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Khaya senegalensis seed: Chemical characterization and potential uses

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ABSTRACT

This study was done to characterize the seed kernel and coat of Mahogany (Khaya senegalensis (Desr.) Juss. A.), indigenous to Sudan, for its proximate composition, minerals, fatty acids, total soluble phenolics, phenolic constituents and tocopherols. The crude fat was 53% in seed kernel and 13% in coat. Oleic was the major fatty acid in seed kernel (79%) and coat (73%). Total soluble phenolics were 2620 mg GAE/100 g DW in seed coat and 920 mg GAE/100 g DW in kernel. The amount of δ-tocopherol was 36 mg/ 100 g DW in seed kernel and 10 mg/100 g DW in coat. These characterizations, in addition to information collected from secondary sources, value the plant seed for a number of potential food, industrial, pharmaceutical and cosmetics uses. However, it is imperative to conduct toxicity and biological studies for any product intended for human or animal use.

Keywords: *Khaya senegalensis*, Proximate composition, Minerals, Fatty acids, Phenolics, Tocopherols.

INTRODUCTION

Mahogany (*Khaya senegalensis* (Desr.) A Juss), belongs to Family *Meliaceae*, is a deciduous tree, 20-30 m high, 9.5 – 15 m long bole and up to 1.5 m in diameter. The tree has a round evergreen crown of dark shiny foliage, pinnate leaves and characteristic round capsules woody fruits [1, 2]. Its fruit is 4-6 cm in diameter and has four to five valves in which up to 6–18 seeds are embedded. Seeds are flat disk-like, 2-2.5 cm long, and weigh 289 g per 1000 seeds. Mahogany occurs in alluvial soils within riverine forests, along seasonal streams and scattered within the high-rainfall savannah.

Mahogany is a multipurpose tree with several environmental, economic and medicinal uses. The tree is famous for its high quality timber which is excellent for furniture, construction, joinery, interior fitting, turnery, plywood and veneers. Also mahogany is very rich in its seed oil content which is reported to be used in some West African countries for cooking, cosmetics and herbal medicine to cure a number of ailments [3, 4, 5]. *Khaya senegalensis* seed oil was mentioned as a potential insecticide in Nigeria [6]. The bark of the tree is extensively used in folk medicine to treat malaria, fever, diarrhea, dysentery and anaemia. Also the bark is used to treat dressing ulcers on the backs of sheep, camels and horses. The flowers are used for treating stomach diseases and as an anti-syphilitic. Furthermore, mahogany is planted as a roadside and an ornamental shade tree.

In the Sudan and many other countries, mahogany is still an underutilized species that has not received the deserved attention for its non-timber products. In this study we aimed to focus on mahogany seed kernel and coat and characterize them for their proximate composition, minerals, fatty acids, total soluble phenolics, phenolic

constituents and tocopherols. Furthermore, we intended to discuss the potential uses of mahogany seed kernel and coat based on findings reported in this or other studies.

EXPERIMENTAL SECTION

Plant material

Mahogany seeds were obtained from National Tree Seed Centre, Forestry Research Centre, Sudan. The seeds were originally collected from El Rashad district (lat. 11° 40` – 11° 55 N` and long. 30° 45` – 31° 25` E), Eastern Nuba Mountains, Southern Kordofan state, Sudan. The area of the seed collection belongs to the low rainfall woodland savanna [7] where the mean annual temperature is about 29.9°C and the mean annual rainfall is 542 mm.

The mahogany seed samples were air dried in shade, mature and free from diseases. Composite sample were milled (using M20 universal grinding mill, IKA work) and the analyses were carried out in triplicates.

Analytical methods

Chemicals and solvents

All reagents used in this study were Sigma-Aldrich products (St. Louis, MO). Alpha-tocopherol and delta–tocopherol standards were 95% and 90% pure, respectively. The purity of fatty acids and phenolic acids standards were > 99%. All the other chemicals were obtained from J.T. Baker (Baker Mallinckrodt, Mexico) and were High Performance Liquid Chromatography (HPLC)-grade. Milli-Qplus purification system (Millipore Corporation, Bedford, MA) was used to prepare HPLC-grade water.

Proximate composition

The proximate composition parameters measured in this study were crude fat, crude protein and crude fiber. These parameters were determined according to AOAC methods [8]. A fat extraction unit (B-810 Soxhlet, Büchi Labortechnik AG, Flawil, Switzerland) was employed for oil extraction using petroleum ether (boiling point 40 - 60°C). Determination of crude protein was done according to Kjeldahl method. A factor equal to 6.25 was used to convert percentage of nitrogen to percentage of crude protein. For crude fiber analyses, filter bag (ANKOM^{2000}) technique was used. A fiber analyzer vessel (ANKOM$^{200/220}$ Fiber Analyzer, ANKOM Technology, NY, USA) was used to perform the samples digestion.

Fatty acids composition

The samples were prepared and analyzed for FAME (Fatty Acid Methyl Ester) as described by Emanuel *et al*. [9]. Briefly, 3 mL of a 3.75 mol L^{-1} NaOH–methanol solution was added to 100 μL of the oil heated in a water bath at 100°C for 25 min and followed by addition of 6 mL of 3.25 mol L^{-1} HCl–methanol and heating in a water bath at 80°C for 10 min. After that, 3.75 mL of a 1:1 mixture of hexane and methyl *tert*-butyl ether was added and the bottom layer was discarded. Finally, 9 mL of 0.3 mol L^{-1} NaOH–water was added and the top layer was used for Gas Chromatograph (GC).

Analyses of FAME were carried out in an HP 5890 series II GC equipped with a split injector and a flame-ionization detector (FID). The injection volume was 0.5 μL and the split ratio was 20:1. The injector temperature was 250ºC and the split ratio was 20:1. The separations were performed on a capillary fused silica column (30 m long, 0.25 mm id. and 0.25 μm film thickness). The column starting temperature was programmed to 180 ºC held for one min and then increased by 1.5ºC/min up to a final temperature of 220ºC which remained for one min. The carrier gas was helium which was supplied at a flow rate of 1.8 mL/min. The auxiliary gas was helium at a flow rate of 22.3 mL/min and 260 ºC FID temperature. For the FID, hydrogen and air flows were 30 and 400 mL min^{-1}, respectively. The peaks were identified by comparing their retention times to those of the FAME standards.

Mineral elements

Concentrations of mineral elements were determined according to the methods described by Gul and Safdar [10] with some modifications. Briefly, the entire needed laboratory wares were first washed with de-ionized water then soaked in 1.0N HNO_3 for 2 h and let to dry. One gram of sample was subjected to an overnight cold digestion with 10 mL of 16N HNO_3 followed with a hot digestion, at about 50-60°C until appearance of white fumes. After cooling to ambient temperature, the aliquot volume was made to 10 mL with 1.0N HNO_3 and filtered. Dilutions of 1:10, 1:100 or 1:1000 with 1.0N HNO_3 were used. Mineral elements were determined using atomic absorption spectrometer (AAnalyst 700 atomic absorption spectrometer, Perkin Elmer, Massachussets, USA).

Total soluble phenolics (TSP) extraction and measurement
The extraction of TSP was done as described by Corral-Aguayo *et al*. [11] with slight modification [12] Briefly, 20 mL of 80% acetone and 2% formic acid (in a ratio of 80% acetone: 20% formic acid) was added to 1 g of sample and homogenized using an Ultra Turrax model T25 (IKA Works, Willmington, NC). The homogenized sample was placed in Bransonic 2510 sonicator (Bransonic Ultrasonic Co., Danbury, CT) for 5 min and then centrifuged using high speed centrifuge HERMLE Z 323 K (LaborTechnik, Wehingen, Germany) for 15 min at 2°C and 19000g. The aliquot of the extract was then filtered through a Whatman no. 1 filter paper (Whatman Inc., Clifton, NJ) and the supernatants were collected. The residues were subjected to re-extraction, repeating the above steps. All supernatants were mixed and concentrated in a rotary evaporator R – 200 (Büchi Labortechnik, Postfach, Switzerland) at 40°C for 45 min. The concentrated samples were diluted with 25 mL of methanol and the volume was completed to 50 mL with HPLC- grade water.

For TSP determination, Folin-Ciocalteu reagent assay was used. The sample extract was diluted by adding HPLC - grade water, in a ratio of 1:10. A 30 µL of the diluted sample was placed in a micro-plate and 150 µL of Folin−Ciocalteu reagent diluted 1:10 followed by 120 mL of Na_2SO_4 (7.5%) were added. The contents were allowed to stand for 3 hrs in the dark at room temperature. The absorbance was measured at 630 nm using Dynex MRX micro-plate reader (Dynex Technol. Chantilly, VA). TSP content was calculated using an equation obtained from standard gallic acid curve and expressed as mg GAE/ 100 g DW (milligrams of Gallic Acid Equivalents per 100 g Dry Weight).

Identification and quantification of phenolic constituents
The identification and quantification of phenolic compounds was done as described by Yahia *et al*. [12]. HP 1100 series HPLC (Hewlett-Packa GmbH, Waldbronn, Germany), equipped with a diode-array detector DAD, at 280 and 320 nm and a 250 × 4.6 mm i.d., 5 µm, X-terra RP 18 column (Waters, Ireland) was employed. For the mobile phase, 1% formic acid/acetonitrile in a ratio 98:2 (v:v), at a flow rate of 0.5 mL/min was used. The phenolic compounds of interest in this study were gallic acid, p-hydroxybenzoic acid, protocatechuic acid and vanillic acids (hydroxybenzoic acids); caffeic acid, chlorogenic acid, cinnamic acid, p-coumaric acid, ferulic acid, 2-hydroxycinnamic acid and sinapic acids (hydroxycinnamic acids); kaempferol and quercetin (flavonols) and catechin and epicatechin (flavan-3-ols). Standards calibration curves were prepared for quantification.

Tocopherols extraction and measurement
Alpha- and delta-tocopherols were analysed in mahogany seed kernel and coat by HPLC. The extraction and determination was performed as described by Yahia and Mondragon-Jacobo [13]. Briefly, a sample weighing 0.5 g was homogenized with 10 mL of methanol, vortexed for 30 sec, incubated in water bath (Reciprocal shaking bath model 25, Precision Scientific Co.) at 30ºC for 15 min and centrifuged using HERMLE Z 323 K (LaborTechnik, Wehingen, Germany) at 5000g and 24ºC for 5 min. A 20 µL of the upper phases of the extracts were filtered using a non-sterile 13 mm Millex syringe filter unit (Millipore Corp., Ireland) and injected into HPLC. A 150 × 4.6 mm i.d., 3.5 µm, Symmetry C18 column (Waters Co., Milford, CT) was employed. For the mobile phase, HPLC- grade methanol (100%) at a flow rate of 0.8 mL/min was used. The stop time was 40 min and the post time was 5 min. The samples were detected for α-and δ-tocopherols employing a model FLD G1321A fluorescence detector (Agilent Technologies Corp., Palo Alto, CA) at an excitation wavelength of 294 nm and emission wavelength of 325 nm. Equations from calibration curves of α-and δ-tocopherols standards were used for quantification. The amounts of tocopherols in the extracts were calculated as mg tocopherols in 100 g dry sample.

Statistical analysis
Statistical analysis was done using StatView statistical program. Results were represented as mean of observations of triplicate samples.

RESULTS AND DISCUSSION

Proximate composition and mineral contents
As shown in Table 1, high contents of fat (53%) and protein (30%) were reported in mahogany kernel. Mahogany seed coat had 13% fat and 4.5% protein. Oil content in the whole mahogany seed reaching 67% was reported [2, 14]. Mahogany seed exhibited paramount properties that may qualify it for several utilizations. The high content of oil and protein might have applications in food if verified free from antinutrient components. In W. Africa the utilization of mahogany seed oil for cooking was reported among local people [2]. Refractive index (1.46),

saponification (195.58 mg KOH/g) and iodine (88.4 g/100 g oil) values of mahogany seed oil falling within the range for edible oils were reported [5]. Another potential reported use was the possibility of using mahogany seed oil as a biodiesel fuel oil [15]. As reported by Aliyu *et al*. [15], the basic properties of the synthesized mahogany biodiesel were in conformity with ASTM D6751-06 standard for biodiesel fuel (B100).

Table 1: Proximate composition (%) and mineral concentrations (mg/100 g DW) of *Khaya senegalensis* seed kernel and coat

Analyses	Mahogany Seed kernel	Mahogany seed coat
Proximate composition		
Crude Fat %	53.17 ± 0.12	13.37 ± 0.01
Crude protein %	29.95 ± 1.11	4.56 ± 0.16
Crude fiber %	5.01 ± 0.03	41.17 ± 1.03
Mineral concentrations		
Ca	228.80 ± 3.14	403.78 ± 5.02
Na	5.50 ± 0.15	7.43 ± 0.01
Fe	1.60 ± 0.00	7.97 ± 0.00
Cu	0.89 ± 0.02	0.28 ± 0.00
Zn	0.60 ± 0.00	0.19 ± 0.01
Mn	0.63 ± 0.01	0.55 ± 0.00
Al	1.63 ± 0.00	10.23 ± 0.10
K	927.54 ± 9.14	776.71 ± 6.85
Mg	340.55 ± 3.23	38.96 ± 0.62

In addition to the high oil and protein contents, mahogany seed kernel and coat exhibited good mineral concentrations (Table 1). Ca contents detected in Mahogany seed coat and kernel were more than 400 and 228.8 mg/100 g DW, respectively. Also the content of K reached 927.5 mg/100 g DW in seed kernel and 776.7 mg/100 g DW in coat. The Mg concentrations were 340.5 mg/100 g DW in seed kernel and 38.9 mg/100 g DW in coat. Calcium, sodium and iron had higher contents in seed coat while potassium and magnesium were higher in seed kernel. In comparison to the 18 oil-bearing seeds and kernels studied by Özcan [16], Ca concentration of our mahogany seed coat was comparable with the highest value which was recorded for cumin seed. Also Mg concentration in kernel was higher than that of cotton seed which had the highest Mg values in the study of Özcan [16].

Fatty acids composition

The fatty acid compositions of mahogany seed kernel and coat are summarized in Table 2. Oleic acid was the predominant fatty acid in mahogany seed coat and kernel. Oleic acid percentages reached 79 % in seed kernel and 72% in coat.

Table 2: Fatty acids composition (%) of *Khaya senegalensis* seed kernel and coat

Fatty acid	Mahogany seed kernel	Mahogany seed coat
Saturated fatty acids		
Palmitic acid $C_{16:0}$	5.60 ± 0.06	7.10 ± 0.01
Stearic acid $C_{18:0}$	10.46 ± 0.02	11.39 ± 0.51
Mono-unsaturated fatty acids		
Oleic acid $C_{18:1}$	79.27 ± 1.04	72.92 ± 2.42
Poly-unsaturated fatty acids		
Linoleic acid $C_{18:2}$	4.67 ± 0.05	8.23 ± 0.07
Linolenic acid $C_{18:3}$	ND	0.36 ± 0.01
SFA	16.06	18.49
MUFA	79.27	72.92
PUFA	4.67	8.59
UFA/SFA	5.23	4.41
PUFA/SFA	0.29	0.46

ND = not detected; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acid; UFA= unsaturated fatty acid.

Okieimen and Eromosele [17] reported 21.39% palmitic, 10.41% stearic and 64.62% oleic while Balogun and Fetuga [18] found 11.3% palmitic, 13.8%, stearic acid and 59.4% oleic in mahogany seed oil. In comparison to vegetable oils, content of oleic acid in mahogany kernel exceeded that of olive oil (72.3%) and rapeseed oils (58.5%) which had the highest oleic oil content in the study of Rafalowski *et al*. [19]. The high content of oleic acid measured in mahogany oil can contribute several beneficial properties in food, pharmaceutical and cosmetics. From

health point of view, the presence of oleic acid in a diet reduces low density lipoprotein content [20]. Also oleic acid is effective in preventing the risk of cardiovascular diseases and lowering the insulin body-requirement and the concentration of glucose in plasma [21]. The amount of oleic acid was found positively correlated to the antioxidant capacity of the oil [22]. In addition to medicinal uses, oil rich in oleic can be employed as a bioactive ingredient in cosmetics to enhance skin moisturization [23]. Mahogany oil potential uses include production of lubricants, soaps and personal care products and topical treatments.

Total soluble phenolics and phenolic constituents

As summarized in Table 3, TSP in mahogany seed coat exceeded 2600 mg GAE/100 g DW while mahogany kernel made more than 900 mg GAE/100 g DW. The amount of TSP detected in mahogany seed coat was higher than the amount reported for berries [24]. From the HPLC analyses we identified and quantified eight phenolic acids in mahogany seed coat whereas mahogany seed kernel exhibited three phenolic acids. Mahogany seed coat was predominantly characterized by the presence of a considerable amount of catechin which reached 99 mg/100 g DW. The major phenolic acid detected in mahogany kernel was cinnamic acid amounting to 57.86 mg/100 g DW.

Table 3: Phenolic compounds (mg/100 g DW), TSP (mg GAE/100 g DW) and δ-tocopherol (mg/ 100 g DW) of *Khaya senegalensis* seed kernel and coat

Phenolic compound	Mahogany seed kernel	Mahogany seed coat
Hydroxybenzoic acids		
p-hydroxybenzoic acid	ND	19.43 ±0.62
Vanillic acid	ND	6.94 ±0.05
Hydroxycinnamic acids		
Caffeic acid	ND	14.77 ±1.24
Chlorogenic acid	ND	26.79 ±2.04
Cinnamic acid	57.86 ±1.30	9.43 ±0.73
Flavanols		
Kaempferol	8.84 ±0.27	16.41 ±0.98
Flavan-3-ols		
Catechin	9.95 ±0.82	99.14 ±2.54
Epicatechin	ND	1.42 ±0.01
TSP	920.96 ±7.15	2620.14 ±11.08
δ-tocopherol		36.5± 0.97

ND= not detected

The high phenolic contents in mahogany seed kernel and coat may serve high antioxidant activity. The positive correlation between phenolic content and antioxidant activity was reported earlier by Cai *et al.* [25] who investigated antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Phenolic acids play a natural antioxidant activity role in reducing the risk of chronic diseases and improving human nutrition and health. Also mahogany seed, which possesses high content of cinnamic acid in seed kernel and catechin in seed coat, might has antioxidant properties. The potential of cinnamic acid as cancer chemo-protective bioactive substances was reviewed [26]. Catechin content was found significantly correlated with antioxidant capacity of tea and green tea [27]. In addition, the importance of phenolic compounds for the oxidative stability of the unsaturated fatty acids of plant edible oils was revealed [28]. The accumulation of high content of phenolics in mahogany seed coats might play significant roles in seed longevity and defense against microorganisms as reported from other seed coats [29].

Tocopherols content

As shown in Table 3, only δ-tocopherol, measured at 325 nm, amounting to 36.5 mg/100 g DW in seed kernel and 10 mg/100 g DW in seed coat was detected. In comparison with the study of Manan [30], which investigated tocopherol contents of Pakistani seed oil, our mahogany seed kernel showed a δ-tocopherol comparable to that of soybean which had the highest value. Also mahogany seed coat exhibited δ-tocopherol higher than that of corn, sunflower, olive and sesame seed oil. The δ-tocopherol content of mahogany seed kernel was found higher than the values reported by Nehdi *et al*. [31] for walnut (13.07 mg/100 g), corn (10.74 mg/100 g) and lower than soybean (77.89 mg/100 g). The presence of high tocopherols in mahogany seed is another important property that may has potential in combating abnormalities linked to development of cancer. The preventive activity of δ-tocopherol against colon cancer was extensively discussed in the study of Guan *et al*. [32]. Also another study [33] showed that δ-tocopherol is more active than α- or γ-tocopherol in inhibiting tumor growth in lung.

CONCLUSION

The characterizations reported in this study revealed good quality of mahogany seed kernel and coat. These characterizations qualify mahogany seed for several food, industrial, pharmaceutical and cosmetics potential applications which would enhance the socioeconomic role of the species non-wood products. However, it is imperative to conduct toxicity studies for any product intended for human or animal use. Further studies on determination and treatment of anti-nutritional substances are needed. Also biological studies are needed to investigate the discussed potential uses.

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