D.D*	*C.P*	*D.D*	*C.P*	*D.D*
EF1	Maceration	Chloroform	2.0	-	-	-	-	-	-	-	-
EF2	Maceration	Ethanol	13.0	17	15	17	15	16	14	17	15.5
EF3	Maceration	Ethanol 70%	16.3	20	18	17	16	16	13	13	11
EF4	Maceration	Methanol	15.7	18	17	18	15	12	10.5	15	12
EF5	Maceration	Methanol70%	16.1	16	14	15	12.5	14	12	16	14
EF6	Fractionation	n-hexane	1.6	-	-	-	-	-	-	-	-
EF7	Fractionation	Ethyl acetate	1.4	10	-	-	-	-	-	-	-
EF8	Fractionation	Chloroform	1.3	-	-	-	-	-	-	-	-
EF9	Fractionation	Ethanol 70%	12.0	11.5	10	10	-	-	-	10	-
EF10	Fractionation	n-hexane	1.9	-	-	-	-	-	-	-	-
EF11	Fractionation	Ethyl acetate	1.5	10.5	-	10	-	-	-	-	-
EF12	Fractionation	Chloroform	1.3	11	-	10.5	-	11	-	-	-
EF13	Fractionation	Methanol	11.0	10	-	11	-	-	-	-	-
EF14	Infusion	Water	4.7	12	10	13	10	11	-	13.5	10
EF15	Soxhlet	Chloroform	2.2	-	-	-	-	-	-	-	-
EF16	Soxhlet	Ethanol	21.3	15	13	14	12.5	15	13	15	13
EF17	Soxhlet	Methanol	19.4	18	15	16	14	13	11	14	12.5

Antifungal susceptibility test

All extracts of both fresh and dry fruits of *Kigelia africana* exhibited no antifungal activity. Fungi are eukaryotes thus the cell is difficult to be penetrated by the extracts due to the presence of cell wall made of cellulose as described by [22].

Minimum inhibitory concentration (MIC)

The MIC of aquatic ethanol extract of the fresh fruits against*S.aureus, B.subtilis,P.aeruginosa*and *E.coli*was 6.25,12.5, 25 and 12.5 mg/ml respectively**.**

The aquatic ethanol extract of *K. africana* fruits was the most active among the others and its highest potency was against *S. aureus* with MIC of 6.25 mg/ml and the least potent was 25 mg/ml against the *P. aeruginosa*.

The concentrations and proportions of the active compounds in plant extracts components depend on the plant variety, origin, time of harvest, solvent used, conditions of processing and storage [12]. For the *K. africana* fruit extracts, the antibacterial potency was observed since they were crude extracts and that was justified by the presence of the active phytoconstituents mentioned in table 4.

Phytochemical screening

The aquatic ethanol extract showed the presence of high contents of tannins and flavonoids and moderate representation of triterpenes, whereas sterols, alkaloids, coumarins and anthraquinones glycosides were present in trace amounts. The antibacterial activity of the extract was attributed to the presence of these phytochemical groups because all have antibacterial activity.[3,8,26]. No cardiac glycosides or cyanogenic glycosides was detected as depicted in table 4.

Cytotoxicity Assay

The mortality percent of different concentrationsof aquatic ethanol extract of *Kigelia africana*fruits using potassium dichromate and sea water as positive and negative controls respectively are showed in table 5.

The LC50 of aquatic ethanol extract was 1.11 mg/ml with regression (R^2) of 0.97 and P value of 0.01. The LC50 of standard potassium dichromate was 0.217mg/ml, a regression (R^2) of 1.0 and a (0.00) P value.

Toxicity studies are very important during the screening of medicinal plants in order to determine their safety. Fortunately, the absence of cardiac glycosides and cyanogenic glycosides approved the safety of *K. africana* extracts as these constituents usually exhibit hazardous effects [12].

Table3: Major phytochemical groups of aquatic ethanol extract of *Kigelia africana* fresh fruits

No.	Constituent	Test/reagent	Result	Observation
1	Tannins	Ferric chloride	+++	Blackish blue color
		Gelatin salt	+++	Precipitation
2	Triterpenes	Acetic anhydride	++	Pink color
3	Sterols	Acetic anhydride	+	Green-blue color
4	Alkaloids	Valser's reagent	+	White precipitate
		Meyer's reagent	+	Cream colored precipitate
		Dragendorff's reagent	+	Red orange precipitate
5	Flavonoids	Aluminum chloride	+++	Yellow color
		Potassium hydroxide	+++	Dark yellow color
6	Saponins	Frothing	-	No observation
7	Coumarins	UV lamb	+	UV florescence
8	Anthraquinone glycoside	Ammonium hydroxide test	+	Pink color on benzene layer
9	Cyanogenic glycoside	Sodium picrate paper	-	No observation
10	Cardiac glycosides	Killer-killiani test	-	No observation

(+++): high content, (++): moderate content, (+): low content, (-): absent

Table4: Mortality of aquatic ethanol extract of *Kigelia africana* fresh fruits against brine shrimp larvae

Test/ Control	Number of survived larvae Mean , n=3				% Nauplii that survived the treatment (St)				Mortality %			
	0.01 mg/ml	0.1 mg/ml	1 mg/ml	2 mg/ml	0.01 mg/ml	0.1 mg/ml	1 mg/ml	2 mg/ml	0.01 mg/ml	0.1 mg/ml	1 mg/ml	2 mg/ml
Aquatic ethanol extract	18.0	15.33	11.0	4.33	90	76.6	55	21.65	10	23.35	45	78.35
Potassium dichromate	17.6	14.3	NA	NA	88	71.5	NA	NA	12	28.5	NA	NA
Natural sea water	20				100				0			

Furthermore, the cell toxicity of the aquatic ethanol extract was determined. The LC50 of extract was 1.11mg/ml indicate non toxicity of the extract according to Meyer's toxicity index; extracts with LC50 < 1000 µg/ml are considered as toxic, while extracts with LC50 > 1000 µg/ml are considered as non-toxicand according toClarkson's toxicity criterion for the toxicity assessment of plant extracts classifies extracts into the following order: extracts with LC50 above 1000 µg/ml are non-toxic, LC50 of 500 - 1000 µg/ml are low toxic, extracts with LC50 of 100 - 500 µg/ml are medium toxic, while extracts with LC50 of 0 - 100 µg/ml are highly toxic [19,27].

CONCLUSION

The results of this study validate the traditional use of *Kigelia africana* fruits in multiple African traditional system to treat bacterial infection, the fresh fruits were more effective than dry fruits. The aquatic ethanol extract of *K. africana* fresh fruits exhibited significant antibacterial activity, also the study primarily prove its safety by studying of its cytotoxicity using brine shrimp lethality assay. Bioactivity driven purification of active constituent and examination of mechanism of action is required.

CONFLICTS OF INTEREST

The authors declare that they have no potential conflicts of interest.

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