African Journal of Biotechnology Vol. 6 (7), pp. 894-896, 2 April 2007
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2007 Academic Journals

Short Communication

Physico-chemical characterization and cytotoxicity studies of seed extracts of *Khaya senegalensis* (Desr.) A. Juss.

R. G. Ayo[1], O. T. Audu[2] and J. O. Amupitan[2]

[1]Samaru College of Agriculture, Division of Agricultural Colleges, Ahmadu Bello University, Zaria, Nigeria.
[2]Department of Chemistry, Ahmadu Bello University, Zaria, Nigeria.

Accepted 22 February, 2007

The seeds of *Khaya senegalensis* were extracted using petroleum ether and methanol with the aim of determining the physico-chemical properties of the seed oil and the cytotoxicity of the two extracts. The refractive index of the oil was found to be 1.458, while the relative density was 0.953. The iodine and saponification values were 88.40 and 195.58, respectively. The peroxide and acid values were 4.6 and 2.69, respectively. Brine shrimp lethality bioassay of petroleum ether and methanol extracts of the seeds showed that the extracts were moderately cytotoxic at high concentration. The LC_{50} values using Probit analysis method for petroleum ether and methanol extracts were 827.39 and 51.79 µg/ml, respectively. Therefore, the seed oil of *K. senegalensis* was found to be non-drying oil and the extracts from the seeds may contain bioactive compounds of potential therapeutic and prophylactic significance.

Key words: *Khaya senegalensis*, Meliaceae, physico-chemical characteristics, seed oil, cytotoxicity.

INTRODUCTION

Khaya senegalensis (Desr.) A Juss (Family: Meliaceae) is a dry-zone mahogany tree, widely distributed in the sub-Sahara savannah from Senegal to Sudan and Uganda. It is recognized by its round evergreen crown of dark shiny foliage, pinnate leaves and characteristic round capsules (Irvine, 1961; Keay et al., 1989). The bark of the tree is very bitter and is extensively used for the treatment of fever and dressing ulcers on the backs of sheep, camels and horses. The flowers are used for treating stomach diseases, and as an ingredient in antisyphlytic prescriptions. The oil from the seeds is used as emmenegogue (Dalziel, 1956; Watt and Breyer-Brandwijk, 1962). Some limonoids have been isolated from the stems, barks, leaves and flowers of *K. senegalensis*. They include phragmalin limonoids named khayanolides D and E, khayanosides, 2,6-dihydrofissinolide and two mexicanolides named khayanone and 2-hydroxyseneganolide (Olmo et al., 1997; Khalid et al., 1998; Nakatani et al., 2001, 2002). Abdelgaleil et al. (2004) also reported the isolation of three other mexicanolide limonoids named seneganolide A, 2-hydroxyseneganolide A and 2-acetoxyseneganolide A. These limonoids have a wide range of biological activities, including insect antifeeding and growth-regulating properties, and medicinal activities in humans and animals. They also possess antiviral, antifungal and bactericidal properties (Abdelgaleil et al., 2001; Abdelgaleil and Nakatani, 2003; Ademola et al., 2004). Traditional healers in some parts of Northern Nigeria use the seed oil to treat diabetes mellitus.

The aim of the present study was to determine the physico-chemical properties of the seed oil from *K. senegalensis* and the cytotoxicity of the extracts of the plant seeds.

MATERIALS AND METHODS

Plant material

The trees of *K. senegalensis* were identified within the premises of the Institute for Agricultural Research, Ahmadu Bello University, Zaria (11^0 10′N, 07^0 38′E), Nigeria. The plant was identified at the Herbarium of the Department of Biological Sciences, Ahmadu Bello University, Nigeria. A voucher specimen (Voucher Specimen Number 900181) has been deposited at the Herbarium of the Depart-

*Corresponding author. E-mail: gbekeayo@yahoo.com. Tel: +2348036371606.

ment. The seeds of the tree were harvested, air-dried at room temperature, powdered and stored in screw-capped plastic containers.

Extraction procedure

Soxhlet extraction of the powdered seeds (250 g) was carried out using petroleum ether (40 – 60°C) and methanol. Each extract was concentrated and evaporated to dryness on a rotary evaporator.

Physico-chemical characterization

The physical and chemical properties of the seed oil (petroleum ether extract), including iodine, saponification, acid, peroxide values, refractive index, density and unsaponifiable matter were determined according to standard procedures (Pa Qurt, 1979; AOAC, 1980; Pearson, 1981).

Brine shrimp lethality bioassay (cytotoxicity)

The brine shrimp lethality bioassay was carried out on the petroleum ether and methanol extracts using standard procedure (Meyer et al., 1982; McLaughlin, 1991). Briefly, brine shrimp (*Artemia salina* Leach) eggs were hatched in a hatching chamber filled with fresh sea water. The chamber was kept under illumination using a fluorescent bulb for 48 h for the eggs to hatch into shrimp larvae. 30 mg of each extract were separately dissolved in 3 ml of DMSO, and from these 1000, 500, 250, 125 and 62.5 µg/ml were prepared by serial dilution. Each concentration was tested in triplicate, giving a total of 15 test-tubes for each sample. A control containing 5 ml of DMSO solvent was used for each solvent. The final volume of the solution in each test-tube was made up to 5 ml with sea water immediately after adding shrimp larvae. The test-tubes were maintained under illumination. Survivors were counted after 24 h and the percentage death at each dose was determined. The LC_{50} values were calculated using the Probit analysis method (Sauders and Fleming, 1971).

Table 1. Physico-chemical characteristics of seed oil (petroleum ether extract) of *Khaya senegalensis*.

Properties of Oil	Values
Colour	Golden yellow
Refractive index (21 ^{0}C)	1.458 ± 0.001
Relative density	0.053 ± 0.002
Iodine value (g/100g)	88.40 ± 0.058
Saponification value (mg KOH)	195.58 ± 0.044
Acid value (mg KOH)	2.69 ± 0.015
Unsaponifiable matter (%)	1.50 ± 0.058

RESULTS AND DISCUSSION

The weights of the petroleum ether and methanol extracts were 71.25 and 82.50 g, respectively. The petroleum ether of the extract contained the seed oil. The results of the physical and chemical properties of the oil are shown in Table 1. The iodine value was 88.40. Since the value was lower than 100, the oil was classified as non-drying oil. Iodine value measures the unsaturation of oil. The fact that the iodine value was lower than 100 showed that the seed oil was of lower degree of saturation. In general, the greater the iodine value, the higher the degree of unsaturation and the tendency of the oil to undergo oxidative rancidity (Pa Quart, 1979; Pearson, 1981).

The saponification value is an indication of the average molecular mass of fatty acids present in oil. The results showed that the seed oil had a high saponification value of 195.58, indicating that it contained mainly fatty acids of high molecular mass. This result compared favourably with saponification values of palm oil (196–205), olive oil (185–196), soy oil (193), cotton seed (193–195) and linseed oil (193–195) as reported by Pearson (1981). The acid value of the oil, found to be 2.69, is a measure of the extent to which the constituent glycerides have been decomposed by lipase action. The acid value has been shown to be a general indication of the edibility of oils (AOAC, 1980; Pearson, 1981). The peroxide value was found to be 4.60 mEq/kg. The peroxide value is frequently used to measure the progress of oxidation of oil. It indicates the oxidative rancidity of oil. The low peroxide value for this oil indicates that it is less liable to oxidative rancidity at room temperature (deMan, 1992).

The refractive index of *K. senegalensis* seed oil was 1.46, which showed that the value fell within the range for edible oils. Edible oils have iodine value of less than 100 and a refractive index of about 1.47 (Williams and Hilditch, 1964; Rossell, 1991). Refractive index is a useful property in the preliminary examination of oil. It shows quickly the degree of unsaturation and whether the oil has unusual components such as hydroxyl groups or conjugated acids (Pearson, 1981). The value for unsaponifiable matter is 1.5. Therefore, the unsaponifiable matter contained in the seed oil may include squalene, waxes, sterols, phospholipids, vitamins, terpenes, steroids and some hydrocarbons (Williams and Hilditch, 1964; AOAC, 1980).

The results of brine shrimp lethality bioassay were summarized in Tables 2 and 3. The petroleum ether and methanol extracts were moderately cytotoxic at high concentration. The two extracts displayed toxicity, with LC_{50} < 1000 µg/ml. The methanol extract showed strong cytotoxicity (LC_{50} = 51.79 µg/ml), and the oil, petroleum ether extract, was less cytotoxic (LC_{50} = 827.39 µg/ml). In toxicity evaluation of plant extracts by brine shrimp lethality bioassay, LC_{50} values lower than 1000 µg/ml are considered bioactive (Meyer et al., 1982). Therefore, the petroleum ether and methanol extracts of *K. senegalensis* may have some biological activity. The results showed that the oil is a non-drying type, while the saponification value of 195.58 ± 0.044 mg KOH indicated that the oil is made up of long chain fatty acids. The brine shrimp lethality bioassay for the methanol extract showed significant bioactivity, while that of the petroleum ether extract (oil) was less. The two extracts were, how

Table 2. Brine shrimp lethality bioassay of petroleum ether extract of the seeds of *Khaya senegalensis*.

Concentration (µg/ml)	1000	500	250	125	62.5
Number of shrimps per test sample	30	30	30	30	30
Number of survivors	3	16	20	24	26
Number of deaths	27	14	10	6	4
Percentage mortality	90	46	33	20	13

LC_{50} = 827.39 ± 5.546 µg/ml.

Table 3. Brine shrimp lethality bioassay of methanol extract of the seeds of *Khaya senegalensis*.

Concentration (µg/ml)	1000	500	250	125	62.5
Number of shrimps per test sample	30	30	30	30	30
Number of survivors	0	0	3	7	10
Number of deaths	30	30	27	23	20
Percentage mortality	100	100	90	77	67

LC_{50} = 51.79 ± 3.672 µg/ml.

ever, considered bioactive. The results of the present investigation may be of value in the elucidation of the potential and medicinal uses of the extracts. Further studies on the analysis of the fatty acid composition of the extracted oil are required.

In conclusion, the seed oil of *K. senegalensis* is a non-drying oil, consisting of long chain fatty acids, and the extracts from the seeds may contain bioactive compounds of potential therapeutic and prophylactic significance.

REFERENCES

Abdelgaleil SAM, Iwagawa T, Doe M, Nakatani M (2004). Antifungal limonoids from the fruits of *Khaya senegalensis*. Fitoterapia 77: 566 – 572.

Abdelgaleil SAM, Nakatani M (2003). Antifeeding activity of limonoids from *Khaya senegalensis*. J. Appl. Entomol. 127: 236 – 239.

Abdelgaleil SAM, Okamura H, Iwagawa T, Sato A, Miyahara I, Doe M, Nakatani M (2001). Khayanolides rearranged phragmalin limonoid antifeedants from *Khaya senegalensis*. Tetrahedron 57: 119 – 126.

Ademola IO, Fagbemi BO, Idowu SO (2004). Evaluation of the antihelminthic activity of *Khaya senegalensis* extract against gastrointestinal nematodes of sheep: *in vitro* and *in vivo* studies. Vet. Parasitol. 122: 151 – 164.

AOAC (1980). Official Methods of Analysis of the Association of Official Analytical Chemistry. 13th Ed., AOAC, Washington DC.

Dalziel JM (1956). Useful Plants of West Tropical Africa. Crown Agents for the Colonies, London, pp. 179 – 183.

deMan JM (1992). Chemical and physical properties of fatty acids. In: Chow CK (ed) Fatty Acids in Foods and Their Health Implications. Marcel Dekker Inc. New York. pp. 18 – 46.

Irvine FR (1961). Woody Plants of Ghana (With Special Reference to Their Uses). Oxford University, London. pp. 523 – 524.

Keay RWJ, Onochie CFF, Stanfield J (1989). A Revised Version of Trees of Nigeria (1964). Clarendon Press, New York. pp. 339 – 340.

Khalid SA, Friedrichsen GM, Kharazmi A, Theander TG, Olsen CE, Bragger CS (1998). Limonoids from the bark of *Khaya senegalensis*. Phytochemistry, 49: 1769 – 1772.

McLaughlin JL (1991). Methods in Plant Biochemistry. In: Hostettmann K (ed) Assays for Bioactivity, Academic Press, London, 6: 1 – 33.

Meyer BN, Ferrigni NR, Putman JE, Jacobson LB, Nicholas DE, McLaughlin JL (1982). Brine shrimp: a convenient general bioassay for active plant constituents. Planta Med., 45: 31 – 34.

Nakatani M, Abdelgaleil SA, Kassem SM, Takezaki K, Okamura H, Iwagawa T, Doe M (2002). Three new modified limonoids from *Khaya senegalensis*. J. Nat. Prod. 65: 19 – 216.

Nakatani M, Abdelgaleil SA, Kurawaki J, Okamura H, Iwagawa T, Doe M (2001). Antifeedant rings B and D opened limonoids from *Khaya senegalensis*. J. Nat. Prod. 64: 1261 – 1265.

Olmo LRV, Da Silva MF das GF, Fo ER, Vieira PC, Fernandes JB, Pinheiro AL, Vilela EF (1997). Limonoids from leaves of *Khaya senegalensis*. Phytochemistry, 44: 1157 – 1165.

Pa Qurt C (1979). IUPAC: Standard Methods for the Analysis of Oils, Fats and Derivatives. Pergamon Press, Oxford. pp. 16 – 162.

Pearson D (1981). The Chemical Analysis of Foods. 7th Ed. Churchill Livingstone, Edinburgh. pp. 504 – 530.

Rossell JB (1991). Vegetable Oils and Fats. In: Rossell JB, JLR Prichard (eds.) Analysis of Oilseeds, Fats and Fatty Foods. Elsevier Science Publishers Ltd. London. pp. 261 – 325.

Sauders L, Fleming R (1971). Mathematics Statistics for Use in the Biological and Pharmaceutical Sciences. 2nd Ed. The Pharmaceutical Press, London. pp. 225 – 286.

Watt JM, Breyer-Brandwijk MG (1962). Medical and Poisonous Plants of Southern and Eastern Africa. EXS Livingstone, London. pp. 1463 – 1469.

Williams PN, Hilditch TP (1964). The Chemical Composition of Natural Fats. 4th Ed. Chapman and Hall, London. pp. 216 – 325.