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Research Article

In vitro antioxidant activity and gas chromatography-mass spectrometry analysis of solvent extracts of *Kigelia africana* stem bark

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ABSTRACT

Objective: *Kigelia africana* is widely used, traditionally, in Africa in the treatment of many conditions and complaints such as kidney and stomach disorders, malaria, wounds, and venereal diseases. In the present study, an attempt has been made to investigate the antioxidant activity of extracted fractions of the stem bark and analyze the non-polar fractions for the presence of various components that may be responsible for their antioxidant properties. **Methods:** The antioxidant activity was evaluated using 1,1-diphenyl-2-picrylhydrazyl, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) radical cation and nitric oxide radical scavenging models. Analysis of the chemical components of the solvent extracts was carried out by gas chromatography-mass spectrometry (GC-MS). **Results:** Strong activity (71-89% inhibition) was found for the ethyl acetate fraction, whereas the non-polar chloroform fraction exhibited a relatively weak activity (24-48%) inhibition, at 25 µg/ml concentration. GC-MS analysis of the non-polar extracted fractions identified 21 and 12 compounds for the hexane and chloroform fractions, respectively, of which α-terpineol (2) (39.21%) was the main component identified for the hexane extract, and α-amyrin (9) (32.54%) was the main component identified for the chloroform extract. **Conclusion:** This study shows that the non-polar extracted fractions of *K. africana* stem bark may be a potential source of natural antioxidants or bioactive agents.

KEY WORDS: Antioxidant activity, Gas chromatography-mass spectrometry, *Kigelia africana*, Non-polar solvent extract, Percentage inhibition

INTRODUCTION

From time immemorial, the control and treatment of diseases have been through the use of natural substances, in particular, plants. Hence, it is not surprising that the practice of traditional medicine in developing countries involves the use of a wide variety of natural products to treat common infections and to maintain good health.[1,2] In fact, it has been reported as far back as 20 years ago that more than 35,000 plant species are being used in various human cultures around the world for medical purposes.[3] Currently, natural products from plants referred to as secondary metabolites, and many of their derivatives with varied chemical structures account for a high percentage of all the drugs in clinical use globally. A typical example of drugs based on traditional medicine leads is paclitaxel (1) (brand name Taxol) which was extracted from the Pacific yew tree *Taxus brevifolia* with anticancer activity.[4,5]

Superoxide anion, hydrogen peroxide, and hydroxyl radicals are examples of free radicals and reactive oxygen species (ROS) which can induce the oxidative damage of cell membranes, DNA, and proteins, thus they have been implicated in over 50 diseases including degenerative processes related to aging, cancer, and atherosclerosis.[6,7] The use of antioxidants to block free radicals and ROS could be beneficial in the prevention of free radical-related diseases.[8]

Kigelia africana (Lam.) Benth – Bignoniaceae (commonly known as sausage tree, African sausage tree, cucumber tree, and pandoro [Western Nigeria]) is widely grown in tropical Africa and found in South

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Received on: 18-05-2017; Revised on: 24-06-2017; Accepted on: 20-07-2017

Africa. It is widely used in Africa in the treatment of many conditions and complaints. All parts of the plant (fruits, leaves, roots bark, stem bark, roots, and wood) have been used in traditional healing systems. As examples, the fruits (ripe and/or unripe/green) are used in the treatment of abscesses, constipation, dysentery, gynecological disorders, hemorrhoids, post-partum hemorrhage (loss of blood estimated to be >500 ml within 24 h following childbirth from the genital tract), lumbago, rheumatism, syphilis, ulcers, and wound.[9-13] A decoction of the fruit is also used for inducing secretion of breast milk.[14] The leaves and the twigs have been used for treating dysentery, epilepsy, headache, infertility, kidney disorder, malaria, rheumatism, snake bite, stomach disorder, and wounds.[12,15]

The stem bark and roots (pounded or an infusion) are used for relieving asthma and for treating dysentery, pneumonia, stomach problems in children, chronic wounds, and sores and venereal diseases.[9,11,13,15]

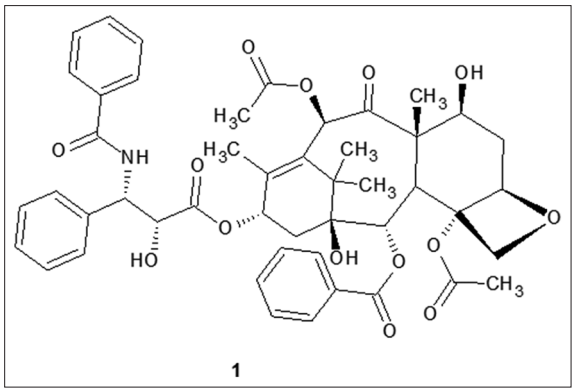
The main bioactive secondary metabolites isolated from the *K. africana* plant include 4-hydroxycinnamic acid (coumaric acid) derivatives and iridoids (such as coumaric acid itself, caffeic acid, catapol, and verminoside), naphthaquinone derivatives (such as lapachol, kigelinone, pinnatal and its isomer isopinnatal, kigelinol and its isomer.), isocoumarins (such as kigellin), sterols (such as stigmasterol and beta-sitosterol), flavonoids (such as quercetin and luteolin), lignans (such as kigeliol), phenylpropanoids, and furanone derivatives.[16]

Scientific studies to verify the ethnomedicinal properties of the plant have been carried out. Solvent extracts of different parts of the plant have been shown to possess anticancer, anti-inflammatory, antimalarial, antimicrobial, antioxidant, and central nervous system stimulant activities.[16,17]

There are reports in the literature on the antioxidant studies of methanol stem bark extract of *K. africana*. For example, the methanol extract exhibited 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity with reported concentration at 50% inhibition (IC_{50}) values of 13.7 µg/ml[18] and 175 µg/ml[19] and scavenged hydroxyl radical (OH•) production in a dose-dependent manner with an IC_{50} for H_2O_2 of 30 µg/ml.[19]

To the best of our knowledge, no study concerning the non-polar extracts of the stem bark of *K. africana* has been reported. Knowledge of the chemical constituents of plants extracts is a useful route to the discovery of new sources of therapeutic agents. Gas chromatography-mass spectrometry (GC-MS) is a method that provides a detailed chromatographic profile of mixtures, giving information on the relative or absolute amounts of the components.

Hence, in this study, we assessed the antioxidant activity and GC-MS analysis of the non-polar n-hexane and chloroform extracts of *K. africana* stem bark.



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MATERIAL AND METHODS

Collection of Plant Material

The stem bark of *K. africana* was purchased from local market of Ilawe-Ekiti, Ekiti State, Nigeria.

Plant Extracts Preparation

The dried stem bark was pounded to small pieces and then ground to fine powder using a pulverizer (490 g). The pulverized sample was extracted with 80% methanol (5 L) at room temperature for 3 days. The aqueous methanol extract was then filtered to give a reddish-brown clear filtrate, and the filtrate was concentrated under reduced pressure to remove methanol. The extract was suspended in water and partitioned with n-hexane, chloroform, and ethyl acetate successively. All the extracts and aqueous layer were separately evaporated to dryness under reduced pressure and transferred to microtubes and stored in a refrigerator at 4°C until required.

DPPH Radical Scavenging Activity

The antioxidant activity of the plant extracts was assessed on the basis of the ability to reduce the stable DPPH free radical.[20] A serial dilution of the hexane, chloroform, ethyl acetate, and aqueous extracts was carried out to achieve 25.00, 12.50, 6.25, 3.125, and 1.563 µg/ml concentrations. A 100 µL aliquot of a methanol solution of 0.16 mM DPPH was added to 200 µL of various concentrations of each extract. A control solution was prepared in the absence of plant sample. Absorbance was measured using a spectrophotometer at a wavelength of 515 nm. All measurements were made in triplicate and averaged. The ability of the extracts to scavenge DPPH radical was calculated using the following equation:

$$\text{DPPH radical scavenging activity}(\%) = \left(1 - \frac{\text{Absorbance DPPH + sample}}{\text{Absorbance DPPH}}\right) \times 100$$

2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) Radical Cation ($ABTS^{+}$) Radical Scavenging Activity

The ABTS•+ decolorization assay procedure, developed by Re *et al.*,[21] was used to determine the free radical scavenging activity of the plant extracts. The ability of antioxidants to quench the long-lived ABTS radical cation, an intensely-colored chromophore with characteristic absorption at 734 nm, in comparison to that of α-tocopherol, is the basis of this method. The reaction of ABTS stock solution (7 mM) with potassium persulfate (7 mM) in a ratio 2:1 was used to produce the ABTS radical cation (ABTS•+), and the reaction mixture was allowed to stand in the dark at room temperature for 16 h before use. Before use, the stock solution was diluted with 70% ethanol to an absorbance of 0.75 ± 0.05, to give ABTS working solution. 1 ml of ABTS working solution was mixed with 1 ml of different concentrations of the extracts and the absorbance was measured after 20 min at 734 nm. Trolox was used as a standard.

Nitric Oxide (NO) Radical Inhibition Activity

The method of Green *et al.*[22] as described by Marcocci *et al.*[23] was used to determine the inhibition of NO radical activity of the extracts. NO, generated from sodium nitroprusside in aqueous solution at physiological pH, interacts with oxygen to produce nitrite ions which was measured by Griess reaction:

A mixture containing 0.1 ml of different concentrations of the extracts and 0.9 ml of sodium nitroprusside (2.5 mM) in phosphate buffer saline was incubated under illumination for 150 min. After incubation, 0.5 ml of 1% sulfanilamide in 5% phosphoric acid was added and +incubated in the dark for 10 min, followed by addition of 0.5 ml of 0.1% N-1-napthylethylenediamine dihydrochloride. The absorbance of the chromophore formed was measured at 546 nm. The percentage inhibition of NO radical formation was calculated and the IC_{50} was determined. Ascorbic acid was used as positive control in the experiment.

GC-MS Analysis

The GC-MS analysis of the extracted oils was performed using Agilent GC/MS (5915/1890N) with an HP-5ms fused silica capillary with a (5% phenyl)-poly-methylsiloxane) stationary phase, film thickness of 0.25 µm, a length of 30 m, and an internal diameter of 0.25 mm. The GC oven initial temperature was 50°C and gradually increased to 100°C at a rate of 5°C/min and finally to 280°C at the rate of 10°C/min. Helium gas at flow rate of 1 ml/min was used as carrier gas and the sample was injected in split mode (50.2:1). The GC was coupled to Mass Selective Detector Transfer Line Heater maintained at 270°C. The extracts were diluted with appropriate solvent (1/100, v/v) and filtered. The particle-free diluted extracts (1 µL) were taken in a syringe and injected into injector with a split ratio 30:1. Identification of compounds was based on comparisons of the relative retention time and mass spectra with those of the Wiley Registry of Mass Spectral Data (John Wiley & Sons, Inc./ Hoboken, NJ, USA) and NIST/EPA/NIH Mass Spectral Library (National Institute of Standards and Technology/Gaithersburg, MD, USA) of the GC-MS. The percentage composition was computed from the peak areas of the GC spectra.

RESULTS

Determination of Antioxidant Activity of the Plant Extracts

Antioxidant property of diverse medicinal plants is related to their therapeutic usefulness. It is often necessary to investigate the major chemical constituents that are involved in biological activities to evaluate the antioxidant activity of natural products.

Antioxidant activity of the solvent fractionated extracts of *K. africana* stem bark of varying concentrations, ranging from 1.563 to 25.00 µg/ml, was evaluated in different *in vitro* models, namely, DPPH, NO, and ABTS. In general, the hexane extract did not display appreciable radical scavenging potentials in the three models at the concentration range evaluated. However, it was observed that the chloroform, ethyl acetate, and aqueous extracts scavenged radicals in concentration dependent manner in all the models (Figures 1-3). The scavenging effect of the extracts were expressed as % inhibition and they were compared with standard antioxidants, ascorbic acid, gallic acid, and trolox for the DPPH, NO, and ABTS assays, respectively.

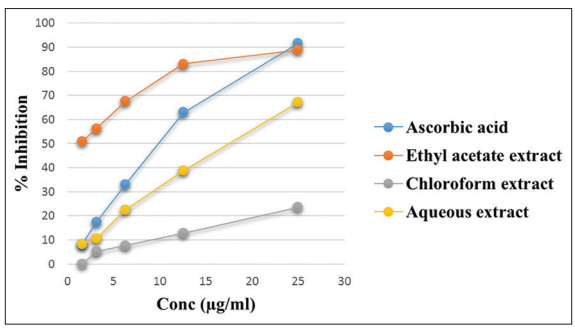


Figure 1: Antioxidant assay of *Kigelia africana* stem bark extracts using 1,1-diphenyl-2-picrylhydrazyl assay

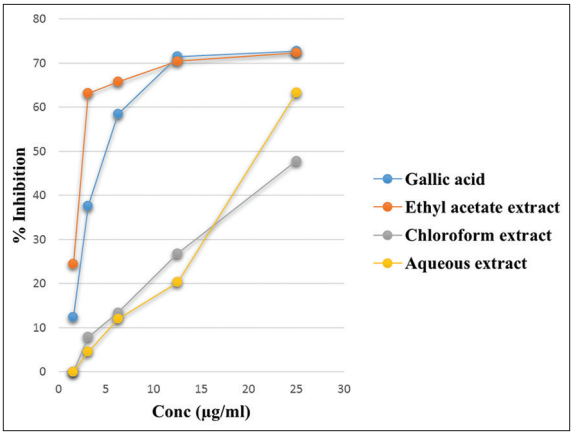


Figure 2: Antioxidant assay of *Kigelia africana* stem bark extracts using nitric oxide assay

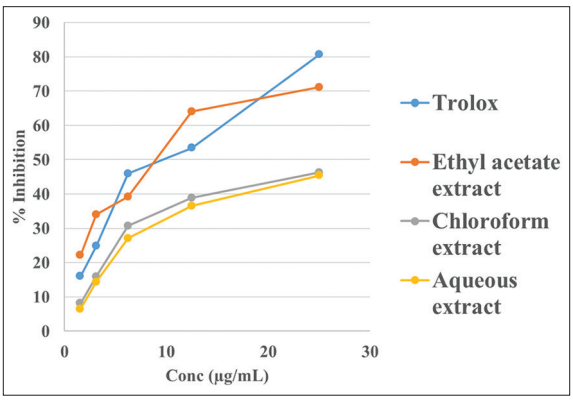


Figure 3: Antioxidant assay of *Kigelia africana* stem bark extracts using 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) radical cation assay

DPPH Free Radical Scavenging Activity

Figure 1 shows the DPPH radical scavenging activities of the polar and non-polar solvent extracts of *K. africana* stem bark. Ascorbic acid, at a concentration of 1.56 µg/ml, exhibited a percentage inhibition of 8.13% and at 25.0 µg/ml, a 91.64% inhibition. The percentage inhibition value of the extracts was found to be in the order of ethyl acetate > ascorbic acid > aqueous >chloroform extract. However, the percentage of inhibition produced by ascorbic acid at a concentration of 25 µg/ml was greater than the scavenging activity of the three extracts at the same concentration. The IC_{50} values (in µg/ml) of the extracts and the standard was found in the order of ethyl acetate (1.4) < ascorbic acid (11.78) < aqueous (17.84) < chloroform (56.41) extract.

NO Radical Inhibition Activity

The results of the NO scavenging activity of *K. africana* extracts as percentage inhibition are summarized in Figure 2. In this test, the extracts and standard effectively reduced the generated NO radicals at 25.0 µg/ml (chloroform [47.87%], aqueous [63.31%], ethyl acetate [72.36%], and gallic acid [72.72%]). At concentrations below 25.0 µg/ml, the chloroform and the aqueous fractions exhibited relatively much weaker scavenging activity. In general, they exhibited strong NO radical scavenging capacity with IC_{50} values of 2.5, 6.88, 21.03, and 25.88 µg/ml for ethyl acetate, gallic acid, aqueous and chloroform extract, respectively.

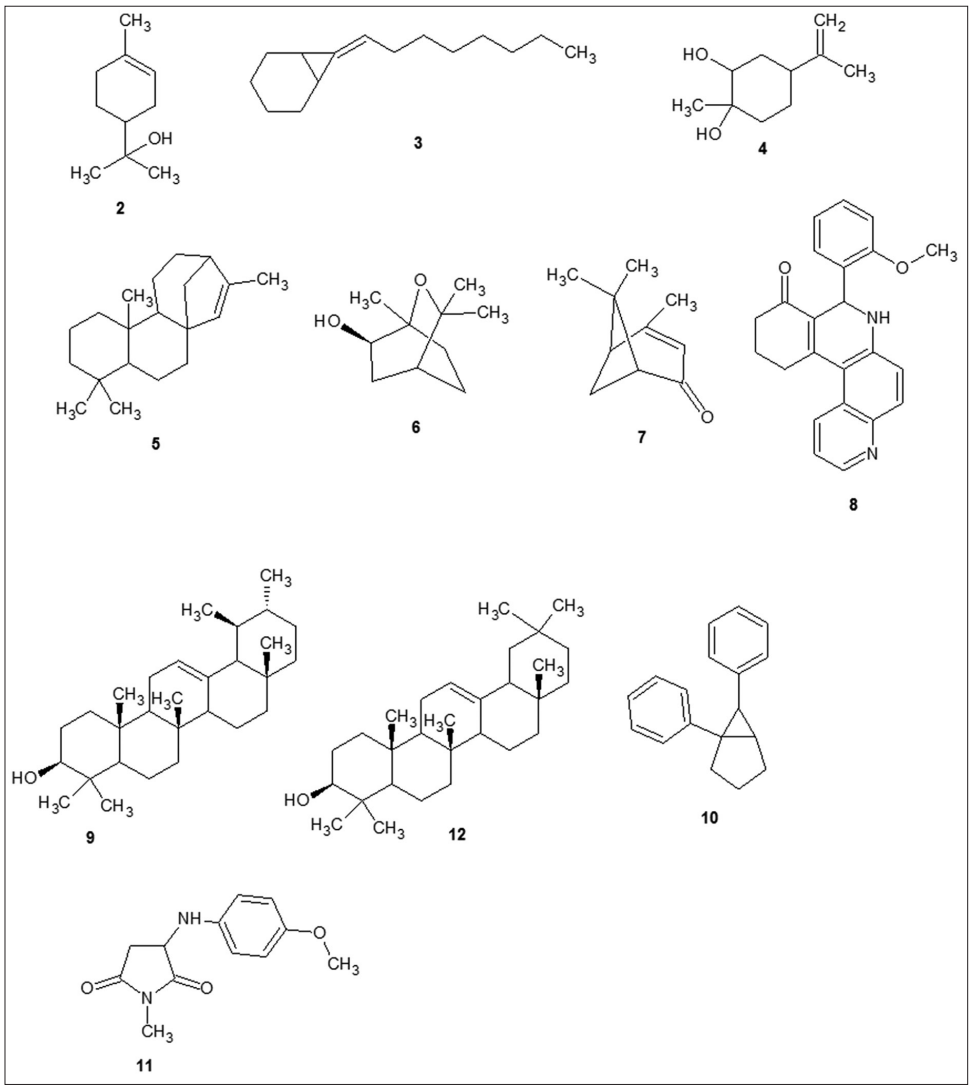
ABTS Radical Cation Scavenging Activity

In the ABTS scavenging assay, the extracts also showed a dose-dependent effect, and the results were compared with the standard antioxidant, trolox (Figure 3). The ethyl acetate extract showed a high scavenging activity of 71.05% at 25.0 µg/ml, whereas the standard trolox showed 80.63% scavenging activity. However, the aqueous and chloroform extracts showed less than average scavenging activity with values of 45.37% and 46.28%, respectively. Ethyl acetate, chloroform, and aqueous fractions showed IC_{50} values of 10.55, 25.79, 26.68 µg/ml, respectively, as compared to that of trolox 11.96 µg/ml.

Chemical Composition of Non-polar Extracts by GC-MS Analysis

The GC-MS analysis was carried out for the hexane and chloroform fractions and the GC-MS chromatograms are as shown in Figure 4 for hexane and in Figure 5 for the chloroform extracts, whereas Tables 1 and 2 show the number and nature of phytochemical constituents found in the hexane and chloroform extract fractions, respectively. The GC-MS analysis of the hexane extract revealed the presence of 24 compounds (Table 1). However, a total of 21 components of the extract were identified, accounting for 87.5% of the total extract. These compounds are comprised mainly of hydrocarbons, alcohols, ketones, fatty acid, and ester. Alpha-terpineol (2) was identified as a major chemical constituent (39.21%) followed by 7-octylidenebicyclo (4.1.0) heptane (3) (8.05%), limonene-1,2-diol (4) (6.82%), kaur-15-ene (5) (4.54%), exo-2-hydroxycineole (6) (4.23%), (D)-verbenone (7) (4.16%), and so on. Monoterpenoids represented the major composition, corresponding to 69.81% of the extract, whereas hydrocarbons accounted for 14.32% composition, only one diterpenoid (4.54% composition), one fatty acid and an ester of a fatty acid (3.65% composition) and an alkaloid, a benzo[a][4,7]phenanthrolin-9-one derivative (8), (1.71%).

The chloroform extract revealed the presence of 16 chemical compounds by GC-MS analysis (Table 2), out of which 12 components were identified, accounting for 75.0% of the total extract. The principal compounds in the chloroform extract were α-amyrin (9) (32.54%), 1,6-diphenylbicyclo[3.1.0]hexane (10) (24.64%), 1-methyl-3-(4-methoxyphenylamino)pyrrolidine-2,5-dione (11) (16.96%), β-amyrin (12) (10.22%), and limonene-1,2-diol (4) (3.87%).



DISCUSSION

Plants are nature's main storehouse of raw materials for the manufacture of traditional and modern medicine, and in particular play a significant role in providing primary health-care services to rural inhabitants. In fact, the World Health Organization has recommended more effective use of medicinal plants in health-care system.[24] Plants contain numerous chemical constituents, many of which are known to be bioactive and are responsible for exhibiting diverse pharmacological activities.[25] It is therefore desirable to have knowledge of the chemical constituents of plants to discover new therapeutic agents and lead compounds that may lead to the synthesis of more potent analogs of great economic value.

Complex mixture of compounds constitute the non-polar extracts of medicinal plant species, including essential oils, monoterpenes, diterpenes, sesquiterpenes, triterpenes, long-chain aliphatics, alicyclics, and their oxygenated derivatives (alcohols, aldehydes, esters, ethers, ketones andoxides) and in some cases, alkaloids and phenols. These phytoconstituents may be present in different plant parts such as the bark, flowers, leaves, roots, seeds, and wood[26] and are believed to play an important role in plant defense system.[27] In the present study, we have examined the *in vitro* antioxidant activity, and GC-MS analysis of the non-polar (n-hexane and chloroform) partition fractions obtained from methanol extract of *K. africana* stem bark. The biological activity exhibited by non-polar extracts (such as n-hexane) has often been attributed to the presence of complex mixtures of triterpenoid and/or steroid compounds.[28] The absence of exhibition of antioxidant activity from the hexane fraction, in the concentration range examined, may be due to the absence of phenolic, steroidal, and triterpenoid compounds in the fractionated extract. This is supported from the results of the GC-MS analysis of the hexane fraction. The analysis revealed the presence of a number of components with highest peak area at 16.48 RT, identified as α-terpineol (2-(4-methyl-1-cyclohex-3-enyl)propan-2-ol) (2).

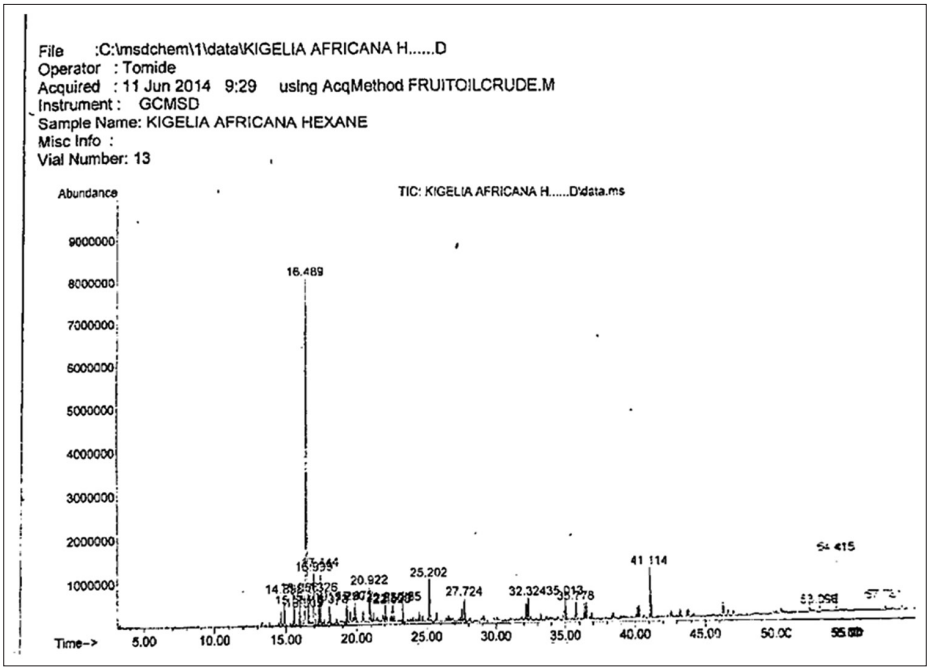


Figure 4: Gas chromatography-mass spectrometry chromatogram of the n-hexane extract of *Kigelia africana* stem bark

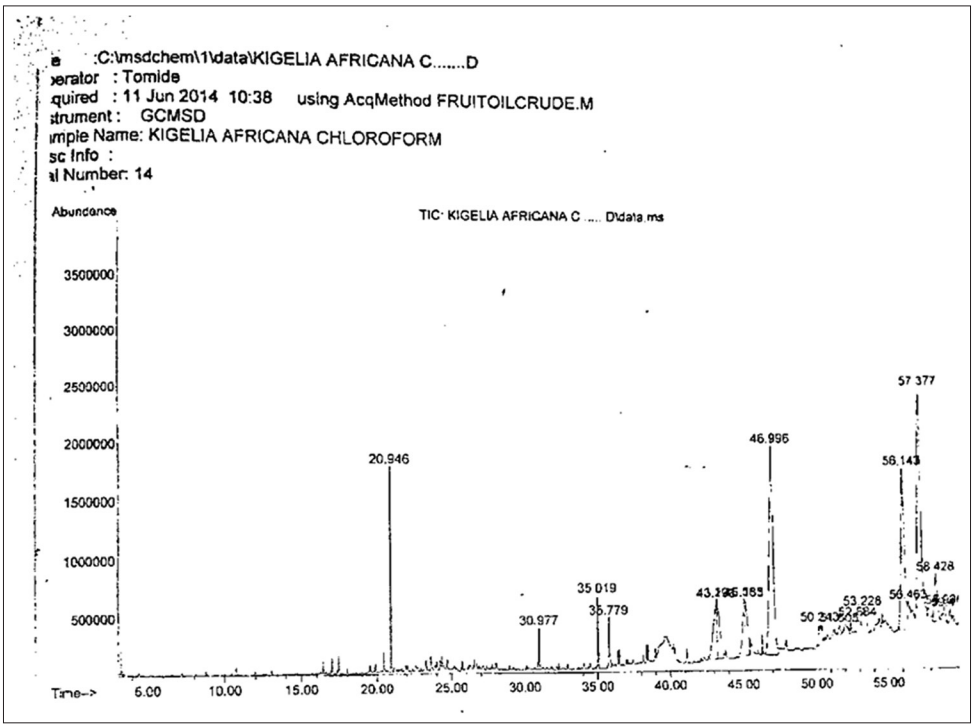


Figure 5: Gas chromatography-mass spectrometry chromatogram of the chloroform extract of *Kigelia africana* stem bark

Alpha-terpineol, a volatile monoterpenoid alcohol, which is a major component of the essential oil of many plants, has been found to show very weak antioxidant activity,[29] but exhibited numerous biological properties including, antibacterial,[30] antifungal,[31] anti-inflammatory (selectively regulating cell function during inflammation) activities,[32,33] ability to exhibit antiproliferative effects on human erythroleukemic cells,[34] and enhance the permeability of skin to lipid soluble compounds[35] and inhibitory effects on the growth of tumor cells.[36] The next dominant phytocompound, 7-octylidenebicyclo [4.1.0] heptane (3) (8.05%) is a non-isoprenoid hydrocarbon which has not been reported to show any biological activity. A study by Ruberto and Baratta[37] found that sesquiterpene- and non-isoprenoid hydrocarbons showed a "low, if any, antioxidant effect." Limonene-1,2-diol (p-menth-8-ene-1,2-diol) (4) (6.82%

Table 1: Phytocompounds present in the hexane extract of *K. africana* stem bark as determined by GC-MS analysis

Compound	Retention time	Molecular formula	% Composition
Monoterpenoids			
2,3-dehydro-1,8-cineole	22.02	$C_{10}H_{16}O$	1.54
Endo borneol	15.58	$C_{10}H_{18}O$	1.48
Terpinen-4-ol	15.96	$C_{10}H_{18}O$	1.48
p-cymenol	16.30	$C_{10}H_{14}O$	1.56
Alpha-terpineol	16.48	$C_{10}H_{18}O$	39.21
(-)-myrtenol	16.61	$C_{10}H_{16}O$	1.92
(D)-verbenone	16.99	$C_{10}H_{14}O$	4.16
Exo-2-hydroxycineole	17.44	$C_{10}H_{18}O_2$	4.23
Cis-carveol	17.32	$C_{10}H_{16}O$	2.21
D-carvone	18.07	$C_{10}H_{14}O$	1.28
Limonen-1,2-diol	20.92	$C_{10}H_{18}O_2$	6.82
s-(+)-5-(1-hydroxy-1-methylethyl)-2-methyl-2-cylohexen-1-one	23.28	$C_{10}H_{16}O_2$	1.58
Myrtenyl acetate	14.89	$C_{12}H_{18}O_2$	2.34
Non-isoprenoid hydrocarbons			
Dispiro [4.2.4.2]tetradecane	53.09	$C_{14}H_{24}$	1.49
Tetradecane	22.59	$C_{14}H_{30}$	2.83
7-octylidenebicyclo [4.1.0]heptane	54.41	$C_{15}H_{26}$	8.05
Hexadecane	27.72	$C_{16}H_{34}$	1.95
Fatty acid and derivative			
Pentadecanoic acid, 14-methyl-, methyl ester	35.01	$C_{17}H_{34}O_2$	1.66
Palmitic acid	35.77	$C_{16}H_{32}O_2$	1.99
Diterpenoid			
Kaur-15-ene	41.11	$C_{20}H_{32}$	4.54
Alkaloid			
8-(2-methyoxy-pheny)-8,10,11,12-tetrahydro-7H-benzo [a] [4,7] phenanthrolin-9-one	57.78	$C_{23}H_{20}N_2O_2$	1.71
Unidentified compounds			
Unidentified	19.29		1.77
Unidentified	19.87		2.14
Unidentified	22.52		1.30
Total	99.24		

GC-MS: Gas chromatography-mass spectrometry, *K. africana: Kigelia africana*

Class of compounds	% Composition
Monoterpenoid	69.81
Diterpenoid	4.54
Fatty acid and derivative	3.65
Hydrocarbon	14.32
Alkaloid	1.71
Unidentified compounds	5.21
Total	99.24

composition), a derivative of bioactive limonene, has been reported to exhibit antitumor activity and inhibit benzo[α]pyrene-induced carcinogenesis in the mouse forestomach[38]

Exo-2-hydroxycineole (exo-2-hydroxy-1,8-cineole, 4.23% composition) (6) is a 2-hydroxy derivative of 1,8-cineole, a compound which Amakura *et al*. reported exhibited a negligible antioxidant activity,[39] but Asanova *et al.* demonstrated that 1,8-cineole had moderate antioxidant and cytotoxic properties and pronounced analgesic and antitumor activity.[40] Verbenone (7), an alpha, beta-unsaturated ketone monoterpenoid, possessed antibacterial and antifungal activities.[41,42]

The tested chloroform fractionated extract, together with the ethyl acetate and aqueous fractions, have a strong antioxidant activity against various oxidative systems *in vitro*. However, the chloroform fraction exhibited the lowest percentage inhibition at 25 µg/ml in all *in vitro* assays, except in the ABTS assay (Figures 1-3). The ethyl acetate fraction exhibited the highest antioxidant activity (lowest IC_{50} values) even exceeding that of the standard antioxidants (ascorbic acid, gallic acid, and trolox) in the DPPH, NO, and ABTS assays. Antioxidant effect of *K. africana* plant was also studied by other researchers. For example, Hussain *et al.*[28] in a comparative analysis of the ethanol extracts of the plant parts, found that the bark extract exhibited the most remarkable antioxidant as well as antibacterial activity as compared to leaves and fruit extracts.[43] In another study, the methanol extract of the leaf and stem bark exhibited antioxidant activity, scavenging DPPH free radical with IC_{50} values of 56.9, and 13.7 µg/ml for the leaves and stem bark extracts, respectively.[18] Similarly, methanol extract stem bark

Table 2: Phytocompounds present in the chloroform extract of *K. africana* stem bark as determined by GC-MS analysis

Compound	Retention time	Molecular formular	% Composition
Monoterpenoid			
Limonen-1,2-diol	20.94	$C_{10}H_{18}O_2$	3.87
Benzene derivative			
Coniferol	30.97	$C_{10}H_{12}O_3$	0.89
Fatty acid and derivative			
Methyl palmitate	35.01	$C_{17}H_{34}O_2$	1.35
Palmitic acid	35.77	$C_{16}H_{32}O_2$	1.48
Triterpenoids			
Stigmast-4-en-3-one	30.24	$C_{29}H_{48}O$	1.29
Beta-amyrin	43.19	$C_{30}H_{50}O$	10.22
Alpha-amyrin	45.08	$C_{30}H_{50}O$	32.54
Lucidumol	52.88	$C_{30}H_{48}O_4$	0.72
Lanosterol	58.99	$C_{30}H_{50}O$	0.95
Alkaloid			
1-methyl-3-(4-methoxyphenylamino) pyrrolidine-2,5-dione	56.14	$C_{12}H_{14}N_2O_3$	16.96
Non-Isoprenoid hydrocarbon			
7-oxabicylo[4.1.0]heptane, 2,2,6-trimethyl-1-(3-methyl-1-,3-butadienyl)-5-methylene	51.60	$C_{15}H_{22}O$	0.53
1,6-diphenyl-bicyclo[3.1.0]hexane	57.37	$C_{18}H_{18}$	24.64
Unidentified compounds			
Unidentified	53.22		0.54
Unidentified	56.46		0.77
Unidentified	58.42		2.38
Unidentified	59.41		0.85
Total			99.98

GC-MS: Gas chromatography-mass spectrometry, *K. africana: Kigelia africana*

Class of compound	% Composition
Monoterpenoid	3.87
Benzene derivative	0.89
Fatty acid and derivative	2.83
Triterpenoids	45.72
Alkaloid	16.96
Non-isoprenoid hydrocarbon	25.17
Unidentified compounds	4.54
Total	99.98

of *K. africana* was shown to possess good antioxidant activity by scavenging hydroxylradical OH• production and stable free radical (DPPH) in a dose-dependent manner, with an IC_{50} values of 30 µg/ml (for H_2O_2 only) and 175 µg/ml, respectively.[19]

Previous GC-MS analysis of the different parts of *K. africana* revealed that methanol extract of the stem bark contained eighteen different active compounds, which in terms of percentage abundance, 9-octadecenoic acid, E-2-octadecadecen-1-ol, 9-octadecenal, Z-10-pentadecen-1-ol, and E-9-tetradecenoic acid were the major constituents, and the crude extract inhibited pro-oxidants induced hydroxyl radicals and lipid peroxidation in rat's testes in a dose-dependent manner,[19] while 19 bioactive phytochemicals were identified in the hexane extract of the root, with 3 major components: Elaidic acid ($C_{18}H_{34}O_2$, 56.12%), palmitic acid ($C_{16}H_{32}O_2$, 18.02%), and stearic acid $C_{18}H_{36}O_2$, 12.80%).[44] Furthermore, linoleic acid, linolenic acid, palmitic acid, arachidic acid, stearic acid, elaidic acid, and oleic acid were the major constituents in both oil and wax from the fruits from Cape Town, South Africa,[45] whereas the main components of some fractions of the hexane extract of the plant leaves from Nigeria were 4,4-dimethyl undecane, methyl nonadecanoate, emery, oleic acid ester (methyl oleate), methyl n-butyrate, and 2,3-hexanediol (66.9% free fatty ester, 27% alkane hydrocarbon, and 6.02% alcohol).[46]

The GC-MS analysis of the chloroform fraction detected 16 components and showed that the major components were triterpenoids, beta-amyrin and alpha-amyrin, (9 and 12) (42.76% composition), non-isoprenoid hydrocarbon, 1,6-diphenyl-bicyclo[3.1.0] hexane (10) (24.64% composition), and an alkaloid, a 3-aminopyrrolidine-2,5-dione derivative (11) (16.96% composition). The minor phytoconstituents include three triterpenoids-stigmast-4-en-3-one (1.29%), lanosterol (0.95%), lucidumol (24,25-dihydroxylanost-8-ene-3,7-dione, 0.72%)-, limonene-1,2-diol (3.87%), palmitic acid and its methyl ester (2.83%) and a phenol derivative, coniferol (coniferyl alcohol, 0.89%), many of which are known to possess several pharmacological activities. Hence, the antioxidant activity displayed by the non-polar (chloroform)

fraction (IC_{50} values: 56.41, 25.88, 25.79 µg/mL in the DPPH, NO, and ABTS assays, respectively) may be partially ascribed to the presence of phenolic and triterpenoid compounds.

Alpha- and beta-amyrins are two promising bioactive natural products (pentacyclic triterpenes) that have been shown to exhibit various pharmacological actitivies such as anti-inflammatory, anti-hyperglycemic, antioxidant, gastroprotective, hepatoprotective, and hypolipidemic effects at non-toxic concentrations.[47-50] The researchers' results indicated the amyrins have potential for development as drug for diabetes and atherosclerosis.[50] However, evaluation of α-amyrin for antioxidant activity using DPPH and ferric reducing antioxidant power assays and its ability to scavenge intracellular ROS induced by UVA irradiation revealed that it does not exhibit direct antioxidant activity.[51]

Pyrrolidine-2,5-dione derivatives have exhibited anticonvulsant,[52,53] potential antitumor[54] activities, and have been demonstrated that some can be used for the treatment of diseases or disorders mediated through α1a and/or α1d adrenergic receptors, benign prostatic hyperplasia and related symptoms, and lower urinary tract symptoms associated with or without benign prostatic hyperplasia.[55]

CONCLUSION

K. africana is a traditional medicinal plant that represents a rich source of diversity of bioactive compounds. Little work has been carried out on the non-polar extracted fractions of the plant, hence extensive research is required to explore their biological properties and identify potential biological compounds of medicinal importance. The results of the present study revealed that the non-polar extracted fractions of *K. africana* stem bark may be a potential source of natural antioxidants or bioactive agents such as alpha- and beta-amyrins, alpha-terpineol, and pyrrolidine-2,5-dione derivative.

ACKNOWLEDGMENT

The authors would like to acknowledge the support of the Central Science Laboratory, Obafemi Awolowo University, Ile-Ife.

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