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Research Article

Antioxidant and Antibacterial potential of Methanol root Extract of *Kigelia africana* (Sausage tree)

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ABSTRACT

Kigelia africana popularly known as the cucumber or sausage tree that possesses medicinal and traditional uses like anticancer, antiulcer, anti-aging, antioxidant, and anti-malarial activity.This study aimed to investigate GCMS, FTIR, Antioxidant and Antibacterial properties of methanolic root extract of *Kigeliaafricana*.The antioxidant activity of root extracts of Kigelia was evaluated by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical assay, Reducing power (PR) assay, Ferric Reducing Power assay and Nitric oxide (NO) radical scavenging assay. Ascorbic acid was used as a standard antioxidant. GCMS analysis reveals the presence of 9-Octadecanoic acid (z)-methyl ester while theFTIR spectral analysis identify some functional group such as hydroxyl group, Carboxyl group, methyl group, carbonyl group, amino group, and phosphate group. In vitro antibacterialactivityof the extracts wastestedagainst4bacterialstrains viz; *Staphylococccus aureus, Escherichia coli, Pseudomonas aeruginosa*, and *Salmonella spp*.The highest antibacterial activity was found against *Staphylococcus aureus* then followed by *Pseudomonas aeruginosa, Salmonella spp* and *Escherichia coli*. The extract showed much higher scavenging activity against DPPH and with most reducing power against ferric cyanide. This study reveals the potency of the root of *K. africana* to be used as a good natural source for antioxidant and antibacterial activity.

Keywords: *Kigelia africana;* anti-oxidant; antibacterial; GCMS; FTIR

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INTRODUCTION

Herbal medicine has received greater attention as an alternative to orthodox medicine in recent times, leading to a subsequent increase in herbal medicine preparations [Sushruta *et al.,* 2007; Ogbonnia *et al.,* 2016]. *Kigelia africana*a member of family of Bignoniaceae is popularly known as the cucumber or sausage tree because of the huge fruits, which hangs from long fibrous stalks [Cragg & Newman, 2001]. The plant possesses medicinal and traditional uses like anticancer, antiulcer, anti-aging, antioxidant, and anti-malarial. It is also widely applied in the treatment of genital infections. The stem bark possessed antidibetic and antibacterial properties [Said *et al.,* 2019 and Abdullahi *et al.,* 2020]. Remedies from root bark are also used for the treatment of venereal diseases, hemorrhoids, and rheumatism [Oliver-Bever, 2004]. A study of the antimicrobial properties of the aqueous stem bark extract of *Kigelia africana* revealed the presence of two naphthoquinoneskigelinone and isopinnatal [Atolani and Olatunji, 2010]. The *K. africana* plant has many medicinal properties due to the presence of numerous secondary metabolites. It has been reported that the major plant constituents in K. africana include polysaccharides, polypeptides,

glycopeptides, triterpenoids, steroids, xanthones, flavonoids, coumarins, phenols, iridoids, alkyl disulphides, inorganic ions, and guanidine [Obianagha *et al.,* 2021]. Some of these products have been shown to exhibit antioxidant properties and as well as antidiabetic activities [Bhushan *et al.,* 2010]. The methanolroot extract of *Kigelia africana* has been widely used in traditional medicine. In African herbal medicine, the root is believed to be a cure for a wide range of ailments, from epilepsy, to relieve a toothache, for treating sores, skin ulcer, cancer, and wound healing. [Houghton. 2002].

MATERIALS AND METHODS

Sample collection

The *Kigelia africana* root sample used were obtained from villages around Dutsin-ma Local Government, Kastina state, Nigeria, on 22nd April 2021.

Extraction of the plant sample

Roots of the plant collected were air-dried under shade at the Biochemistry laboratory. The dried root of the plant was pounded using mortar and pestle, and then sieved into powder using a sieve. 200g of the powdered sample was dissolved in 1000ml of ethanol and allowed to stay for 48 hours with periodic stirring. The solution was filtered using whatman filter paper, the filtrate was then placed in the ovum at 80^0 C for 8 hours for complete drying.

In vitro antioxidant studies.

Test for: 2-2-diphenyl l-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 *et al.* (2008). 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. These solutions were mixed and incubated in the dark for 30 minutes at room temperature. The absorbance was read at 517 nm against blank samples lacking scavenger.

Test for: Ferric ion (Fe^{3+}) reducing antioxidant power (FRAP)assay. The total reducing power of the extracts was determined using the FRAP method of Oyaizu (1986) with slight modifications. The assay was performed using, 1 mL of each extract 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 minutes. Thereafter, 1 mL of 10% trichloroacetic acid was used to acidify the reaction mixtures. After the acidification, 1 mL of the sample were mixed with 1 mL of distilled water and 200 µL of 0.1% $FeCL_3$. The absorbance of the resulting solution was read at 700nm 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 % =Absorbance of sample**/** Absorbance of ascorbic acid **X** 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 nitric oxide (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 [Kurian *et al*., 2010]. 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 at 37°C for 2 hours. Thereafter, 500 µL of Griess reagent was transferred to the reaction mixture. Diazotization of nitrite with sulphanilamide produced a chromophore which are measured at 546 nm. The percentage inhibition of NO generated was measured by comparing 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 nitric oxide radicals scavenging assays were calculated by using the following formula:

Scavenging activity % = (1− As / Ac) x 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 well diffusion method [Akinyemi *et al.,* 2006]. Mueller hinton agar was poured on the petri plate and allowed to solidify, agar surface of each plate was then streaked with pure culture of *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and*

Salmonella spp using a sterile wire loop, the agar plate will then be punched with a sterile cork borer of 4mm size with each plate having wells each for methanol root extract of *Kigelia africana*, and a plate containing a well which serve as the control which is ciprofloxacin. These bacterial strains were propagated in the microbiology laboratory at Federal University Dutsin-ma, Katsina state.

GC-MS analysis

GC-MS analysis is a common confirmation test. It is best used to make an effective chemical analysis. This analysis provided 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 Gas chromatography (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 performed by one way analysis of variance (ANOVA) followed by Tukey multiple comparison tests. Pvalues< 0.05 were considered as significant.

Bacterial Strains

Bacterial strains that were collected from the Federal Medical Center Katsina, include
The result of FTIR has shown the presence of some functional group present in the plant root extract to be hydroxyl group, Carboxyl group, methyl group, carbonyl group, amino group, and phosphate group. The frequencies of peaks were compared to the reference literature to evaluate the functional groups present in the methanolic root extract of *Kigelia africana*. The infrared spectroscopic study of the representative spectra for the root extract of *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Salmonella spp.*

RESULTS

DPPH Free Radical Scavenging Activity

The scavenging activity of DPPH are presented in Figure 1, with the findings of the study revealing increasing scavenging activity with increase concentration. The least and highest activities were recorded in 100 100µg/ml and 1000µg/ml respectively.

Ferric ion (Fe^{3+}) Reducing Antioxidant Power

Figure 2 shows a significant increase in ion reducing antioxidant activity as the concentration increases. The lowest concentration of 100 µg/ml shows the lowest antioxidant activity while the highest concentration of 1000 µg/ml shows the highest antioxidant activity.

Reducing Power (PR)

Figure 4 shows a significant increase in reducing power activity as the concentration increases. The lowest concentration of 50µg/ml shows the lowest reducing power activity while the highest concentration of 1000 µg/ml shows the highest reducing power activity.

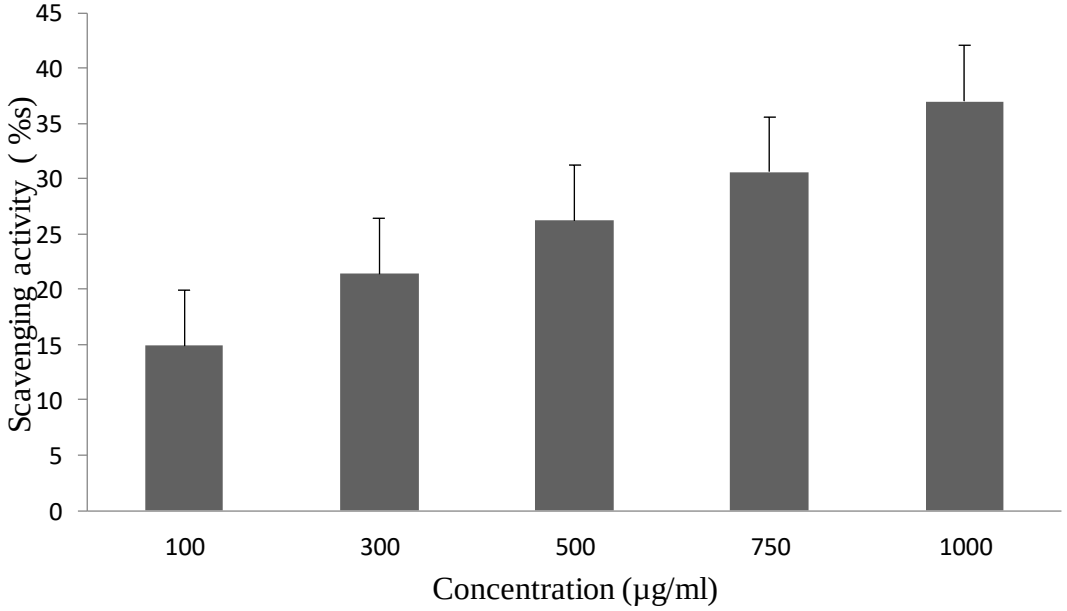
Gas Chromatography Mass Spectrometer (GCMS)

The result obtain showed the presence of only 9-Octadecenoic acid (Z)-methyl ester in the methanol root extract.

Fourier Transform Infrared Spectroscopy (FTIR)

Kigelia africana as shown in figure 5and table 2 had characteristic peaks seen in the spectra with specific functional groups.

Fig 1. Free radical scavenging activity (%) of methanolic root extracts of *K. africana* against DPPH



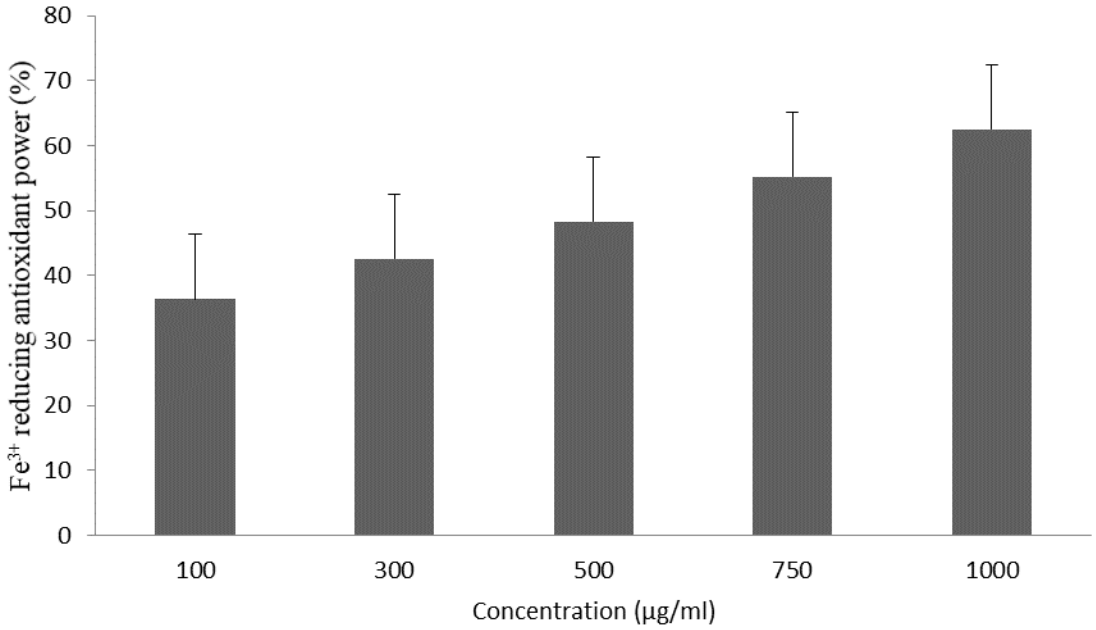


Fig 2. Ferric (Fe^{3+}) reducing antioxidant power (%) of methanolic root extracts of *K. africana*

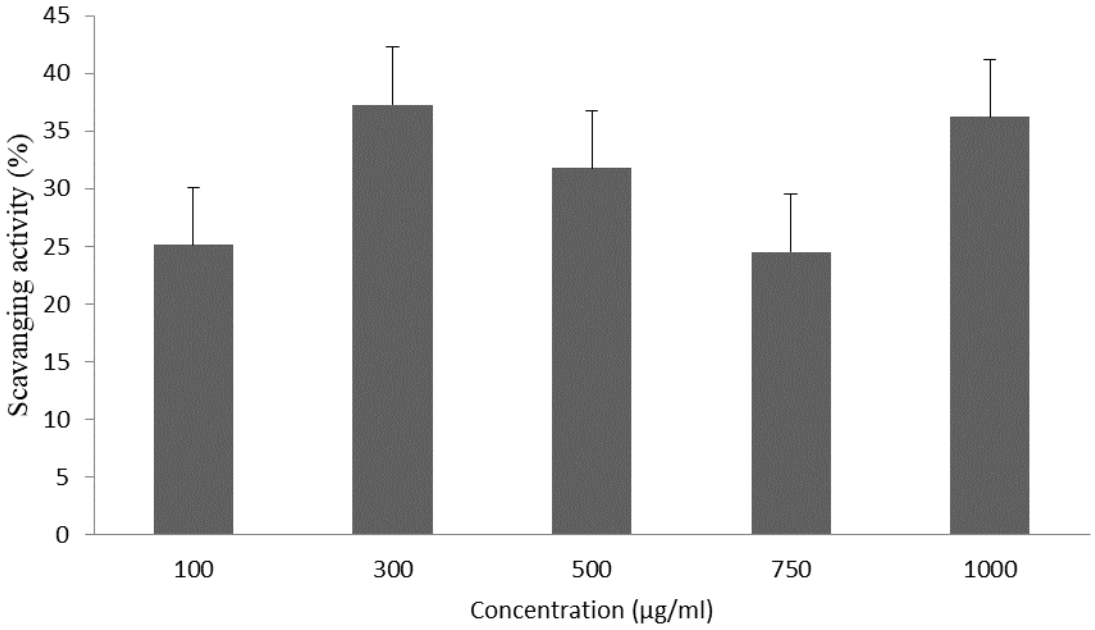


Fig 3: Free radical scavenging activity (%) of methanolic root extracts of *K. africana* against nitric oxide (NO)

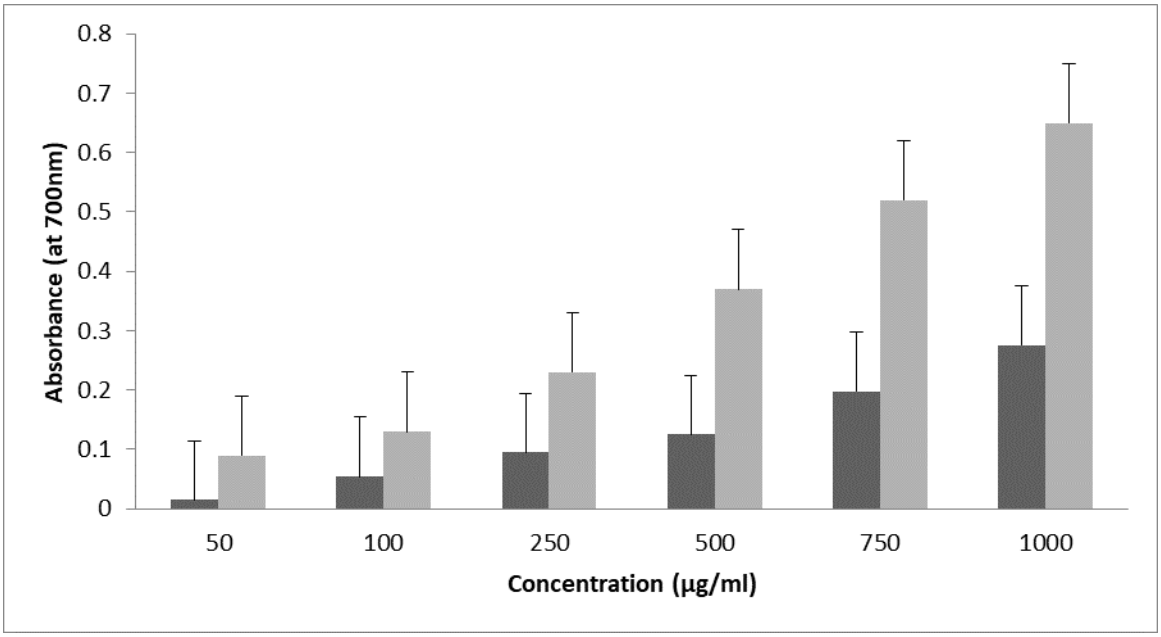


Fig 4: Reducing power of methanol root extracts of *K. Africana* and Standard (Ascorbic acid) against potassium ferricyanide.

Table 1. Gas Chromatography Mass Spectrometer analysis result

NO	RT	NAME OF COMPOUND	MF	WM	PEAK AREA
1	17.2626	9Octadecanoic acid (z)-ester	$C_{19}H_{35}O_2$	280	55.0

Where: RT- retention time, MW – molecular weight and MF- molecular formula

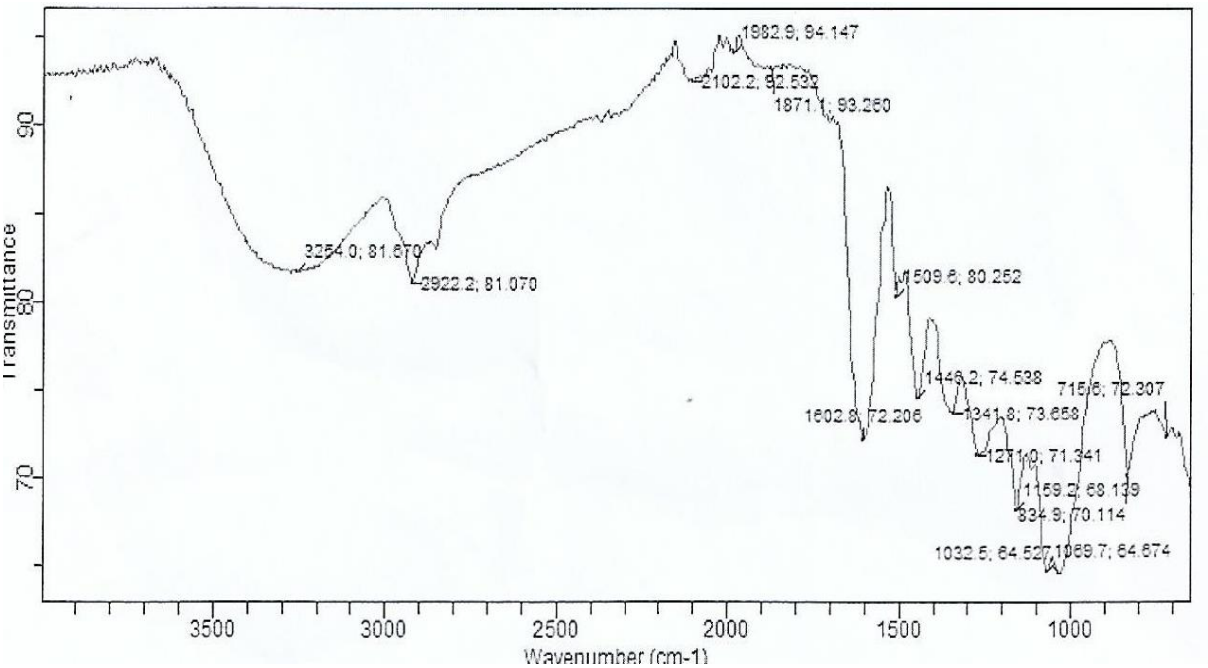
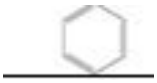


Fig 5. Fourier Transform Infrared Spectroscopy (FTIRS): Graph showing the frequency at which sample was absorb by infrared light

Table 2. FTIRSpectral analysis result

Position (cm^{-1})	**Group**	**Inference**
3500– 300		Strong intensity, very broad band
2950 – 2500		C is Sp3 hybridized, 3000 cm^{-1} is a convineint dividing line between this type of C – H bond and the preceeding type
2450 – 2000		Medium intensity
1950 – 1500		Strong intensity, exact position depends on substituents
1550 – 1000		Two strong intensity band
950 - 500		Strong intensity

Antibacterial Result

Root extract of *K. africana* possess good antibacterial activity against *Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella spp,* and *Escherichia coli*. The activity of the extract against the organisms was compared with standard antibiotic ciprofloxacin as a standard. In this study, bacterial zone of inhibition was seen to be 6.00±3.61, 10.70±2.08, 7.30±1.05, 8.00±3.61 for *E. coli, Staphylococcus aureus*, *Salmonella spp*, and *Pseudomonas aeruginosa* respectively, while the zone of inhibition of standard drug ciprofloxacin for *E. coli*, *Staphylococcus aureus*, *Salmonella spp*, and *Pseudomonas aeruginosa* was found to be 5.70±2.10,9.00±1.00, 10.00±2.30,and 10.00±3.00, respectively.

Table 2. Antibacterial Activity of Methanol Root Extract of *K. africana*

Bacteria	Methanol root extract (nm)	Ciprofloxacin (nm)
Escherichia coli	6.00±3.61[a]	5.70±2.10[a]
Staphylococcus aureus	10.70±2.08[a]	9.00±1.00[a]
Salmonella spp	7.30±1.05[a]	10.00±2.30[b]
Pseudomonas aeruginosa	8.00±3.61[a]	10.00±3.00[b]

Values are mean ± SD. Values> 6mm indicate some activity. Values in the same raw with different superscript differs significantly ($p < 0.05$), n = 3

DISCUSSION

The combination of Gas chromatography and mass spectrometry seems to be an effective measure for chemical analysis [Krishnaveni *et al.,* 2014]. The GCMS analysis of methanol root extract of *Kigelia africana* reveals the presence of 9-Octadecanoic acid (z)-methyl ester which have a great potentials as antimicrobial agent against some microorganisms and found to be effective against fungi.

DPPH is a stable free radical which can accept and react with a hydrogen or electron from any donor molecule thereby resulting in the bleaching of DPPH absorption. DPPH is a dye which is purple in colour and absorbs maximum at 517nm. This dye when reacted with a hydrogen the purple colour disappears or reduces as a result of conversion of DPPH to 2, 2-diphenyl-1-picryl hydrazine resulting in the decrease in its absorbance [Abdu *et al.,* 2018].

The reducing ability of the extracts increases with increase in sample concentration. High absorbance suggests high antioxidant activity of the extract [Abdu *et al.,* 2018]. In reducing power assay, the presence of the reductants in the solution causes the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form. Therefore, Fe^{2+} can be monitored by absorbance measurement at 700 nm. It is known that the reducing properties of an extract could serve as a measure of its antioxidant action by donating hydrogen atom to break the free radical chain [Sarkar *et al.*, 2012]. Increasing absorbance at 700 nm might indicate an increase in reducing ability. The antioxidants present in the fractions of *K. Africana* caused the reduction of Fe^{3+}/ferricyanide complex to the ferrous form, and thus proved their reducing power. Nitric oxide is a free radical with a short half-life and its action is independent which may cause neuronal damage, especially in conjunction with other ROS such as superoxide radical to form peroxynitrite radical [Oboh and Rocha 2008]. However, the result revealed that methanol root extracts of *K. africana* had both high NO and DPPH scavenging potentials. Hence, this extract may elicit inhibitory action against NO-induced cellular damage. All the results show dose-dependent increase in their antioxidant activities except nitric oxide (NO) radical scavenging assay.

The antibacterial result of root extract of *K. africana* possesses good antibacterial activity against *P. aeruginosa S. aureus*, *Pseudomonas aeruginosa, and Salmonella spp*. One Gram-positive bacteria, *Staphylococcus aureus*and three Gram-negative bacteria, *Pseudomonas aeruginosa, Salmonella spp* and *Escherichia coli*, are used as test microorganisms with the standard. For *Escherichia coli,* the zone of inhibition (mm) of methanol root extract is greater than that of the standard which signifies a greater activity than the standard, also the extract posses greater antibacterial activity against *Staphylococcus aureus* than the standard drug, also against *Salmonella spp* when compared with standard there is a slight significant difference and with *Pseudomonas aeruginosa* the extract posses greater antibacterial activity against the organism when

compared with the standard drug, this might suggest the methanolic root extract of *Kigelia africana* possess good antibacterial activity against some selected microbial agent. Hence, the anti-microbial activity might be attributed by the presence of carboxylic compound 9-Octadecanoic acid (z)-methyl ester identified by the Gas Chromatography machine. The results revealed that the methanol of root of *K. africana* possess good antibacterial activity as compared to the standard drug (Table 2), which is in support of previous studies which have also reported antibacterial properties for *K. Africana* extracts [Akunyili *et al*, 1991; Jeyachandran and Mahesh, 2007].

CONCLUSION

In accordance with the results obtained, *K. africana* root extract was found to contain some basic functional groups as well as a chemical compound identified by FTIR Spectral and GCMS respectively in which these compounds might attribute to the good antioxidant and antibacterial activity of such extract as evident in this study.

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