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Pharmacognostic, antioxidant and antimicrobial activity of fractions of the leaf extract of *Kigelia africana* Lam. Benth (Bignoniaceae).

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ABSTRACT: *Kigelia africana Lam. Benth (Bignoniaceae) leaves are used in ethnomedicine for the management and treatment of various infectious diseases. This study was aimed at investigating the Pharmacognostic, antioxidant and antimicrobial activities of the plant to aid standardization and continuous usage.*

Evaluation of fresh, powdered and anatomical sections of the leaves were carried out to determine Pharmacognostic profile. Chemical tests were employed in phytochemical investigations. The antioxidant activities were carried out using DPPH radical scavenging method. The polyphenolic contents were also evaluated. Inhibitory activity of the crude methanol extract and fractions against clinical strains of Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella aerogenes, Candida albicans and Candida parasilopsis were compared with ciprofloxacin and nystatin for bacteria and fungi respectively.

Pharmacognostic data which can aid sample identification are provided. Phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, and saponins. The fractions showed significant antioxidant activity when compared to the standard with n-butanol fraction having the highest activity. The crude methanol extract and the fractions inhibited the growth of E. coli, B. subtilis, S. aureus, P. aeruginosa, C. albicans and C. parasilopsis to varying degrees, except the aqueous fraction that was devoid of activity.

Extract and fractions of K. africana have demonstrated antimicrobial activity against clinical strains of selected microorganisms and showed significant antioxidant activity. These results showed that K. africana has a role in the treatment of infectious diseases.

KEYWORDS: *Kigelia africana, Pharmacognostic, antioxidant, antimicrobial.*

INTRODUCTION

Medicinal plants are commonly used in treating and preventing specific ailments and diseases and are generally considered to play a beneficial role in healthcare. Within the last few decades, many plants have been screened for their biological and pharmacological properties by researchers. These efforts are continually being taken to examine the merits of traditional medicine in the light of modern science with a view aimed at adopting effectively beneficial medical practice and discouraging harmful ones [1].

The genus *Kigelia* comprises one specie, *Kigelia africana* which occurs throughout tropical Africa [2]. Common names include Sausage tree (English), Um Vunguta (Zulu), Muvevha (Venda), Worsboom (Afrikaans), Rahaina (Hausa), Pandoro (Yoruba) and Ishi (Igbo) [3].

K. africana is widely used to treat gynaecological disorders. Aqueous preparations of the leaves, roots, fruits and flowers are administered orally or as a virginal pessary while the fruits and barks are used to promote breast development in young women or in contrast to reduce swelling and mastitis of the breast [4]. The wound healing activity of the aqueous extract of shade-dried bark of *K. africana* in rats was attributed to epithelization [5]. A crude ethanol extract exhibited antibacterial and antifungal activities against *Staphylococcus aureus* and *Candida albicans* [6]. The leaf extract formulated as shampoo also exhibited antimicrobial activity [7]. The extracts of the plant has been shown to possess potential anticancer agents [8, 9]. Butanol extract of the stem bark exhibited *in vitro* antiamoebic activity when tested against HK-9 strain of Entamoeba histolytica (micro dilution method) using metronidazole as reference drug [10]. The present study was designed not only to establish the Pharmacognostic and antioxidant profile of *K. africana*, but also to investigate the antimicrobial activities of the fractions as a prelude to isolating the active compounds responsible for the claimed biological activities.

Materials and Methods

Preparation of plant extract

Fresh leaves of *K. africana* Lam. Benth were collected in Ediaken-Uselu area of Benin City, Edo State, Nigeria. The plants were authenticated by the curator at the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Benin City where voucher specimens were deposited. The fresh leaves were air–dried for 72 h and powdered using an electric mill.

Macroscopy

The following macroscopic characters for the fresh leaves were noted: size and shape, colour, surfaces, venation, presence or absence of petiole, the apex, margin, base, lamina, texture, odour and taste [11, 12].

Microscopy

The outer epidermal membranous layer (in fragments) were cleared in chloral hydrate, mounted with glycerin and observed under a compound microscope. The presence/absence of the following were observed: epidermal cells, stomata (type and distribution) and epidermal hairs (types of trichomes and distribution). The transverse section of the fresh leaves through the lamina and the midrib as well as a small quantity of the powdered leaves were also cleared, mounted and observed [13].

Chemomicroscopic examination

Examination of the powder for starch grains, lignin, mucilage, calcium oxalate crystals, cutin and suberin were carried out using standard techniques [11].

Phytochemical studies

Screening for secondary plant metabolites was carried out according to previously described methods [11, 14, 15, 16]. These include chemical tests for tannins, alkaloids, cardiac, saponin, anthracene and cyanogenetic glycosides.3

Extraction and partitioning

The dried leaves of *K. africana* (3.5 kg) were extracted with MeOH-H_2O (80: 20). Evaporating the solvent yielded an extract (0. 83 kg) which was subsequently resuspended in water and successively partitioned into Chloroform (3 X 2L) and n-BuOH (3 X 2L). The fractions were investigated for antioxidant and antimicrobial activity.

Determination of antioxidant activity

The scavenging effect of the crude (80% methanol extract) and the various fractions on DPPH radical was determined [17]. A 1.0mL solution of 0.1mM of DPPH was mixed with 3.0mL of the crude extract, fractions and ascorbic acid (0.05-0.2mg/ml concentration). The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 minutes. The absorbance of the mixture was measured spectrophotometrically at 517nm. Abscorbic acid was used as reference standard. The ability to scavenge DPPH radical was calculated by the following equation:

DPPH radical scavenging activity (%) = $[(A_0 – A_1)/(A_0)] \times 100$

Where; A_0 was the absorbance of DPPH radical + methanol,

A_1 was the absorbance of DPPH radical + sample extract/standard [18].

The 50% inhibitory concentration value (IC_{50}) which was indicated as the effective concentration of the sample that was required to scavenge 50% of the DPPH free radical [17] was also determined.

Determination of polyphenolic content

Total phenol contents in the extracts were determined by the method described by Kim [19]. The extract solution (0.5ml) with concentration of 1000ug/ml was added to 4.5ml of deionized water and 0.5ml of FolinCicalteu's reagent (previously diluted with water 1: 10 v/v) which was added to the solution. After mixing the tubes, they were maintained at room temperature for 5 minutes followed by the addition of 5ml of 7% sodium carbonate and 2ml deionized water. The samples were incubated for 90 minutes at room temperature and the absorbance was measured with a spectrophotometer at 750nm. The total phenolic content was expressed as milligrams of gallic acid equivalents (GAE) per gram of extract (mg GAE/g extract). The standard curve was prepared with gallic acid in six different concentrations (12.5, 25, 50, 75, 100 and 150mg/L).

Determination of Total Flavonoid

Total flavonoid contents were estimated using the method described by Ebrahimzadeh [20]. 0.5ml of the crude extract and fractions (1 mg/ml) were mixed with 1.5ml of methanol and 0.1ml of 10% aluminum chloride was added, followed by 0.1ml of 1M potassium acetate and 2.8ml of distilled water. The mixture was incubated at room temperature for 30 minutes. The absorbance was measured by a spectrophotometer at 415nm. The results were expressed as milligrams quercetin equivalents (QE) per gram of extract (mg QE/g extract). The standard curve was prepared with quercetin in six different concentrations (12.5, 25, 50, 5, 100, and 150mg/L).

Antimicrobial assay

Clinical strains of five bacteria comprising three gram negative bacteria *(Escherichia coli, Pseudomonas aeruginosa and Klebsiella aerogenes*) and two gram positive bacteria (*Staphylococcus aureus and Bacillus subtillis*) were used for the antibacterial assay. Two yeasts (*Candida albican and Candida parasilopsis*) were used for the antifungal assay. The organisms were obtained from the department of Pharmaceutical Microbiology Laboratory, Faculty of Pharmacy, University of Benin, Benin City, Nigeria. The purity of the culture prior to use was confirmed by conventional cultural, morphological and biochemical methods. The microbial cultures were maintained in Nutrient Agar and Sabouraud Dextrose Agar for bacteria and fungi respectively at 4°C.

Preparation of inoculum

An overnight culture was used for the preparation of microbial suspension with a turbidity equivalent to that of 0.5 McFarland's standard.

Agar well diffusion method

The media were prepared and sterilized at 121°C for 15 mimutes. A total of 30ml Nutrient agar was seeded with bacterial culture and allowed to solidify and on each plate wells of 10mm in diameter were made. The open wells were filled with different concentrations of the extract ranging from 20mg/ml to 100mg/ml, and incubated at 37°C for 24 h. For antifungal assay, Sabouraud agar was used in place of Nutrient agar and the medium incubated at 28°C for 2 days. All test were carried out in triplicates. The inhibition zone diameter were measured and compared with ciprofloxacin and nystatin for antibacterial and antifungal assays respectively [21].
Statistical analysis: All the data were expressed as mean $\pm$ SEM (Standard error of mean) and Statistical significance was evaluated using the student's t-test. P-value of <0.05 was considered statistically significant.

Results

Macro-micro morphology of *K. africana* leaf

Macroscopically, the leaf is simple in composition, arranged in whorls of three with an eliptico-oblong-lanceolate shape. Venation is pinnate, apex is apiculate and margin is entire, slightly dentate-serrate. Fresh leaves are green in colour, odourless and with a slightly acid taste.

Micromorphological features revealed that anticlinal walls are thick and wavy (Fig. 1) and contains numerous calcium oxalate crystals. Each stoma is surrounded by two (2) subsidiary cells with their common walls at right angles to the long axis of the guard cells (Diacytic arrangement) (Fig. 2). Uniseriate covering trichomes are present on both surfaces. Transverse section of the leaf across the mid-rib shows a prominent cuticle as well as a radial wall. The upper epidermis consists of polygonal cells and a lower epidermis of smaller cells (It has an isobilateral arrangement). There are vascular bundles with pericyclic fibres. Collenchyma is present below the upper and lower epidermi (Fig. 3).

Chemomicroscopic examination of the leaves revealed the presence of lignin, starch, calcium oxalate crystals, mucilage, tannins and cellulose.

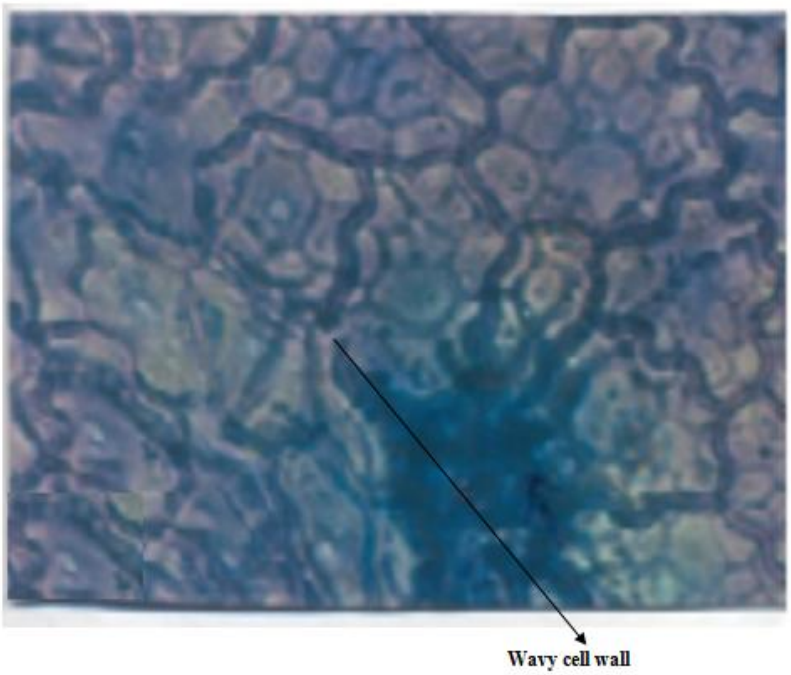

Fig 1: Epidermal cell wall

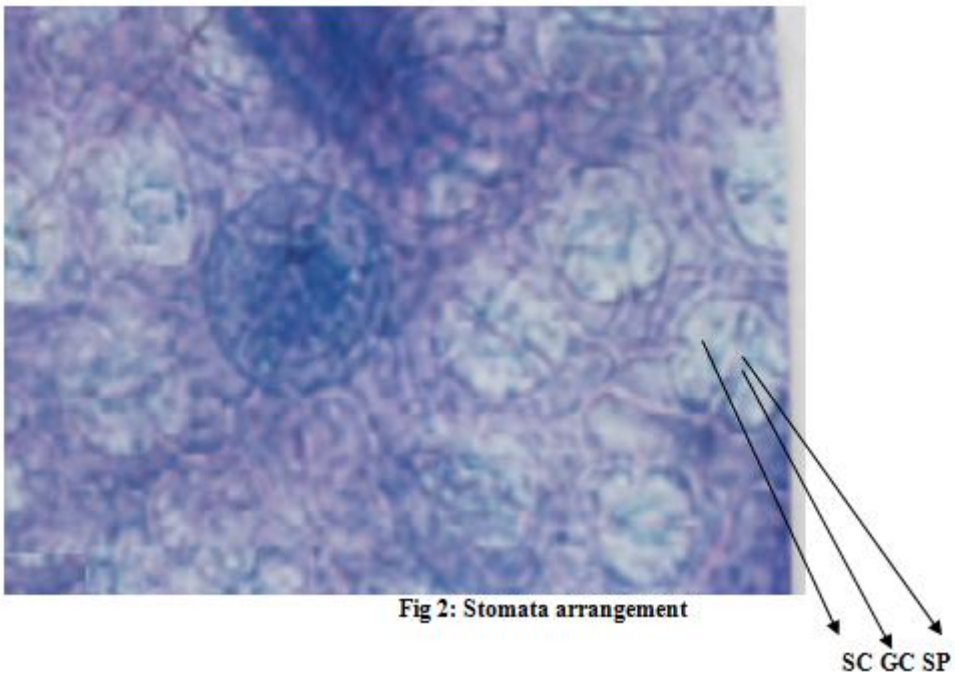

Fig. 3: Transverse section of *K. africana* through the midrib and lamina under high power magnification. (Co-Collenchyma, Xy-Xylem, Pf-Pericyclicfibre, Me-Mesophyll (spongy parenchyma), UE-upper epidermis, LE-lower epidermis)

Phytochemical screening

Phytochemical screening of the leaves of *K. africana* for secondary plant metabolites revealed the presence of alkaloids, tannins, flavonoids, saponins and cardiac glycosides (Table 2).

Table 1: Phytochemical constituents of *K. africana* leaves

Classes of secondary metabolites	inferences
Alkaloids	+
Tannins	+
Flavonoids	+
Anthracene derivatives	-
Saponin glycosides	+
Cardiac glycosides	+
Cyanogenetic glycosides	-

Key: = absent; + = present

Antioxidant activity

There was concentration dependent increase in the scavenging activity of the crude extract and fractions ranging from 5 µg/ml to 20µg/ml. The n-Butanol fraction demonstrated the highest activity.

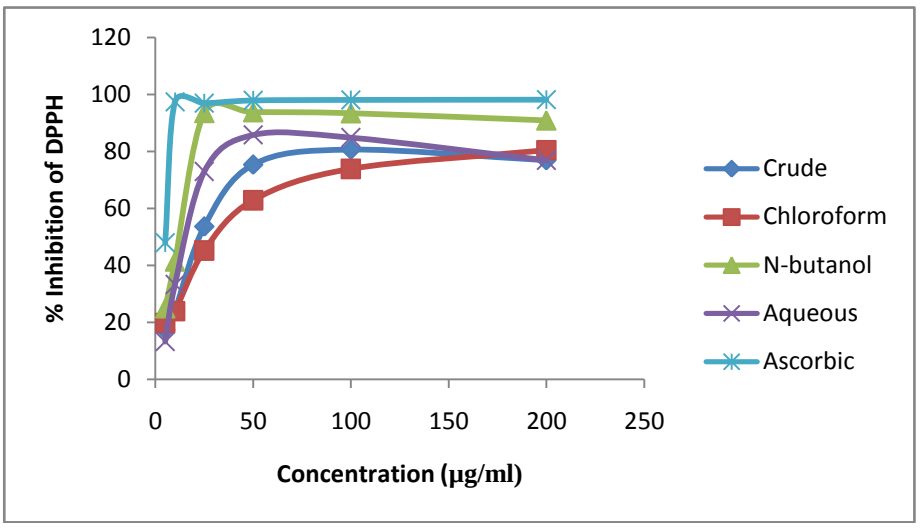

Fig 4: 2,2-diphenyl-1-picryl hydraxyl radical (DPPH) scavenging activity of the crude extract and fractions of *K. africana* compared with the standard (Ascorbic acid).

Table 2: IC_{50} values of crude extract and fractions

Samples (Fractions)	IC_{50} (µg/ml)
Crude	25.630
Aqueous	18.294
Chloroform	31.088
n-Butanol	9.886
Ascorbic acids	0.595

Total phenolic content and flavonoid assay

Table 3 Shows the total phenolic content while Table 4 shows total flavonoid content of the crude extract and fractions of *Kigelia africana*. The total phenolic content was expressed as mgGAE of extract with reference to a standard curve (y=0.0023x + 0.0058 R^2 = 0.9989) (fig 5). The total flavonoid content of the extract was with reference to a standard curve (y = 0.0073x – 0.0216, R^2 = 0.995) (fig 6)

Table 3: Results of total phenolic content

Samples (Fractions)	Phenolic content ± SEM
n-butanol	116.03±3.00
Chloroform	77.19±3.23
Crude	61.54±2.19
Aqueous	79.07±1.05

Table 4: Results of total flavonoid content

Samples (Fractions)	Flavonoid Content ± SEM
n-butanol	28.71±0.49
Chloroform	94.74±6.01
Aqueous	27.75±5.34
Crude	58.30±4.67

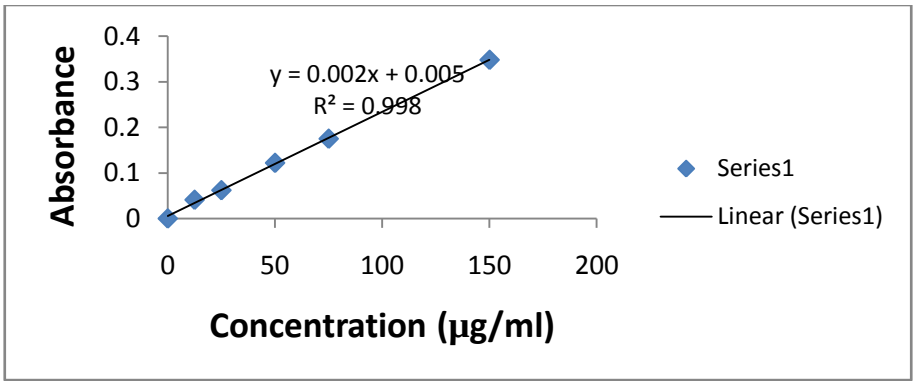

Fig 5: Calibration plot for Gallic acid

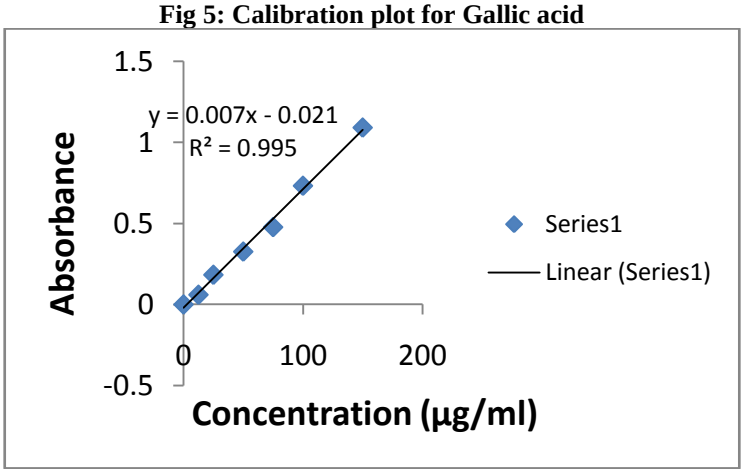

Fig 6: Calibration plot for Quercetin.

Table 5: Antimicrobial activity of 80% methanol extract of *K. africana*

Organisms	Diameter of zones of inhibition (mm)						
	80% methanol extract (mg/ml)					Cp(µg/ml)	Nys(µg/ml)
	20	40	60	80	100	10	10
E. coli	G	12±0.01	13±0.02	14±0.02	15±0.01	34±0.03	ND
P. aeruginosa	14±0.22	16±0.01	18±0.03	20±0.04	22±0.03	32±.16	ND
Kleb.aerogenes	G	G	G	G	G	20±0.75	ND
B. subtilis	12±0.24	13±0.03	16±0.01	18±0.01	20±0.04	30±0.33	ND
S. aureus	G	12±0.01	14±0.01	16±0.01	18±0.01	28±0.23	ND
C. albican	12±0.32	14±0.02	16±0.02	18±0.02	20±0.01	ND	22±0.12
C. parasilopsis	11±0.11	13±0.02	14±0.04	15±0.02	16±0.02	ND	21±0.23

Values are expressed as mean ±SEM, (G) indicates no inhibition zone, (ND) indicates Not Determined, (Cp) indicates ciprofloxacin P<0.05, (Nys) indicates Nystatin P<0.05

Table: 6: Antimicrobia activity of n-Butanol fraction

Organisms	Diameter of zones of inhibition (mm)						
	n-Butanol fraction (mg/ml)					Cp(µg/ml)	Nys(µg/ml)
	20	40	60	80	100	10	10
E.coli	G	G	G	13±0.01	14±0.01	31±0.68	ND
P.aeruginosa	11±0.01	13±0.01	15±0.03	17±0.01	18±0.03	30±0.67	ND
Kleb. aerogenes	G	G	G	G	G	22±0.33	ND
B.subtilis	12±0.03	13±0.02	15±0.01	16±0.05	20±0.03	34±0.54	ND
S.aureus	12±0.04	20±0.02	23±0.01	25±0.04	26±0.02	29±0.43	ND
C.albican	14±0.02	14±0.03	17±0.03	23±0.01	24±0.01	ND	23±0.22
C.parasilopsis	12±0.02	14±0.01	15±0.02	17±0.02	22±0.01	ND	24±0.63

Values are expressed as mean ±SEM, (G) indicates no inhibition zone, (ND) indicates Not Determined, (Cp) indicates ciprofloxacin P<0.05, (Nys) indicates Nystatin P<0.05

Table: 7: Antimicrobia activity of Chloroform fraction

Organism	Diameter of zones of inhibition (mm)						
	Chloroform fraction (mg/ml)					Cp(µg/ml	Nys(µg/ml)
	20	40	60	80	100	10	10
E.coli	11±0.01	16±0.01	17±0.03	18±0.04	19±0.01	30±0.67	ND
P.aeruginosa	12±0.02	15±0.01	13±0.02	16±0.01	16±0.01	28±0.83	ND
Kleb. Aerogenes	G	G	G	G	12±0.02	20±0.65	ND
B.subtilis	G	12±0.03	16±0.01	17±0.01	24±0.01	34±0.42	ND
S.aureus	13±0.01	18±0.01	18±0.01	19±0.02	21±0.04	32±0.53	ND
C.albican	11±0.01	13±0.02	14±0.01	18±0.02	20±0.01	ND	24±0.23
C.parasilopsis	14±0.03	14±0.02	16±0.03	17±0.03	19±0.01	ND	22±0.45

Values are expressed as mean ±SEM, (G) indicates no inhibition zone, (ND) indicates Not Determined, (Cp) indicates ciprofloxacin P<0.05, (Nys) indicates Nystatin P<0.05

Table: 8: Antimicrobia activity of Aqueous fraction

Organism	Diameter of zones of inhibition (mm)						
	Aqueous fraction (mg/ml)					Cp(µg/ ml)	Nys(µg/ ml)
	20	40	60	80	100	10	10
E.coli	G	G	G	G	G	30±0.22	ND
P.aeruginosa	G	G	G	G	G	34±0.21	ND
Kleb. Aerogenes	G	G	G	G	G	26±0.12	ND
B.subtilis	G	G	G	G	G	28±0.31	ND
S.aureus	G	G	G	G	G	32±0.33	ND
C.albican	G	G	G	G	G	ND	26±0.02
C.parasilopsis	G	G	G	G	G	ND	24±0.03

Values are expressed as mean ±SEM, (G) indicates no inhibition zone, (ND) indicates Not Determined, (Cp) indicates ciprofloxacin, (Nys) indicates Nystatin.

Discussion

The standardization of a crude drug is an integral part of establishing its correct identity. Before ant crude drug can be included in a herbal pharmacopoeia, Pharmacognostic parameters and standards must be established. The macro- and micro-morphological features of the leaf described could therefore, serve as a basis of proper identification, collection and investigation of *Kigelia Africana*.

Antioxidants are one of the most essential ingredients of today's therapy since they reduce *in vivo* oxidative damages. Plants are good sources of natural antioxidants [22]. DPPH radical scavenging method is widely used to investigate the total antioxidant activities in plants due to the fact that it is simple, rapid and inexpensive [23]. The method is based on scavenging of DPPH free radicals by the antioxidant. While *K. africana* showed concentration dependent increase in scavenging activity, the extracts and fractions had lower antioxidant activities compared to the standard, ascorbic acid. The 50 % inhibitory concentration value (IC_{50}) is the effective concentration of the sample that is required to scavenge 50 % of the DPPH free radicals. Among the various fractions, n-butanol had the highest phenolic content and maximum antioxidant property. This could serve as a guide in structural activity related experiments.

Phenolic compounds are crucial for plant growth and reproduction, and are produced as a response to environmental factors such as light and pollution and to defend injured plants [24]. Phenolic acids are secondary metabolites extensively spread throughout the plant kingdom. Phenolic compounds confer unique taste, flavor and health promoting properties found in vegetables and fruits [25]. In recent years, the importance of antioxidant activities of phenolic compounds and their potential usage in processed foods as natural antioxidant compounds has reached a new level [26]. Hence, *K. africana* is a potential source of natural antioxidant for the prophylaxis of diseases in which free radicals are implicated.

Flavonoids are well known for their antioxidant activity [27]. The extract showed a dose-dependent response. Significant flavonoids content of crude extract and fractions showed correlations with observed antioxidant activity.

The inhibition of growth of the organisms by the crude extract and fractions, except for the aqueous fraction can be attributed to the presence of biologically active complex organic chemicals (secondary plant metabolites) in the tissues. Plants containing phenolic compounds such as tannins and flavonoids have been known to possess antimicrobial activities [28]. The antimicrobial activity of the n-butanol and chloroform fractions (both containing phenolic compounds) could serve as a guide in structural activity relationship experiments.

Conclusion

The results obtained from this study showed that *Kigelia africana* Lam. Benth possesses unique Pharmacognostic parameters that can aid its standardization and quality control. It has also been shown that it could be effective in the management of infectious diseases.

Post Script

One of the authors, Asemu A. Kenneth died after the manuscript has been prepared. May his gentle soul rest in perfect peace.

REFERENCES

[1]. Sofowora A. The state of medicinal plant research in Nigeria. Ibadan University Press, Nigeria. 1986. 101.
[2]. *Kigelia.* http://en.wikipedia.org/wiki/kigelia.
[3]. Nigerian Natural Medicine Development Agency. Medicinal plants of Nigeria: North-East Nigeria. 1st ed. 2009. 32.
[4]. Gabriel OA and Olubunmi A. Comprehensive scientific demystification of *Kigelia africana*: A review. African Journal of Pure and Applied Chemistry, 2009; 3 (9): 158-164.
[5]. Sharma UK, Singh A, Sharma U, Kumar M, Rai D, Agrahari P. Wound healing activity of *Kigelia pinnata* bark extract. Asian Journal of Pharmaceutical and Clinical Research. 2010; 3 (4): 73-75.
[6]. Owolabi OJ, Omogbai KI. Obasuyi. Antifungal and antibacterial activities of the ethanolic and aqueous extract of *Kigelia africana* (Bignoniaceae). Afr. J. Biotechnol. 2007; 6 (5): 582-585.
[7]. Abere TA, Olusanya OE and Uti SI. In-vitro antimicrobial activity of the leaf extract of *Kigelia africana* Lam. Benth (Bignoniaceae) formulated as shampoo. Journal of Pharmaceutical and Allied Sciences. 2012; 9 (2): 1500-1506.
[8]. Carey MW, Babud MJ, Rao VN, Mohan KG. Anti-inflammatory activity of the fruit of *Kigelia pinnata* DC. Pharmacol. Online Journal, 2008; 2: 234-245.
[9]. Khan MR. Cytotoxicity assay of some Bignoniaceae. Fitoterapia, 1998; 69: 538-40.
[10]. Neelam B, Shailendra S, Fehmida N, Amir A. Isolation and in vitro antiamoebic activity of iridoids isolated from Kigelia pinnata. General Papers. ARKIVOC (X). 2006; 69-76.
[11]. Evans WC. Trease and Evans Pharmacognosy. 15th ed. WB Saunders, Toronto. 2006, 512, 545-547.
[12]. Wallis TE. Textbook of Pharmacognosy. Published by SK Jain. 1985. 572-575.
[13]. African Pharmacopoeia. General method for Analysis. OAU/STRC Scientific Publications, Lagos, vol. 2. No. 2 1st Ed. 1986. 1-5, 137-149, 223-237.
[14]. Brain KR and Turner TD. Practical evaluation of Phytopharmaceuticals. 1st ed. Wright-Scientechnica, Bristol. 1975, 144.
[15]. Ciulei I. Methodology for analysis of vegetable drugs. United Nations Industrial Development Organisation. Romania. 1981, 17-25.
[16]. Harborne JB. Phytochemical methods. A guide to modern technique of plant analysis. 1st ed. Chapman and Hill, London. 1992, 279.
[17]. Jain SC, Jain R, Sinh R. Antioxidant activity of some medicinally important Arid Zone plants. Asian J. Exp. Sci., 2009; 23 (1): 215-221.
[18]. Elmatase M, Isildak, Turkeku I and Temur N. Determination of antioxidant activity and antioxidant compounds in wild edible mushrooms. Journal of Food Composition and Analysis. 2007; 20 (1): 337-345.
[19]. Kim D, Jeong SW and Lee CY. Antioxidant capacity of phenolic phytochemicals from various arthivars of plums. Food Chemistry. 2003; 81: 321-326.
[20]. Ebrahimzadeh MA, Pourmorad F and Bekhradina AR. Iron chelating activity, phenol and flavonoids content of some medicinal plants from Iran. African Journal of Biotechnology. 2008; 7 (18): 3188-3192.
[21]. Collins CH, Lynes PM and Grange JM. Agar-well diffusion method. In: Microbiological methods. 7th Ed. Butterwort-Heinmann Ltd, Britain. 1995, 175-190.
[22]. Krishnaiah D, Sabartly R and Nithyanandan RR. A review of the antioxidant potential of medicinal plant species. Food and Bioproducts processing. 2011; 88: 217-233.
[23]. Sakat SS, Juvekar AR, Gambhire MN (2010). *In vitro* antioxidant and anti-inflammatory activity of methanol extract of *Oxalis corniculata* Linn. International Journal of Pharmacy an Pharmaceutical Sciences, 2010; 2 (1): 146-155.
[24]. Valentine IK, Maria VK and Bruno B. Phenolic cycle in plants and environment. J. Mol. Cell Biol., 2003; 2: 13-18.
[25]. Tomas-Barberan F and Espin JC. Phenolic compounds and related enzymes as determinants of quality of fruits and vegetables. J. Sci. Food Agric., 2001; 81: 853-876.
[26]. Elzaawely AA, Xuan TD and Tawata S. Essential oils, kava pyrones and phenolic compounds from leaves and rhizomes of Alpini azerambet and their antioxidant activity. Food Chem., 2007; 103: 486-494.
[27]. Kukic J, Petrovic S and Niketic M. Antioxidant activity of four endemic *Stachys taxa*. Biol. Pharmaceut. Bull., 2006; 29: 725-729.
[28]. Musa KY, Ahmed A, Ibrahim H, Arowosaiye G and Olonitola OS. Phytochemical and antimicrobial studies of leaves of *Acalypha racemosa*. Nig. Journal of Natural Products and Medicine, 2000; 4: 67-69.