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RESEARCH ARTICLE

In vitro Antioxidant Analysis and Antibacterial Screening of the Methanolic Leaf Extract of *Kigelia africana* (Lam.) Benth.

S.S. Said, M. A. Abdullahi, M. Aliyu

Department of Biochemistry and Molecular Biology, Faculty of Life Science, Federal University, Dutsin-Ma, Nigeria

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ABSTRACT

Kigelia africana is a medicinal plant with several attributes and considerable potentials, various parts of the plant are used locally to treat cancer, ulcer, skin diseases, diabetes, bacterial and fungal infections, etc. To investigate the invitro Antioxidant and Antibacterial Screening of methanolic leaf extract of kigelia *africana*. The percentage of antioxidant activity of leaf extracts of *Kigelia* was assessed by 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical assay, reducing power (PR) assay, ferric reducing/antioxidant power assay, and nitric oxide radical scavenging assay. Ascorbic acid was used as a standard antioxidant. Antibacterial activity of the leaf extract was tested against four bacterial strains; *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa,* and *Salmonella* spp. The comparative analysis revealed that DPPH with extract exhibited the most remarkable antioxidant activity. The leaf extract showed maximum activity on *P. aeruginosa, S. aureus,* and *Salmonella* spp. and moderate activity on *E. coli*. The extracts were found to contain alkaloids, saponins, flavonoids, and tannins which were present in high amount. Steroids were present in moderate amount. Cardiac glycosides and anthraquinone were present in trace amount, while volatile oils were totally absent. The gas chromatography–mass spectrometry results reveal the presence of nine compounds in the methanolic leaf extract.

Keywords: Antibacterial, antioxidant, gas chromatography–mass spectrometry, *Kigelia africana*

INTRODUCTION

Kigelia africana is a member of family Bignoniaceae popularly known as the cucumber or sausage tree because of the huge fruits, which hangs from long fibrous stalks.[1] The stem bark possessed antidiabetic and antibacterial properties.[2,3] Remedies from root bark are also used for the treatment of venereal diseases, hemorrhoids, and rheumatism.[4] Traditional remedies prepared from crushed dried fruits are used for emollient, anti-eczema, anti-psoriasis, as dressing for ulcers and wounds, treatment of skin cancer, as an aphrodisiac, and also as an active ingredient in skin lightening and breast firming formulations.[5]

***Corresponding Author:**
M. A. Abdullahi
E-mail: abdulmabdu@gmail.com

However, little is known about the pharmacological properties of its leaves previously, preliminary data from our laboratories have indicated that the ethanolic leaf extract of *K. africana* has antiulcerogenic potential against aspirin-induced ulcer.[6]

Free radicals are produced during the various metabolic processes in our body. Their excessive production causes cell death or cell dysfunction as they are capable of attacking the healthy cells, leading to structure deformation and creating the conditions of oxidative stress. Natural plants containing phytonutrients have been explored for assessing their antioxidant potential. Antioxidants participate in oxidative stress defense and act as free radical stabilizers which significantly help in treating various diseases such as cardiovascular, cancer, and other neurodegenerative disorders.[7,8]

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METHODOLOGY

Collection and Identification of *K. africana*

Kigelia africana leaf sample used were obtained from villages around Dutsin-ma, Local Government, Katsina state, Nigeria, on April 22, 2021. They were authenticated at the Department of Biochemistry and Molecular Biology, Federal University Dutsin-Ma, Katsina State by Mr. S. S. Said.

Extraction of the Plant Sample

K. africana leaves were dried under shade for 5 days. They were pulverized into powder form using an electric blender. A 250 g of the powder was macerated in 1 L of methanol. The solution was occasionally stirred for 48 h. Then, it was filtered using cheese cloth and refiltered using filter paper. It was then concentrated using water bath at 70°C and the slurry obtained was kept in a desiccator containing desiccant (silica gel) for the complete absorption of its water content until a completely dry solid granules was obtained. The semi-solid extract was stored in the refrigerator for further use.

In vitro Antioxidant Studies

Test for 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging ability

The free radical scavenging ability of the extracts against DPPH free radical was evaluated using a slightly modified method as described by Tuba.[9] A 0.3 mM solution of DPPH was prepared in methanol and 500 μL of the DPPH solution was added to 1 mL of the extracts at various concentrations (15–240 μg/mL). These solutions were mixed and incubated in the dark for 30 min at room temperature. The absorbance was read at 517 nm against blank samples lacking scavenger.

Test for ferric cyanide (Fe^{3+}) reducing antioxidant power (FRAP) assay

The total reducing power of the extracts was determined using the FRAP method[10] with slight modifications. The assay was performed using 1 mL of each extract (15–240 μg/mL) and was incubated with 1 mL of sodium phosphate buffer (0.2 M, pH = 6.6) and 1% potassium ferricyanide at 50°C for 30 min. Thereafter, 1 mL of 10% trichloroacetic acid was used to acidify the reaction mixtures. After the acidification, 1 mL of the sample is mixed with 1 mL of distilled water and 200 μL of 0.1% $FeCL_3$. The absorbance of the resulting solution was read at 700 nm in a spectrophotometer. The absorbance of the samples is proportional to the reduction capability of the extracts. The results were expressed as a percentage of the absorbance of the sample to the absorbance of ascorbic acid.

Ferric reducing antioxidant power (FRAP) % = Absorbance of sample/Absorbance of ascorbic acid × 100

Nitric Oxide (NO) Radical Scavenging Assay

This assay is based on the ability of aqueous solution of sodium nitroprusside at physiological pH to spontaneously produce NO, which could interact with oxygen to generate nitrite ions that can be measured using Griess reagent. All agents that can scavenge NO compete with oxygen, resulting in decreased NO generation.[11] The assay was carried out by incubating 500 μL of 10 mM sodium nitroprusside in sodium phosphate buffer (pH = 7.4) and 500 μL of different extract concentrations (15–240 μg/mL) at 37°C for 2 h. Thereafter, 500 μL of Griess reagent was transferred to the reaction mixture. Diazotization of nitrite with sulfanilamide produced a chromophore which is measured at 546 nm. The percentage inhibition of NO generated was measured by compared with the absorbance value of a control (10 mM sodium nitroprusside in phosphate buffer).

All assays were carried out in triplicate. The scavenging activities of the seed extracts in the case of DPPH and NO radicals scavenging assays were calculated using the following formula:

$$\text{Scavenging activity } \% = (1 - As/Ac) \times 100$$

Where, As: Absorbance in the presence of the sample and

Ac: Absorbance of the control

Determination of Antibacterial Test

Antibacterial test was carried out using the agar diffusion method. Bacterial strains collected from the Federal Medical Center Katsina, include *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa,* and *Salmonella* spp. These bacterial strains were propagated in the microbiology laboratory in Federal University Dutsin-ma, Katsina state.

Phytochemical Screening (Qualitative)

Saponins

- Frothing test: A 2 cm^3 of each extract was pipetted into five test tubes and each of the test tubes was shaken vigorously for 2 min. Fronting indicated the presence of saponins.
- Emulsion test: Five drops of olive oil were added to cm^3 of each extract in four test tubes and the mixtures were vigorously shaken. A stable emulsion formed in the extract tested, indicated the presence of saponins.

Flavonoids

A 1 cm^3 of 10% NaOH was added to 3 cm^3 of each extract in five test tubes. The presence of yellow coloration indicated the presence of flavonoids.

Tannins

1. A 1 cm^3 of freshly prepared 10% KOH was added to 1 cm^3 of each extract in five test tubes. Appearance of a dirty white precipitate indicates the presence of tannins.
2. Two drops of 5% $FeCl_3$ were added to 1 cm^3 of each extract in five test tubes. A greenish precipitate observed indicated the presence of tannins.

Alkaloids

A 1 cm^3 of 1% HCL was added to 3 cm^3 of each extract in five test tubes. The mixture was heated for 20 min. It was cooled and filtered. The filtrate was used for the test using Wagner's reagent. Drops of Wagner's reagent were added to 1 cm^3 of each extract. A reddish-brown precipitate indicates the presence of alkaloid in the extract.

Cardiac Glycosides

A 1 mL of the extract was pipetted in five different test tubes. Then, 2 mL of 3.5% ferric chloride solution was added and allowed to stand for 1 min. One milliliter of concentrated H_2SO_4 was then carefully poured down the wall of the tube so as to form a cover layer. A reddish-brown ring at the interface with the upper layer becoming green to blue indicated the presence of cardiac glycosides containing 2-deoxy sugar.

Volatile Oils

A small quantity of each of the extracts was shaken with dilute HCL. The absence of a white precipitate which was to be performed indicated the absence of volatile oils.

Steroids

The 1 ml of the extract was dissolved in 2 mL of chloroform, sulfuric acid was carefully added to form lower layer. A reddish-brown color at the interface indicated the presence of steroidal ring (i.e., aglycone portion of the cardiac glycoside).

Anthraquinones

Five grams of the plant extract were shaken with 10 mL benzene, filtered and 5 mL of 10% ammonia solution was added to the filtrate. The mixtures were shaken and the presence of a pink, red, or violent color in ammonical (lower) phase indicates the presence of free anthraquinones.

GC–MS Analysis

GC–MS analysis is a common confirmation test. It is best used to make an effective chemical analysis. The analysis provides a representative spectral output of all the compounds that got separated from the sample. The first step of GC–MS was started by injecting the sample to the injected port of the GC device. The GC instrument vaporized the sample and then separated and analyzed the various components. Each component ideally

produced a specific spectral peak that was recorded on a paper chart electronically. The time elapsed between elution and injection is called the "retention time." Differences between some compounds were identified using the retention time. The peak was measured from the base to the tip of the peak.

Statistical Analysis

The values were expressed as mean ± SEM. Statistical analysis was analyzed by one-way analysis of variance (ANOVA) followed by Tukey multiple comparison test. $P < 0.05$ was considered as statistically significant.

Table 1: Qualitative phytochemical analysis for methanolic leaf extract of *K. africana*

Phytochemicals	Ethanol extraction
Flavonoids	+++
Alkaloids	+++
Tannins	+++
Cardiac glycosides	+
Anthraquinone	+
Saponins	+++
Steroids	++
Volatile oils	-

Not present, +: Low, ++: Moderate, +++: High

Table 2: Antibacterial assay of *Kigelia africana* leaf extract and their different zone of inhibition

Bacterial parameters	Methanol leaf extract	Standard drug (ciprofloxacin)
Escherichia coli	6.00±1.20 mm	5.70±2.10 mm
Staphylococcus aureus	8.70±2.08 mm	9.00±1.00 mm
Salmonella spp.	9.30±1.50 mm	10.00±2.30 mm
Pseudomonas aeruginosa	7.40±2.51 mm	10.00±3.00 mm

Values are mean±standard deviation. Values>6 mm indicate some activity. Values in the same column differ significantly ($P < 0.05$)

Table 3: The antioxidant activity of the methanol leaf extract of *Kigelia africana*

Concentration of extract (µg/mL)	DPPH scavenging activity (%)	Fe^{3+}reducing antioxidant power (%)	NO radical scavenging (%)
Control	0.000	0.000	0.000
100	11.3±4.16*	33.4±7.04*	37.5±3.37
300	18.5±1.61*	41.1±4.71*	48.2±1.60
500	23.8±5.18*	53.8±5.18*	43.3±1.04
750	29.6±5.43*	58.4±5.43*	36.5±2.70
1000	34.4±3.58*	61.4±3.42*	46.2±1.35

Data are expressed as mean±SD (*n*=3); *Dose-dependent increase from 100 to 1000 (µg/mL). DPPH: 1,1-diphenyl-2-picrylhydrazyl, NO: Nitric oxide

RESULTS

Phytochemical (Qualitative) Analysis

The flavonoids, Alkaloids, Tannins and Saponins were present in high amount, Steroids in moderate amount, Cardiac glycosides and Anthraquinones were present in low amount while Volatile oils were totally absent [Table 1].

Antibacterial

Leaf extract of *K. africana* possesses antibacterial activity against *P. aeruginosa*, *Salmonella* spp., and *S. aureus* when tested and has little effect on *E. coli* [Table 2].

The result shows that the different concentration against the absorbance, the concentration is at 100 (µg/mL) up to 1000 (µg/mL), as the concentration increased, the activity also increased [Table 3 and Figure 1].

This result shows that as concentration increased, the reducing power also increased when compare with ascorbic acid. The red color is representing ascorbic acid, while the blue color is for the extracts to show reasonable activity compared to the standard [Figure 2].

The result shows that the different concentration against ferric cyanide (fe^2) reducing antioxidant power assay, as the reducing power assay increased, the concentration also increased from 100 to 1000 (µg/mL) [Figure 3].

This result shows that the concentration against the absorbance, the concentration is at 100–1000 (µ g/ml), the differences were showed and do not follow the common rate by the increases the concentration and activity which is punctuating [Figure 4].

Result of GC–MS Analysis of *K. africana* Leaf Extract

Table 4 shows the GC–MS analysis of *K. africana* leaf extract, the methanolic leaf extract is rich in hydrocarbons with nine compounds detected. Since most of the compounds are hydrocarbons, they contain these functional groups, alkene (=), and alkyne (≡), which were detected in the spectrum.

DISCUSSION

The antibacterial activity of *K. africana* leaf extract against *E. coli*, *Salmonella* spp., *P. aeruginosa,*

Table 4: The hydrocarbon compositions of *Kigelia africana* leaf extract

No.	RT	Name of compound	MF	Area	Quality
1	17.1772	(Z)-9,17-Octadecadienal	$C_{18}H_{32}O$	0.029	94
2	27.1343	Stannane, tetraethyl-	$C_8H_{20}Sn$	0.131	91
3	28.1515	3-(3,4-Dimethoxyphenyl) propylamine, PFP	$C_{11}H_{17}NO_2$	17.167	41
4	28.3886	3-(3,4-Dimethoxyphenyl) propylamine, PFP	$C_{11}H_{17}NO_2$	40.974	35
5	28.4181	3-(3,4-Dimethoxyphenyl) propylamine, PFP	$C_{11}H_{17}NO_2$	12.871	35
6	28.5075	3-(3,4-Dimethoxyphenyl) propylamine, PFP	$C_{11}H_{17}NO_2$	15.151	38
7	28.7466	1H,3H-Furo[3,4-c] furan, 1,4-bis (3,4dimethoxyphenyl)	$C_{22}H_{26}O_7$	13.053	93
8	28.833	1H,3H-Furo[3,4-c] furan. 1,4-bis (3,4dimethoxyphenyl)	$C_{22}H_{26}O_7$	0.5394	95
9	30.887	3-Buten-2-one, 4-(3-hydroxy-6,6-dimethyl-2 methylenecyclohexyl)-	$C_{13}H_{20}O_2$	0.0847	35

RT: Retention time, MF: Molecular formula

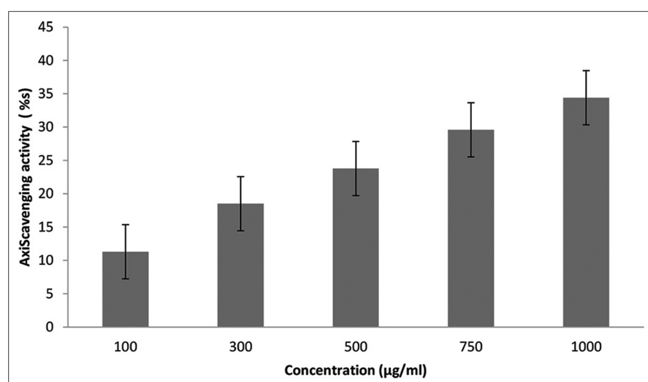

Figure 1: Scavenging activity (%) of the methanolic leaf extracts of *Kigelia africana* against 1,1-diphenyl-2-picrylhydrazyl

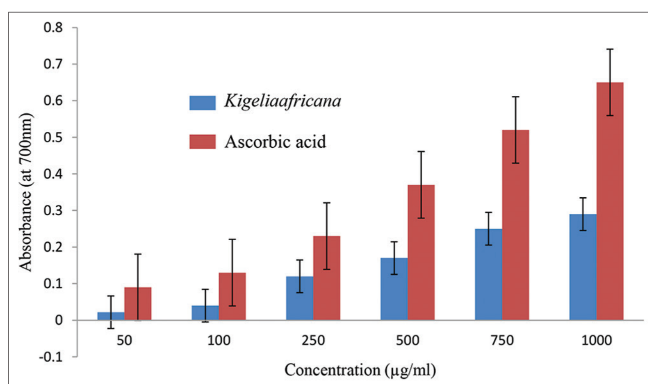

Figure 2: The reducing power of the methanolic leaf extracts of *Kigelia africana* and standard (ascorbic acid)

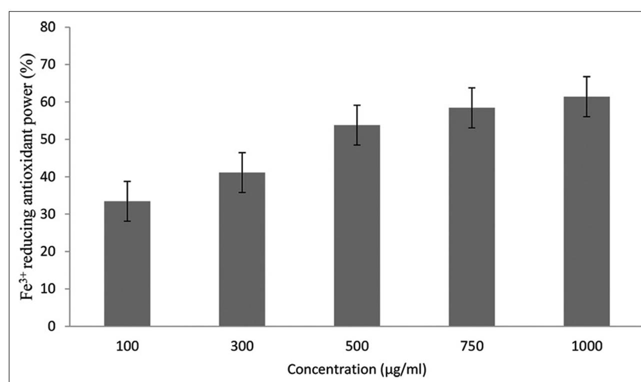

Figure 3: Ferric cyanide (Fe^{3+}) reducing antioxidant power (%) of the methanolic leaf extracts of *Kigelia africana*

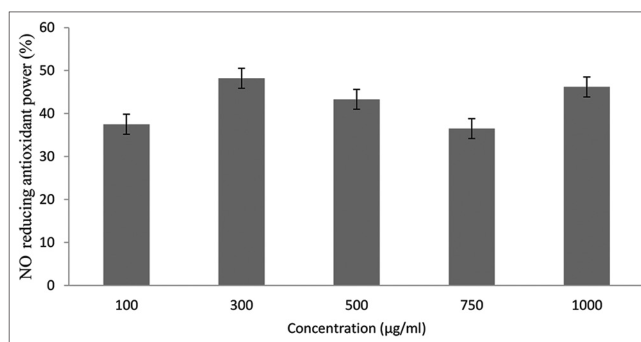

Figure 4: Scavenging activity (%) of the methanolic leaf extracts of *Kigelia africana* against NO radical

and *S. aureus* showed that the methanolic leaf extracts were found to exhibit different antibacterial activities. The methanolic leaf extract showed moderate activity against *E. coli* and maximum activity against *P. aeruginosa, S. aureus,* and *Salmonella* spp. These results were compared with a standard antibiotic drug, ciprofloxacin as a standard. Methanolic extract of leaf of *K. africana* showed remarkable activity against the bacterial strains. The results revealed that the methanolic leaf extract of *K. africana* possesses good antibacterial activity as compared to the standard drug, which is in support of the previous studies which have also reported antibacterial properties for *K. africana* extracts from other plant parts. In the microtiter plate bioassay, the stem bark and fruit extracts showed similar antibacterial effects against Gram-negative and Gram-positive bacteria.[12,13] Hence, extracts of *K. africana* may be used as the alternative source for treating several infectious diseases caused by

various pathogens.[14,15] The leaf extract showed the presence of flavonoids, alkaloids, tannins, and saponins at high amount, steroids moderate amount, cardiac glycosides, and anthraquinones low amount while volatile oil total absent.

The antioxidant studies revealed that *K. africana* leaf extract with DPPH showed good antioxidant activity. The DPPH is a stable free radical, due to the delocalization of the spare electron on the whole molecule. Thus, DPPH does not dimerize, as happens with most free radicals. When DPPH reacts with a hydrogen donor, the reduced (molecular) form (DPPH) is generated, accompanied by the disappearance of the violet color. Therefore, the absorbance diminution depends linearly on the antioxidant concentration. Ascorbic acid is used as standard antioxidants. Reducing power (PR) assay with extract possess moderate antioxidant activity. Ferric cyanide (Fe^{3+}) reducing antioxidant power assay (FRAP) assay combined with extract showed the poor antioxidant activity. The FRAP relies on the reduction by the antioxidants, of the complex ferric ion. NO radical assay combined with extract does not follow the common rate by the increased of the concentration and activity. Ascorbic acid was used as a standard antioxidant.

The antioxidant activity may be due to the inhibition of the formation of radicals or scavenging of the formed radical.[16] Diet plays a key role in the production of the antioxidant defense system by providing essential nutrient antioxidants such as Vitamin C, Vitamin E, and β-carotene. Plants are considered as one of the most important and interesting subjects that should be explored for the discovery and development of newer and safer drug candidates. The scavenging of the stable DPPH radical is widely used to assess antioxidant activity in a short time compared with other methods. This assay allows comparison of the reactivity of powerful antioxidants such as BHT and ascorbic acid with those present in extracts against DPPH radical.[6] The herbal remedies have become more popular in the treatment of minor ailments.[17,18] *Kigelia* is one of the most important sources of new bioactive compounds but there is a need for more such approaches to find out more effective chemical compounds.

The GC–MS analysis carried out [Table 5] which showed that nine compounds were present in the methanolic leaf extract of *K. africana,* namely, (Z)-9,17-octadecadienal, Stannane, tetraethyl-, 3-(3,4-dimethoxyphenyl) propylamine, PFP, 3-(3,4-dimethoxyphenyl) propylamine, PFP, 3-(3,4-dimethoxyphenyl) propylamine, PFP, 3-(3,4-dimethoxyphenyl) propylamine, PFP, 1H,3H-Furo(3,4-c)furan, 1,4-bis(3,4-dimethoxyphenyl), 1H,3H-Furo[3,4-c] furan, 1,4-bis(3,4-dimethoxyphenyl), and 3-Buten-2-one, 4-(3-hydroxy-6,6-dimethyl-2-methylenecyclohexyl).

The methanolic extract of the leaf of *K. africana* has been widely used in traditional medicine. In African herbal medicine, the leaf is believed to cure a wide range of ailments, from epilepsy to relieving toothache, for treating sores, skin ulcer, and cancer. The plant has many medicinal properties because of the presence of many secondary metabolites which include iridoids, flavonoids and naphthoquinones, and volatile constituents.[19] Antibacterial compounds from plants represent a potentially novel source of antibacterial substances since they act against bacteria through mechanisms that are different from those of currently used antibiotics and may thus have a clinical value in the treatment of antibiotic-resistant antibacterial strain.[20]

CONCLUSION

The leaf extract of *K. africana* has antibacterial and antioxidant properties. The compounds present in the leaf extract support the medicinal application of the plant as an antibacterial and antioxidant. Therefore, the plant extracts have great potentials as antimicrobial principles against microorganisms and that can be used in the treatment of infectious diseases cause by resistant microorganisms.

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