



Characterization and Determination of Antioxidant Activity of *Kigelia* (*Kigeliaafricana*, L.) Seed oil

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Abstract

The objectives of this study were to investigate the physicochemical characteristics and antioxidant activity of *Kigeliaafricanaseed* oil. The seeds were cleaned, crushed and the oil extracted by n-hexane. The physicochemical properties were determined according to the American Oil Chemists Society (AOCS) official methods. The fatty acid composition of the oil was determined by GC-MS, using the fatty acid methyl esters. Also the antioxidant activity was determined by DPPH assay. The results showed that the oil content was 17%; moisture content was 5.1%, crude fiber was 13.43%, crude protein was 45.12% and ash was 4.25%. The physicochemical properties were: the refractive index, color, free fatty acids, acid value, peroxide value, anisidine value, saponification value, unsaponifiable matter were found to be (1.47, red:3.4-yellow:70, 14.8%, 26.3mg/g, 22.8mg/kg, 10.05, 196.4mg/g and 1.03) respectively. The GC-MS showed that the total saturated fatty acids 29.74 %, (palmitic acid 14.93%, stearic acid 9.56%, arachidic acid 2.67% and behenic acid 1.28%) and the unsaturated fatty acids were 69.11% (linoleic acid 23.18% and linolenic acid 44.2%). The antioxidant activity of *Kigeliaafricanaseed* oil was 19%.

Keyword: *Kigeliaafricana*, Physicochemical, Antioxidant Activity, Seed oil.

Introduction

Almost half of all drugs in clinical use in the world find their origin from natural products and their derivatives. According to World Health Organization estimates, about 80 percent of people living in developing countries rely on wild plants for some part of their primary health care (Siddiquiet *al.*, 2015). One of these plants is *Kigelia africana*. It is commonly known as cucumber or sausage tree and belongs to the family Bignoniaceae, and is widely distributed in Southern, Central and Western Africa. In Sudan *Kigelia* is distributed in central and Southern states (El Gazaliet *al.*, 2004). The tree has been introduced to some countries of South-East Asia like India, Pakistan, China, Philippines and Iraq where it is mainly cultivated as an ornamental tree. Also, it is domesticated in Miami, Florida, where it is grown as a flamboyant tree and it provides shade from the scorching sun for the inhabitants (Bello *et al.*, 2016). *Kigelia* possesses a long history of use by rural communities, particularly for its medicinal properties. These properties are found in every part of the tree, including fruit, bark, and roots, which are employed for medical purposes (Saini *et al.*, 2009). The leaves are not one of the parts of the plant which feature prominently in its traditional use. There are some uses which are widespread but some are more localized. The use of the stem bark extract for curing dysentery and



for sexually-transmitted diseases such as syphilis is reported from many different areas. Another common use of the bark is as a powder or a water macerate to treat sores or fungal infections on the skin (Houghton, 2002). In Southern Africa, the bark is used for ulcers, pneumonia and toothache. It is also,used for curing malaria, diabetes, worm infestations, venereal diseases, convulsions, and as antidote for snakebite. While the fresh fruit is toxic and inedible to humans, it is believed to be a remedy for essentially all gynecological complications (Bello *et al.*, 2016). Also, the fruits are extensively used, probably because of their shape and size, for a large number of reasons associated with indigenous religious and religious-medical practices (Houghton, 2002). Sausage tree trunk makes good shelves, fruit boxes and dugout canoe used for fishing. Besides it is use as a traditional medicinal plant, it is also used as fodder for animals. In this research we attempt to characterize the physiochemical properties of seed oil and identify its chemical constituents and antioxidant activity.

Materials and Methods

Sample collection

The samples of Kigelia seed were kindly provided by Dr. Adil A. Abdullah, National Center for Research, Khartoum, Sudan.

Oil extraction

The fixed oils were obtained using the soxhlet extraction method. Ten grams of Kigelia seeds powder was taken into a thimble after grinding and drying in oven at 103 °C for one hour. A 500 ml soxhlet apparatus and n-hexane as solvent were used. A round bottom flask containing known volume (500ml) of hexane were fixed to the end of the apparatus and a condenser was tightly fixed at the bottom end of the extractor in a heating mantle for six hours followed by solvent removal with rotary evaporator. And the oil content was determined as the ratio of the weight of the extracted seed oil to the weight of the oilseed powder sample as described below:

$$\text{oil content\%} = \frac{\text{Weight of oil}}{\text{Weight of sample}} \times 100$$

Characterization of seed oil

The proximate analysis and physiochemical properties of Kigelia seed oil were determined according to the American Oil Chemists' Society (AOCS) official methods, 2004.

Fatty acid composition

Preparation of fatty acid methyl ester (FAME)

Two ml of Kigelia seed oil were taken in a test tube, 7 ml of alcoholic NaOH were added (prepared by dissolving two grams of NaOH in 100 ml methanol) and 7 ml of alcoholic H₂SO₄ 1% (prepared by mixing one ml conc H₂SO₄ + 99 ml methanol) and shaken by vortex for three minutes, all the contents were left overnight. Two ml of saturated NaCl, two ml of n-hexane were added, shaken for three minutes, n-hexane layer was collected. 5 µl of collected hexane layer were taken and diluted with 5ml diethyl ether, one gram of sodium sulphate was added as drying agent, filtered through syringe filter 0.45 µm and filtrate was transferred directly to the GC-MS vial.

GC-MS method for analysis of FAME

Fatty acid composition was determined at the Medical University of Science and Technology, Khartoum. The GC-MS analysis of the extracted oils was performed using GC-MS model (GC.MS-QP22010 Ultra), Shimadzu company, Japan with (Rtx-



5MS) silica capillary column stationary phase, film thickness 0.25 μm , Length 30m and internal diameter 0.25 m. the GC oven initial temperature was 60 $^{\circ}\text{C}$ and increased to 300 $^{\circ}\text{C}$ at rate of 10 $^{\circ}\text{C}/\text{min}$. Carrier gas was Helium at flow rate of 50ml/min and the sample was injected in split mode. The GC coupled to mass selective detector transfer line heater maintained at 250 $^{\circ}\text{C}$, and ion source temperature 200 $^{\circ}\text{C}$. Identification of compounds was based on comparison of the relative retention time and mass spectral data and NIST mass spectral library of the GC-MS.

Determination of antioxidant activity

The radical scavenging assay was performed to study the antioxidant activity potency of oil extract from *Kigelia*. This method was based on the reduction of 2,2-Di (4-tert-octylphenyl)-1-picryl-hydrazyl (DPPH) in absolute ethanol solution, in which color is changed from pale green to purple due to formation of DPPH-H (reduced form) (Shimada et al., 1992). The concentration of DPPH was kept as (300 μM), and the test samples were dissolved in Dimethyl sulfoxide (DMSO). The free DPPH was measured spectrophotometrically at 517nm wave length. The percentage of radical scavenging activity (RSA) was calculated as shown in equation below:

$$\text{RSA\%} = \frac{\{\text{Control} - (\text{sample} - \text{Blank})\} * 100}{\text{Control}}$$

Results and Discussion

Proximate Analysis

The proximate analysis of *Kigelia* seed included moisture content, oil content, crude fiber, crude protein and ash content are presented in table (1). The oil content of *Kigelia* was much lower than the findings of Chivandi et al, (2011) who reported that the oil content of *Kigelia* was 49.22%. The fiber and protein content of *Kigelia* seed were 13.34% and 45.12% respectively, higher than those reported by Chivandi et al., (2011) as 8.77% and 35.73% respectively. A similar result was previously reported by Chivandi et al., (2011) who stated that the ash content of *Kigelia* seeds was 4.25%.

Table (1): The proximate analysis of *Kigelia africana* seed

No.	Name of test	Result
1	Moisture Content	5.1%
2	Oil Content	17%
3	Crude Fiber	13.34%
4	Crude Protein	45.12%
5	Ash Content	4.25%

The physiochemical properties of *Kigelia* seeds oil are presented in table (2). The color and refractive index of oil were Red: 3.4-yellow: 70 and 1.47 at 20 $^{\circ}\text{C}$ respectively. The free fatty acids were 14.8%. The acid value was 26.3mg KOH/g. The peroxide value was 22.8meq/kg. The total oxidation was 55.8. The saponification value was 196.4mg/g and the unsaponifiable matter was 1.03%.

Table (2): The physiochemical properties of *Kigelia* seed oil

No.	Name of test	Result
1	Refractive Index	1.47
2	Color	Red: 3.4 Yellow: 70
3	Free Fatty Acids	14.8%



4	Acid Value	26.3mg\g
5	Peroxide Value	22.8mg\kg
6	Anisidine Value	10.05
7	Total Oxidation	55.8
8	Saponification Value	196.4mg\g
9	Unsaponifiable Matter	1.03

Table (3): The fatty acids profile of *Kigelia* seed oil by GC-MS

Fatty acids	Retention time	Area %	Structure
Saturate			
Myristic acid	14.181	0.07	C15H30O2
Pentadecanoic	15.3	0.07	C16H32O2
Palmitic acid	16.40	14.93	C17H34O2
Margaric acid	17.4	0.23	C18H36O2
Stearic acid	18.4	9.56	C19H38O2
Arachidic acid	20.26	2.67	C21H42O2
Heneicosanoic acid	21.13	0.10	C22H44O2
Behenic acid	21.9	1.28	C23H46O2
Lignoceric acid	23.5	0.83	C25H50O2
TSFA	-	29.74	-
MONOUNSATURATED			
7-hexadecenoic acid	16.17	0.08	C17H32O2
palmitoleic acid	16.19	0.29	C17H32O2
Cis-10-heptadecenoic acid	17.2	0.14	C18H34O2
Cis-11-Arachidic acid	20.06	0.45	C21H40O2
TMUSFA	-	0.96	-
POLYUNSATURATED			
9,12-Linoleic acid	18.1	23.18	C19H34O2
9,12,15-Linolenic acid	18.2	44.2	C19H32O2
γ.Linolenic acid	19.9	1	C19H30O2
11,14,17-Arachidic acid	20.11	0.22	C21H36O2
Cis-11,14-Arachidic acid	20.03	0.51	C21H38O2
TPUSFA	-	69.11	-
Phenol	21.27	0.19	C23H32O2

The GC-MS analysis of the *Kigelia* seed oil was carried out to know the composition of the oil, A total of 18 fatty acids were found in oil. There were 9 saturated fatty acids (SFA): myristic acid (C14:0), pentadecanoic acid (C15:0), palmitic acid (C16:0), margaric acid (C17:0), stearic acid (C18:0), arachidic acid (C20:0), heneicosanoic acid (C21:0), behenic acid (C22:0), and lignoceric acid (C24:0). Samples contained 9 unsaturated fatty acids (USFA), out of which 4 were monounsaturated fatty acids (MUFA): palmitoleic acid (C16:1), 7-hexadecenoic acid (C16:1), cis-10-heptadecenoic acid (C17:1), 10-eicosenoic acid (C20:1). The 5 polyunsaturated fatty acids were linoleic acid (C18:2), linolenic acid (C18:3), γ-linolenic acid (C18:3), cis-11,14-eicosadienoic acid (C20:2) and 11,14,17-eicosatrienoic acid (C20:3). The common percentage fatty acids in *Kigelia* seed oil are linolenic acid (C18:3, 40 - 60%), linoleic acid (C18:2, 15 - 25%), stearic acid (C18:0, 1 - 6%) and palmitic acid (C16, 5 - 8%).(Chairman and Maury., 2006). In general, the total concentration of saturated, monounsaturated, and polyunsaturated fatty acids was 30%, 1% and 69% respectively (Table 1 and Fig.1).

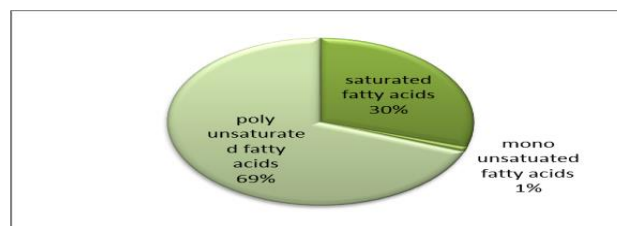


Figure (1) Total Saturated (SFA), Monounsaturated (MUFA) and Polyunsaturated Fatty Acids (PUFA)

Antioxidant activity

The percentage of antioxidant activity of *Kigelia* seed oil was assessed by DPPH free radical assay and found to be 19%. According to Atolani, et al., (2011), the highest antioxidant activity was found in the bark extract (67.33%) while leaves extract had the lowest (59.66%). It was also found that the ethyl acetate fraction of the plant root has higher antioxidant activity at (0.25 mg/mL) against DPPH than the hexane and methanol extract.

Conclusions

Studies on *Kigelia* have emphasized on the ethno medicinal and pharmacological potential of the root, bark, leaf and fruit extracts but have neglected seeds despite being potential sources of macro- and micronutrients, oils and protective phytochemicals. The exploitation of seed as food and feed ingredients justified our evaluation of the nutritive potential by determining the seed proximate, mineral, amino acid, vitamin E, fiber content and by profiling the fatty acid content of the seed oil. It can be concluded that the *Kigelia africana* seeds oil content is 17%. Also, the fatty acid composition of *Kigelia africana* seed oil consists of saturated fatty acids mainly, palmitic 14.93 % and stearic 9.56%. The unsaturated fatty acids were mainly linoleic 23.18 % and linolenic 44.2%. It should be used in industrial application as bio lubricant and bio fuel feed stock.

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