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Antimicrobial and pharmaceutical properties of *Khaya senegalensis* seed oil

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Abstract
The fruits of *Khaya senegalensis* plant, family Meliaceae, were collected early in the year and appropriately identified. The seed oil was extracted just before the rains came in the month of March using traditional methods of extraction of the Higgi (Kamue) people in Michika L.G.A., Adamawa State of Nigeria. The seeds were removed from the fruits and dried under sunlight until crisp and then roasted on earthenware pot, pounded to a smooth paste in a wooden mortar and pestle mixed with water (pH 8) and boiled for about 3 hours. The oil settling on top was skimmed off and heated to remove excess water and then filtered. The oil was subjected to antibacterial and anti fungal testing, then formulated into a lotion and subjected to stability testing. The oil was found to possess some activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The oil has potential as a cosmetic product due to its easy emulsification, good spreadability, consistency, emollient property and being easily washed off from the skin.

Keywords: *Khaya senegalensis*, formulation, *Staphylococcus aureus*, lotion.

Introduction

The various parts of *Khaya senegalensis* (Ders) A. Juss (family Meliaceae) has found application in traditional medicine by many tribes in Nigeria and used for both human and veterinary purposes. (Effraim *et al.*, 1998; Dutta, 1979; Dalziel, 1956). Among its parts used for various medicinal purposes as listed by Ashivini (2001) include the stem bark extract, used as remedy for fever and as a vermifuge, taenicide and for treating syphilis. It was also used for treating jaundice, dermatoses, scorpion sting, allergies, and infection of the gums, hookworms, bleeding wounds and as a laxative. Thioune *et al.* (2003), found the stem bark extract to have anti-inflammatory activity, which can be used to develop an anti-inflammatory ointment. The root extracts are used for treating mental illness, leprosy and used as an aphrodisiac; while the seeds and leaves are used for treating fever and headache. In addition, to the above uses in humans, Dalziel (1956) also found the use of the bark in traditional veterinary practice for cattle suffering from liver fluke, for ulcers in camels, donkeys and in horses for intestinal ailments associated with mucoid diarrhoea. Effraim *et al.* (1998) established the antidiabetic property of the aqueous stem bark extract of the plant while Okieimen and Eromosele (1999) elucidated the constituents

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of the oil by gas chromatography and infrared spectroscopy and found out that it contains fatty acids made up of stearic acid (10.4%), palmitic acid (21.3%), oleic acid (64.62%) and an unidentifiable acid (3.58%). They confirmed the presence of unsaturation in the oil by infrared peaks at 1642 and 3003 cm^{-1} attributed to olefinic C=C and C-H stretching vibrations respectively. Lompo *et al* (1998) studied the topical anti-inflammatory effects of the petroleum ether and chloroform extracts of the stem bark, and was said to have a dose- dependent inhibitory effects.

The seed oil is traditionally used by the Higgi (Kamue) people of Michika L.G.A. of Adamawa State, Nigeria as a cosmetic skin emollient, and for treating various skin infections like rashes, open wounds, skin ulcers and as insect repellant. Dutta (1979) had also listed the seed oil as possessing insect repellant and anti microbial properties. This has stimulated the present investigation with a view to formulating an acceptable dosage form for use as a cosmetic product.

Experimental

Materials. Polysorbate 80, gentamicin injections (80mg/2ml) (Dizpharm, Nigeria), clotrimazole vaginal tablets 100mg, peptone water (Acumedia, England), Oleic acid (BDH chemicals England), triethanolamine, glycerol (May and Baker Ltd, England), chlorocresol, emulsifying wax, perfume, *Khaya senegalensis* seed, 96% alcohol, nutrient broth (Biotin), nutrient agar and sabouraud dextrose agar (Biotin). Test Organisms (all pure cultures) were *Staphylococcus aureus, Pseudomonas aeruginosa, Trichophyton mentagrophyte, Candida albicans* and *Aspergilus niger.*

Collection of fruits. The fruits were collected in February and early March from the wild variety of trees growing on the Mandara hills in Michika L.G.A of Adamawa State, Nigeria and identified at School of Forestry, Jos. The fruits were spread to dry for two weeks under sunlight.

Extraction of the oil. The fruits were opened with a sharp knife and the seeds removed .The thin covering on the seeds were peeled off to expose the creamy white seeds. The seeds were sun dried until crisp and then roasted on an earthenware pot on firewood. Two kilogram of the seeds was pounded in a wooden mortar with pestle into a smooth paste. One kilogram wood ash was soaked in 1.5 L of water and filtered (filtrate pH was 8). The paste was thoroughly mixed with about 5.0 L of water and 1.0 L of the wood ash filtrate and boiled on firewood with intermittent stirring for 3 hours. With time the oil floats on top and is skinned off. The oil was heated to remove any residual moisture and filtered and kept in an airtight container for subsequent use.

Culturing and harvesting fungi test organisms. Each fungal test organism - *Trichophyton mentagrophyte, Candida albicans* and *Aspergilus niger* was obtained from the Microbiology laboratory stock of University of Jos, Nigeria. Sterile wire loop was used to transfer a loopful of fungi spore to the surface of sabouraud dextrose agar (SDA) and streaked. They were then incubated at room temperature (28 °C) until they grew and produced spores (5-7 days). The spores were harvested and used for sensitivity test.

Preparation of culture media. Nutrient broth was prepared. 5ml volumes were distributed into small bottles and sterilized by autoclaving at 121 °C for 15 min. The nutrient agar was similarly prepared following the manufacturer's instructions. This was distributed into 20ml universal bottles and sterilized in an autoclave at 121 °C for 15 min. The SDA was also prepared following the manufacturer's instructions. It was also distributed into 20ml aliquots into universal

bottles and sterilized in an autoclave at 121 °C for 15min.

Preparation of standard drug solution. A tablet of clotrimazole vaginal tablet containing 100mg of the drug was powdered in a sterile porcelain mortar with pestle and dissolved in 100ml of 96% ethanol to make a 1mg/ml i.e.1000μg/ml solution. This was serially diluted to 10μg/ml and used as the standard drug for the fungal isolates. 0.5ml of Gentamicin injection (80mg/2ml) was measured into a volumetric flask using 1.0ml pipette and diluted to 200μg/ml by making up the volume with sterile distilled water to 50ml.

Preparation of overnight cultures for antimicrobial screening. Each bacterial organism (*Staphylococcus aureus* and *Pseudomonas aeruginosa*) in the stock was subcultured into freshly prepared nutrient broth aseptically using a flamed wire loop and then incubated at 37°C for 24 hours in the incubator (Harrow Scientific Ltd, England. Model 115).

Preparation of Khaya senegalensis oil for sensitivity test. 1.0ml of the oil was pipetted into a small bottle and 4.0ml of 0.5% polysorbate 80 solution added to it. The bottle was closed and shaken vigorously to obtain an emulsified system containing 20% of the oil. Double serial dilution was done with this to obtain a 10% and 15% oil concentration respectively. The sensitivity test for each bacterial isolate was done by seeding each 20ml of the molten sterile nutrient agar, cooled to about 48-50°C, with 0.1ml of the overnight culture of the test organism. This was thoroughly mixed and poured into a sterile Petri dish and allowed to set. Five cups were bored into it using sterile cork borer number 5.Three of the cups were filled with 5%, 10% and 20% of the emulsified oil while the fourth cup was filled with 200μg/ml of Gentamicin to serve as the drug standard. The fifth cup was filled with 0.5% polysorbate 80 to serve as the control. The plate was left for 30 minutes at room temperature to allow for the diffusion of the emulsion, and then incubated at 37°C for 24 hours. The zones of inhibition around the cups were measured using a transparent plastic ruler. For the fungal isolates, the same procedure was repeated but clotrimazole (10μg/ml) was used as the standard drug, while 96% ethanol was the diluent control. The plates were incubated at room temperature (28°C) for 48 hours. Their zones of inhibition were similarly measured.

Preparation of Khaya senegalensis lotion

Formulation I

	Ingredient.	Conc. in 100ml.
A	*Khaya senegalensis* seed oil	32.0
	Oleic acid	2.0
	Emulsifying wax	8.0
B	Triethanolamine	1.0
	Glycerol	4.0
	Water	52.9
	Chlorocresol	0.1
C	Fragrance	2 drops

Formulation II

	Ingredient.	Conc. in 100ml.
A	*Khaya senegalensis* seed oil	32.7
	Emulsifying wax	8.2
B	Triethanolamine	1.0
	Glycerol	4.1
	Water	53.9
	Chlorocresol	0.1
C	Fragrance	2 drops.

For each formulation, Part A was first prepared by melting the emulsifying wax, and then the Khaya oil was added when temperature was lowered to 60 ° C. Oleic acid was added in the case of formulation I, and then the whole part A was kept at 60 ° C. Part B was then prepared by heating the water to 60 ° C on water bath and chlorocresol was added with vigorous shaking until it was completely dissolved . Glycerol and triethanolamine were then added, maintaining

the temperature at 60 ° C. Part A was then added to part B with constant agitation using a glass stirrer until the lotion was formed. When the mixture was sufficiently cooled to room temperature, the perfume was added and stirred continuously to homogenize it in the lotion.

Evaluation of emulsion stability.

1. Macroscopical evaluation. 10ml of each of the prepared lotion was poured into 10ml measuring cylinders, covered with aluminum foil and allowed to stand at room temperature. They were observed visually daily to note the time that separation occurred for any change in the general appearance, colour, odour and consistency.

2. Microscopic globule size determination. Using a compound microscope and after calibration, the microscope stage was fitted with a micrometer by means of which the sizes of the globules were estimated. One drop of the prepared lotion was mounted on a slide and placed on the stage using the objective lens of 40mm.

Results

Physical appearance of lotion. The lotion appeared rich, soft. had good spreadability on skin and can be easily washed off. The general appearance was elegant and good looking. The colour was creamy white, with pleasant odour of the perfume. The texture was soft and smooth. Formulation I had a thicker consistency and looked more whitish than formulation II.

Lotion stability. Physical appearance. The lotions in the measuring cylinders had thin visible separation of the oily phase from the aqueous phase on the fourth day for formulation II and on the fifth day for formulation I. The lotion in the beaker on the other hand retained their physical properties for four days after which the colour darkened to brown. There was no change in the odour and texture of both formulations. Table 3 shows that frequency of lower globule sizes decreases with time while that of bigger globule sizes increase with time. Therefore the mode of globule size on 1st day was at class range of 40-79μg but by the 8th day, it was at 40-119μg. Most globule sizes on the 1st day were between 0-119 μg but by the 8th the globule size were almost equally distributed except at class mark of 80-159μg where frequency was significant. The Table therefore indicates increased coalescence of globules sizes with time and suggests the need to stabilize the lotion for better product shelf-life. Table 4 shows the size of mode on the 1st day was at class range of 80-119 μg, but by the 8thday, it increased to 120-159μg. Again the result shows the need for viscosity stabilizing agent so that globule size does not increase at a very rapid rate. The increase in globule size of the emulsion can be better observed with the average globule size of the emulsions shown on table 5 below.

Table 1. Diameter of zones of inhibition (mm) of bacterial isolates by different concentrations of the oil.

Microorganism	Concentration of oil (%)			0.5% polysorbate 80	Gentamicin (200μg/ml)
	5	10	20		
Staphylococcus aureus	20	18	16	10	34
Pseudomonas aeruginosa	13	15	18	10	48

Table 2. Diameter of zones of inhibition (mm) of fungal isolates by different concentration of the oil

Microorganism	Concentration of oil (%)			0.5% polysorbate 80	Clotrimazole (10μg/ml)
	5	10	20		
Candida albicans	10	10	10	10	46
Aspergilus niger	10	10	10	10	34
Trichophyton mentagrophyte	10	10	10	10	32

Note: Diameter of cork borer was 10mm.

Table 3. Microscopical globule size analysis over eight days for formulation I.

Globule size (μm)		Day 1		Day 2		Day 3		Day 4		Day 6		Day 8	
R	c	F	f.c.	f	f.c.	F	f.c	f	f.c.	f	f.c.	f	f.c.
0-39	19.5	13	253.5	18	351	12	234	8	156.0	8	156.0	5	97.5
40-79	59.5	18	1071.0	20	1190	15	892.5	15	892.0	11	654.5	6	357.5
80-119	99.5	13	1293.5	6	597	12	1194.0	11	1094.5	13	1293.5	16	1592.0
120-159	139.5	2	279.0	3	418.5	6	837.0	9	1255.5	9	1255.5	10	1395.0
160-199	179.5	3	538.5	1	179.5	2	359.0	3	538.5	3	538.5	5	897.5
200-239	219.5	1	219.5	2	439.0	3	685.5	4	878.0	6	1317.0	8	1759.0
		$\sum f$ = 50	$\sum fc$= 3655.5	$\sum f$= 50	$\sum fc$= 3175.0	$\sum f$= 50	$\sum fc$= 4175.0	$\sum f$= 50	$\sum fc$= 4815.0	$\sum f$= 50	$\sum fc$= 5215.0	$\sum f$= 50	$\sum fc$= 6098.0

Note: r = class range, c = class center and f = frequency.

Table 4. Microscopical globule size analysis over eight days for formulation II.

Globule size (μm)		Day 1		Day 2		Day 3		Day 4		Day 6		Day 8	
R	c	F	Fc	F	fc	f	fc	f	Fc	F	fc	F	fc
0-39	19.5	13	253.5	13	253.5	3	58.5	9	175.5	4	78	4	78
40-79	59.5	14	833.0	13	773.5	7	416.5	9	535.5	9	535.5	7	416.5
80-119	99.5	15	1492.5	11	1094.5	14	1393.0	16	1592.0	16	1592.0	11	1094.5
120-159	139.5	3	418.5	7	970.5	12	1674.0	10	1395.0	11	1534.5	12	1674.0
160-199	179.5	2	359.0	3	538.5	9	1615.5	4	718.0	7	1256.5	10	1795.0
200-239	219.5	3	658.5	3	658.5	5	219.5	2	439.0	3	658.5	6	1317.0
		$\sum f$ = 50	$\sum fc$= 4015	$\sum f$ = 50	$\sum fc$= 4295.0	$\sum f$ = 50	$\sum fc$= 5377.0	$\sum f$ = 50	$\sum fc$= 4855.0	$\sum f$ = 50	$\sum fc$= 5655.0	$\sum f$ = 50	$\sum fc$= 6375.5

Note: r = class range, c = class center and f = frequency

Table 5. Average globule sizes for formulations I and II over eight days.

	Mean globule sizes in μm					
Formulation	Day 1	Day 2	Day 3	Day 4	Day 6	Day 8
I	73.1	63.5	83.5	96.3	104.3	122.0
II	80.5	85.9	107.5	97.1	113.1	127.5

Discussion

From the investigation carried out on the anti-bacterial activity of the oil (Table 1), the oil was found to have a higher activity against *Staphylococcus aureus*, a Gram-positive bacterium, at lower concentration of 5% than at 20%. However, the sensitivity increases with increasing concentrations for the Gram-negative bacteria, *Pseudomonas aeruginosa.* This could be explained by the fact that Gram-positive bacteria have higher percentage of peptidoglycan molecules that are polar in nature and so more polar substances can cross the barrier more easily than non-polar ones; and so responds more to the 5% emulsion that is more polar than the 20% emulsion. The Gram-negative bacteria on the other hand have a lipid bi-layer surrounding their peptidoglycan molecules and so are more polar and would respond more to the activity of the less polar. The traditional use of the oil for treating skin

diseases, especially those implicating *Staphylococcus aureus* such as boils and abscesses, and those implicating *Pseudomonas aeruginosa* such as infected wounds, are therefore justified. The oil did not show activity against the fungal organisms used in this study (Table 2).

Tables 3 and 4 show the microscopical globule size analysis of formulations I and II over eight days and Table 5 show the average globule sizes over those days. These tables show that globule sizes of lotion increase as the lotion ages. With proper choice of viscosity stabilizing agents like microcrystalline cellulose and ethyl cellulose, better stability could be achieved for longer shelf life of the lotion. The formulation of the oil into lotion form has great potential as evidenced by their softness and smoothness and their easy spreadability and also being easily washed off the skin. The traditional use of the oil 'as it is' has the disadvantage of being too greasy, which soil clothes easily, and being too odoriferous with a bitter taste. Its formulation into an elegant pharmaceutical product helps overcome these problems while still retaining its anti-bacterial properties with its emollient properties as well.

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