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Evaluation of the Phytochemical Constituents of Extracts of *Kigelia africana* Fruit and *Sorghum bicolor*, Stalk in Lagos Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Authors NFO, CJO, OAO and SOO designed the study, performed the statistical analysis and wrote the protocol. Authors NFO and CJO wrote the first draft of the manuscript. Authors UC and EIO managed the analyses of the study. Authors HTH and OHS managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The use of herbal medicines and phytonutrients or nutraceuticals with numerous secondary metabolites continues to expand rapidly across the world, with many people now resorting to these products to treat various health challenges in different national healthcare settings. Therefore, the

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study is aimed at evaluating the phytochemical composition in the fruit extract of Kigelia africana and Sorghum bicolor stalks bought from the Mushin market in Lagos, Nigeria to ascertain its numerous pharmacological activities and identify the various chemical compounds responsible for these activities. Ethanol extracts of identified fresh fruits of K. africana and S. bicolor stalks (L) were prepared for phytochemical screening using established methods for Alkaloids, Tannins, Phenols, Flavonoids, and Terpenoids testing as well as Gas Chromatography-Mass Spectrometry Analysis (GC-MS) for the analysis of the extracts. The chemical constituents present in both extracts were tannins, phenols, flavonoids, and saponins as well as alkaloids found only in K. africana. Also, the chromatogram of K. africana revealed the presence of forty-four (44) phytochemical constituents. At the same time, that of S. bicolor were twenty-nine (29) phytochemical constituents that could contribute to the medicinal quality of the plant with 9, 12-Octadeadienoic acid (Z, Z), and 13-Docosenoic acid, methyl ester, (Z) found as the major compounds respectively. The K africana and S. bicolor African indigenous plants in Lagos, Nigeria, possess different phytocomponents of scientific importance, biological action, and potential medicinal properties. There is a need for more standardization and purification of this herbal formulation for the treatment of diseases.

Keywords: Kigelia africana; Sorghum bicolor; Phytochemical; Gas chromatography.

1. INTRODUCTION

There has been a rapid increase in the usage of herbal medicines and phytonutrients across the world, with many people now resorting to these products to treat various health challenges in different national healthcare settings [1]. The fact that orthodox drugs have been reported to be restricted by their secondary failure rates and accompanying undesirable side effects made herbal drugs gain even more popularity [2,3] Consequently, the use of herbal remedies has now been widely embraced in many developed countries, with complementary and alternative medicines (CAMs) now becoming mainstream[4,5]. Thus, exploring plants and their constituents to develop plant-based medicine is key to achieving a successful modern herbal practice breakthrough. Different parts of African indigenous plants with potential medicinal properties like K. africana and S. bicolor have been used by herbal medicine practitioners to treat various diseases in Nigeria [6]. The Kigelia plant has medicinal properties not only because of its perceived characteristics such as bitterness, astringent taste, or smell but also because of forces that it seems to emit in connection with its location, orientation, and association with other plants[7]. Traditional healers commonly used it to treat a wide range of skin ailments like fungal infections, boils, psoriasis, and eczema. It also has internal applications, including the treatment in dysentery, ringworm, tapeworm, post-partum hemorrhage, malaria, diabetes, pneumonia, and toothache [7]. The Shona people tend to use the bark or root as powder or infusion for application

to ulcers, drunk or applied in the treatment of pneumonia, as a gargle for toothache and the leaves in a compound applied for backache. In West Africa, the root and unripe fruit is used as a vermifuge and treatment for hemorrhoids and rheumatism [7,8]. The bark is traditionally used as a remedy for syphilis and gonorrhea. The fruits and bark ground and boiled in water are also taken orally or used as an enema in treating children's stomach ailments - usually worms. The unripe fruit is used in Central Africa as a dressing for wounds, hemorrhoids, and rheumatism. Venereal diseases are commonly treated with tree extracts, usually in palm wine as oral medication [7].

The K. africana plant has many medicinal properties due to the presence of numerous secondary metabolites. Some of these compounds include iridoids, flavonoids, and naphthoquinones [9]. Other workers [10] isolated a furan one derivative, 3 -(2"-hydroxyethyl) -5-(2"-hydroxypropyl) -dihydrofuran -2 (3H) - one and four iridoids, 7-hydroxy viteoid II, 7hydroxyeucommic acid. 7-hydroxy-10deoxyeucommiol 10-deoxyeucommiol and together with seven known iridoids, together with ten known phenylpropanoid and phenylethanoid derivatives and a flavonoid glycoside from the fruits. The structures of the isolated compounds were characterized by different spectroscopic methods.

On the other hand, Sorghum is very important in the world's human diet, with over 300 million people dependent on it [11]. It is grown for grain, forage, syrup, and sugar with the Grain sorghum as a staple cereal and the large juicy stems containing as much as 10% sucrose. Apart from using the seed as food, other industrial uses of the stems and fibers can be brewing beer and corn malt.

Also, all sorghum and Sudan grass-related species have the potential to smother weeds, suppress nematode species, and penetrate compacted subsoil [12]. Other workers reported bicolor Sorghum as an anti-abortive. cyanogenetic, demulcent, diuretic, emollient, intoxicant, and poison [13,14]. The root is used for malaria in southern Rhodesia: the seed has been used for breast disease and diarrhea: the stem for tubercular swellings [14]. In China, the seeds are used to make alcohol. The leaves primarily consist of carotenoids, flavonoids, and phenolic acids with small amounts of chlorophyll (a and b), lycopene, and β -carotene. The leaves' fatty acid profiles revealed palmitic, stearic, oleic, and linoleic acid as predominant, with each having greater than 5% of the total fatty acid identified. These findings' nutritional implication is that diets prepared with the leaves provide natural antioxidant and essential fatty acids that could fight cardiovascular-related diseases [8]. The various phytochemical constituents of all the plants' parts make them so relevant in clinical practice to treat human beings' multiple diseases over thousands of years across the world. Thus, Complementary and Alternative Medicine (CAM) has been able to apply the knowledge to managing many diseases in Nigeria. Aside from proven efficacy, the general public believes that traditional medicinal products are safer because they are non-processed. However, there is a need for a scientific basis for developing a standardized herbal product or new drug formulation. Though research is being done on other parts of the plants (stem, bark, and leaves), there is very little published in Nigeria regarding K. africana fruit and S. bicolor stalk. Therefore, the study aims to evaluate the phytochemical composition of the fruit extract of Kigelia africana and S. bicolor stalk bought from the Mushin market in Lagos, Nigeria, ascertain its numerous pharmacological activities and identify the various chemical compounds responsible for these activities.

2. MATERIALS AND METHODS

2.1 Plant Materials

The fresh fruits of *K. Africana* (Lam.) Benth (Fam. Bignoniaceae) and *S. bicolor* stalks (L) Moench (Fam. Poaceae) were bought from

different sellers in the Mushin market in Lagos suburb, Nigeria, in November 2014. The fruits and stalks were identified and authenticated by the Department of Botany, Faculty of Science, University of Lagos, Nigeria. The specimens were given voucher no LUH 6487 (*K. africana*) and no LUH 6488 (*S. bicolor*) respectively and were deposited in the Department's Herbarium. The plant materials were washed with a copious amount of clean tap water and spread to drain, then cut into small pieces and dried in an oven at a temperature of 45^oC for seven days.



Fig. 1. *Kigelia africana* fruit (Pandoro in Yoruba and Ohi in Ibo Language)

2.2 Preparation of Extracts

The dried materials were pulverized to a coarse powder with an electric grinder. The powdered materials of *K. Africana* fruits (3200 g) and *S. bicolor* stalks (3150 g) were macerated with 25 liters of hydroethanol (2:8) respectively and allowed to stand for seven days, with regular stirring. The extracts were clarified by filtration (Sofowora, 1993) using What man no.4 filter paper. They were then concentrated using a rotary evaporator then dried in a laboratory oven (45°C) to a dry weight of 243.12 g (7.60 %w/w yield) for *K. africana* and 174.94 g (5.60 %w/w yield) for *S. bicolor*, respectively.

2.3 Phytochemical Screening

The dried extracts of *K. africana* and *S. bicolor* were respectively tested for the presence of various phytochemicals – alkaloids, tannins, saponins, flavonoids, terpenoids, and cardiac glycosides according to established methods [15].

2.3.1 Techniques for detection of phytochemical groups in extracts

i. Tests for Alkaloids

- A. Mayer's reagent test: Few drops of Mayer's reagent (Potassium mercuric iodide solution) were added to 2 ml of extracts (0.1 % w/v acidic solution), and a cream precipitate showed the presence of alkaloids [16].
- B. Dragendorff reagent test: 3 drops of Dragendorff's reagent (Potassium bismuth iodide) was added to 2 ml of extracts (0.1 % w/v acidic solution), and an orange brick precipitate appeared, showing the present of alkaloids [16].
- C. Wagner reagent test: Few drops of Wagner reagent (I₂ (sublimed) in KI) were added to 2ml of the extracts (0.1 % w/v acidic solution), and a brown precipitate appeared, showing the presence of alkaloids [16]
- ii. Test for Tannins

Ferric chloride test: 0.5 g of the extracts were boiled in 20 ml of distilled water in a test tube and filtered. 0.1% $FeCl_3$ was added to the filtered samples, and observed was a blue-black coloration which shows the presence of tannins [17]

iii. Test for Phenols

Ferric chloride test: About 500 mg of the extracts was dissolved in 5 ml of distilled water. To this, few drops of neutral 5% ferric chloride solution were added. A dark green colour indicated the presence of phenols. [18]

- iv. Test for Flavonoids
 - A. The sulphuric acid test: To the extract (0.2% w/v), a few drops of concentrated H₂SO₄were added; flavones and flavonols were dissolved, producing a deep yellow coloured solution, Chalcones and aurones had a red-bluish solution. Flavanones gave orange to red colors.[15,19]
 - B. Shinoda test: Magnesium ribbon piece was added to the extract's ethanolic solution, followed by a few concentrated HCI drops. Flavones, flavonols, the corresponding 2, 3-dihydro derivatives, and xanthones produced orange colour with this test.[15]
- v. Test for Terpenoids
 - A. Liebermann Burchard test: A combination of 1mL acetic anhydride and

1mL of chloroform (CHCl₃) was cooled to 0° C, and one drop of concentrated H_2SO_4 was added. The sample was dissolved in CHCl₃, a greenish blue colour was observed with maximum intensity in 30 mins, indicating the presence of terpenoids.[15]

- B. Saponins: To 10 ml of the plant extract, 3 ml of distilled water was added and shaken well to obtain froth. To the froth formed, a few drops of olive oil were added. The formation of emulsion indicated the presence of saponins.[15]
- vi. Cardiac Glycosides
- A. Kedde test:

Solution I: 2% of 3, 5-dinitrobenzoic acid were dissolved in methanol.

Solution II: 5.7% aqueous of Potassium Hydroxide.

Procedure: One drop of each solution was added to 0.4 mL of the sample solution, and a bluish to purple color appeared after 5 min. the solution did not contain acetone, which gives deep bluish color.[15,19]

B. Keller – Kiliani test

To 5 ml of the aqueous extract, 2 ml of glacial acetic acid containing a drop of Ferric chloride was added. This was followed by the addition of 1 ml of concentrated sulphuric acid. The brown ring, thus obtained, yield a positive result for the test [20].

2.3.2 Gas Chromatography-Mass Spectrometry analysis (GC-MS)

GC-MS analysis of the extracts was performed using GCMS-QP2010SE SHIMADZU system. The charged fragments were detected, and the subsequent spectra obtained were used to identify the molecule. Helium gas was used as the carrier gas with a constant flow rate of 1 ml/ min with an injection volume of 2 µl (split ratio of 10:1) and injection temperature of 25°C. The oven temperature was programmed from 60°C -(10°C/min) to 160°C (2 min) - (15°C/min) to 400°C (4 min). The column type DB-5ms was 30 m x 0.25 µm x 0.25 mm. The gaseous compounds analyzed interact with the column walls, which is coated with different stationary phases. This causes different compounds to elute at a different time, known as the compound's retention time. Identification was based on molecular structure, molecular mass, and calculated fragments. Interpretation on mass

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spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST), having more than 62,000 patterns. The name, molecular weight and structure of the components of the test materials were ascertained. Each component's relative percentage amount was calculated by comparing its average peak area to the total areas. The spectrum of the unknown component was compared with the component stored in the NIST Library [21].

3. RESULT

The results obtained in this study are shown in Tables and Figures below.

Table .1 Shows the result of the phytochemical screening of *K. Africana* fruit extract and *S. bicolor* stalk extract, which revealed the presence of five compounds, namely alkaloids, tannins, phenols, flavonoids, and saponins in *K. africana*, while in *S. bicolor* only four compounds namely tannins, phenols, flavonoids, and saponins were present.

Table 2 shows the GC-MS analysis of hydroethanolic fruit extract of *K. Africana* with the identification of phytochemical constituents confirmed based on peak area %, retention time, molecular formula and molecular weight, with 9, 12-Octadeadienoic acid (Z, Z) found as the major compound.



Fig. 2. Sorghum bicolor leaves stalks (local name: Poroporo in the Yoruba Language)

Table 1. Results of the phytochemical screening of K. africana fruit and S. bicolor stalk
extracts

	Tests	KA	SB
1	Alkaloids		
	✓ Mayer's Reagent	+	_
	 Dragendroff's Reagent 	+	_
	✓ Wagner's Reagent	+	_
2	Tannins		
	✓ Ferric chloride test	+	+
3	Phenols		
	✓ Ferric chloride test	+	+
4	Flavonoids		
	✓ Sulphuric acid test	+	+
	 Shinoda test 	+	+
5	Terpenoids		
	✓ Liebermann- Buchard test	+	+
	 ✓ Saponins (froth test) 	+	_
	✓ Cardiac glycosides	_	_
	✓ Kedde test	_	_
	✓ Keller –kiliani test	_	_

+ Present; – Absent; KA = Kigelia africana; SB= Sorghum bicolor

Table 3 shows the phytochemicals identified through GC-MS analysis of hydroethanolic stalk extract of *S. Bicolor* with relevant biological activities listed with 13-Docosenoic acid, methyl ester, (Z) as the major compound.

The chromatogram of K; africana revealed fortyfour (44) phytochemical constituents that could contribute to the plant's medicinal quality.

The chromatogram of *S. bicolor* revealed twentynine (29) phytochemical constituents that could contribute to the medicinal quality of the plant.

4. DISCUSSION

Herbal medicine has received greater attention as an alternative to orthodox medicine in recent times, leading to a subsequent increase in herbal medicine preparations [3,22]. In rural communities, the exclusive use of herbal medicines prepared with single or combinations of different plant species parts and dispensed by herbalists without formal training for the management of various diseases is still a widespread practice. This reauires that experimental research be conducted to ascertain these herbal products' safety and efficacy and establish their active components [23]. Herbal medicines, also since the prehistoric era, have been recognized and acknowledged to be effective in the treatment of both pathological and pathogenic diseases. Their use in the treatment of certain conditions where orthodox drugs could only serve as palliatives is widespread now [24].

Herbal medicines' pharmacological and physiological activities could be attributed to

myriads of various classes of secondary products present in them. They could be employed in the treatment of various disease states. The phytochemical screening carried out on the two extracts revealed the presence of phenols, flavonoids, and saponins as the chemical constituents present in both extracts as well as (alkaloids in K. africana only). Although, Inspection of the published reports[25] revealed а significant variation in the chemical composition of K. africana fruits, the constituents identified in this study aggress with a previous study done in Nigeria[6] where the constituents were essentially the same. It is important to note that most of the constituent isolated, which were plant constituents such as polysaccharides, polypeptides, glycopeptides, triterpenoids. steroids, xanthones, flavonoids, coumarins, phenols, iridoids, alkyl disulphides, inorganic ions, and guanidine, has been reported to exhibit antioxidant properties and as well are associated with antidiabetic activities [26]. Other workers [10] also reported the presence of phenyl propanoid identified as 6-p-coumaroyl-sucrose phenylethanoid and flavonoids glycosides in the fruits of K. africana. Flavonoids and phenols have since been associated and reported to have measurable antioxidant activities [27].

Similarly, the five major compounds isolated from *S. bicolor* agreed with the works of Kwon and Kim [28],who also isolated Five major compounds, namely methyl ferulate (1), methyl-hydroxycinnamate (2), p-hydroxybenzaldehyde (3), tricin (4), and quercetin 3,4'-dimethyl ether (5). Among these compounds, methyl ferulate exhibited an intense, free radical scavenging activity. The stalks were also found to contain

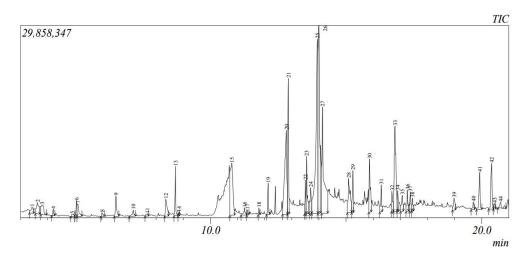


Fig. 3. GC-MS Chromatogram Analysis of K. africana crude extract

Table 2. Major components in hydroethanolic fruit extract of K. africana by GC-MS analysis

RT	Compound name	Peak Area %	Molecular Formula	Molecular Weight	Compound nature
13.943	9, 12-Octadeadienoic acid (Z, Z)	17.03	C ₁₈ H ₃₂ O ₂	280	Linoleic acid
12.798	n-Hexadecanoic acid	8.18	$C_{16}H_{32}O_2$	256	Palmitic acid
12.872	Hexadecanoic acid, ethyl ester	4.99	C18H36O2	284	Ester compound
3.787	Glycerin	1.09	C3H8O3	92	Alcoholic compound
12.128	1H-2-Benzopyran-1-one, 3,4-dihydro-6,8- dihydroxy-7-methoxy-3-methyl-	1.02	$C_{11}H_{12}O_5$	224	Flavonoid fraction
7.156	Eugenol	0.43	$C_{10}H_{12}O_2$	164	Phenyl propanoids
11.784	1H-2-Benzopyran-1-one, 3,4-dihydro-8- hydroxy-6-methoxy-3-methyl-,	0.22	C ₁₁ H ₁₂ O ₄	208	Flavonoid fraction

Table 3. Major components in hydroethanolic stalk extract of S. bicolor by GC-MS analysis

RT	Compound name	Peak Area %	Molecular Formula	Molecular Weight	Compound nature
15.801	13-Docosenoic acid, methyl ester, (Z)-	18.95	$C_{23}H_{44}O_2$	352	Erucic acid
12.388	Hexadecanoic acid, methyl ester	13.54	C ₁₇ H ₃₄ O ₂	270	Fatty acid ester
14.724	cis-11-Eicosenoic acid, methyl ester	11.93	$C_{21}H_{40}O_2$	324	Omega 9 fatty acid
12.841	Hexadecanoic acid, ethyl ester	0.63	$C_{18}H_{36}O_2$	284	Ester compound
9.123	Dodecanoic acid, methyl ester	0.34	$C_{13}H_{26}O_2$	214	Lauric acid

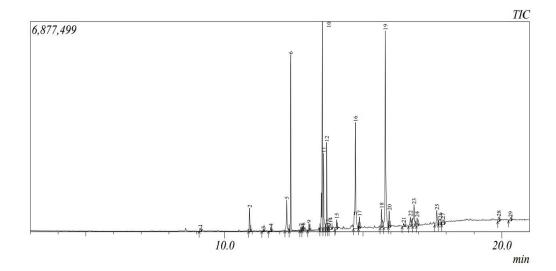


Fig. 4. GC-MS Chromatogram Analysis of S. bicolor crude extract

butyric-, formic-, myristic-, palmitic-, and stearicacids, maltose, emulsine, and were also rich in vitamin B. This study confirmed the presence of flavonoids and polyphenolic derivatives, suggesting that the plant extract is endowed with antioxidant properties, as has been previously reported [7,28].

Furthermore, the GC-MS analysis revealed the presence of forty-four (44) phytoconstituents in K. Africana and twenty-nine (29) in S. bicolor with the identification of phytochemical constituents confirmed based on peak area %, retention time, molecular formula, and molecular weight. The most abundant phytoconstituents in K. Africana obtained from this study were Linoleic acid, Palmitic acid, Ester compound, Alcoholic compound, Flavonoid fraction Phenylpropanoids. It is of note that most of these compounds have been reported to have significant medical importance. Linoleic plays a unique role in heart health support, as shown in Randomized clinical trials[29]. Also, palmitoleic acid (POA) has demonstrated anti□inflammatory and lipidlowering effects in preclinical and human epidemiological and intervention trials [30]. Organic esters are used in the manufacture of painkillers e.g. Aspirin while Alcoholic compounds in their various forms can be used as an antiseptic, disinfectant, and antidote [31].On the other hand, flavonoid fraction has been documented as an oral phlebotropic drug [32] and Phenyl propanoids for medicinal use as an antioxidant, UV screens, anticancer, anti-virus, anti-inflammatory. wound healing. and antibacterial agents [33].

Likewise, the most abundant phytoconstituents in S. bicolor stalk obtained from this study were Erucic acid, Fatty acid ester, and Omega 9 fatty acid. Ester compound and Lauric acid. Every one of these compounds has also been reported to play a critical role in medical science. hexadecanoic acid ethyl ester has the same functions outlined in Κ. africana. as hexadecanoic acid methyl ester has been reported as an antioxidant, 5-Alpha reductase inhibitor, as hypocholesterolemic[21], cis-11-Eicosenoic acid methyl ester as an antiinflammatory, antioxidant, antiarthritic[34] then dodecanoic acid methyl ester as an antioxidant, antibacterial and antiviral [35].

5. CONCLUSION

The hydroethanolic extracts of *K. Africana* fruit and *S. bicolor* stalks, African indigenous plant from Lagos Nigeria, possess different Phyto components of medicinal importance and biological action potential therapeutic properties. There is a need for more standardization and purification of these herbal formulations for the treatment of diseases.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The College of Medicine, University of Lagos Health Research Ethics Committee approved the study (Ethical Approval No: CM/HREC/10/16/101)

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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