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Antimicrobial qualities, phytochemistry and micro-nutritional content of *Khaya senegalensis* (Desr.) A. Juss seed oil

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Abstract

Khaya senegalensis seed oil is a non-drying oil, which consist of long chain fatty acids. The antimicrobial attributes of the seed oil was investigated using disc diffusion and broth dilution methods. The test microbial cultures used in the study were; *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Candida albicans*, *Penicillium notatum*, *Mucor mucedo* and *Aspergillus niger*. Routine procedures were utilized in the determination of the phytochemical and mineral constituents of the seed oil. *B. subtilis* was the most sensitive of the isolates exposed to varying concentrations of the undiluted seed oil whilst *E. coli* was the most resistant amongst the bacterial isolates. The seed oil did not exhibit any antifungal activity. Varying amounts of alkaloids, tannins, flavonoids, saponins, phytates and oxalates were detected in *K. senegalensis* seed oil whilst glycosides were absent. Amongst the micro-nutrients present in seed oil, manganese had the least value (0.05 mg/ml) while potassium had the highest concentration (3.33 mg/ml). There is a need to conduct further studies aimed at determining the percentage yield of antimicrobial compounds and the antibacterial activity of the seed oil on multiple drug resistant bacteria.

Keywords: *Khaya senegalensis* seed oil; Phytochemical; Antimicrobial, Micro-nutrients.

Introduction

Plants are one of the most important sources of medicines. Medicinal plants are rich in secondary metabolites which are potential sources of drugs and of therapeutic importance.[1] The effectiveness of traditional herbs against microorganisms has been reported.[2, 3] The evaluation of various plant products according to their traditional uses and medicinal value based on their therapeutic efficacy leads to the discovery of newer and recent drugs for treating various ailments. This fact forms the basis for the development of new drugs from various plant sources.[4]

Khaya senegalensis A. Juss (Meliaceae) commonly known as African mahogany, is a popular medicinal plant among the Nupes and Yorubas in Nigeria. It belongs to the family Meliaceae (mahogany).The stem bark aqueous extract is traditionally used by Yoruba and Nupe tribes to treat malaria, jaundice, edema and headache.[5] Khaya senegalensis is a tree with shiny foliage up to 25 m or more with exfoliating barks, young branches with dark, grayish-brown lenticels and leaves of 15-60 cm or more. It has pinnate leaves, glabrous with 6 to 12 alternate or opposite elliptical oblong leaflets.

At the flowering, *K. senegalensis* twigs carry at their ends panicles of small white flowers consisting of successive whorls of four floral parts. Its fruits are capsules with thick and woody seed coat. The bark of this tree is very thick, scaly and dark brownish-gray color. In section it oozes reddish exudates.[6] In its natural range, *K. sengalensis* provides cattle fodder, edible and cosmetic oils, medicinal products, shade and shelter. *K. Senegalensis* seeds have been reported to contain about 67% oil content by weight.[7] This oil is quite rich in oleic acid (66%) and is used in West Africa for cooking as well as in cosmetics.[7]The presence of several khivorine based compounds in K. Senegalensis seeds; 3-diacetylkhivorine, 7-diacetyl-7-oxokhivorine, 3, 7-diacetyl-7-oxokhivorine, 3-destigloyl-6-dioxy-swietenine and 3-B-acetoxy-swietenine have also been reported.[6] Traditional doctors used the oil in combination with other substances to treat diseases such as syphilis, stomach upset and on cuts after making incisions on the body of patients.

There is increasing interest in the use of plant extracts as therapeutic agents, particularly the capacity for these extracts to inhibit the growth of pathogenic microorganisms.[8] Ayo *et al*. stated that the seed oil of *K. senegalensis* is a non-drying oil, which consist of long chain fatty acids, and the seed extracts may contain bioactive compounds of potential therapeutic and prophylactic significance.[9] Consequently, this study was aimed at evaluating the phytochemical composition and antimicrobial properties of *K. senegalensis* seed oil.

Materials and Methods

Sample collection

The prepared *K. senegalensis* seed oil sample was purchased from a local market in Adamawa State.

Test organisms

Several test isolates were used in this study; *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Candida albicans*, *Penicillium notatum*, *Mucor mucedo* and *Aspergillus niger*. The bacterial and yeast pure cultures were sourced from the Medical microbiology laboratory, University of Benin Teaching Hospital. The filamentous fungal isolates were obtained from Edo Environmental Consults and Laboratory, Benin City. The identity of the bacterial pure cultures was confirmed by conducting several routine morphological and biochemical tests as described by [Ref. 10-13].[10-13] The results of the tests were compared with reference tables as stated by [Ref. 14-17].[14-17]

Standardization of the microbial inocula

The procedures as described by [Ref. 12, 13] were adapted in the standardization of the microbial test pure cultures All the test bacterial and fungal isolates were sub-cultured on freshly prepared Nutrient agar and Sabouraud Dextrose agar plates and incubated for 24 h and 48 h respectively.[12, 13] Portions of the streaked bacterial, yeast and sub-cultured filamentous fungal colonies were transferred into test tubes containing 8ml of sterile nutrient broth and incubated for 12 h and 48 h at 37ºC. The growth of bacterial and fungal suspension obtained was compared to that of freshly prepared Barium sulphate opacity standard ;0.5ml of 1% Barium chloride to 99.5 ml of 1% H_2SO_4 (0.36 Normal).The turbidity was adjusted by adding more sterile nutrient broth to match 0.5 McFarland standards (106 cfu/ml and 106 spores /ml).

Determination of antimicrobial activity of the undiluted *K. senegalensis* seed oil

The disc diffusion method as described by [Ref. 20] was adapted in evaluating the antimicrobial properties of the undiluted seed oil.[20] Labeled prepared Mueller Hinton agar plates were seeded with an aliquot; 0.2 ml of the standardized microbial inocula using the spread plate method.[10] The seeded plates were allowed to dry for 15-20 mins. The respective filter paper discs were impregnated with differing volumes of seed oil; 1ml and 5 ml. commercially available gentamicin discs (30 µg) were utilized as positive control for the bacterial isolates. The fungal isolates were exposed to filter paper discs soaked with a single concentration of nystatin (50 mg/ml). This was derived by dissolving 0.1 g of the drug in 2 ml of DMSO. The discs were placed on the seeded plates with the aid of a sterile forceps and inoculating needle. The triplicate agar plates were incubated at room temperature (26±2ºC) for 16 h. However, the filamentous fungal culture plates were incubated for 48 h. Resultant zones of inhibition were measured using a transparent meter rule and the mean zones of inhibition were calculated.

Evaluation of the Minimum Inhibitory Concentrations (MIC) of the diluted seed oil, Tween 80 and antibiotics

The MIC procedure as described by [Ref. 21, 22] were adapted in determining the MIC of the seed oil.[21, 22] The seed oil was prepared to the highest concentration of 1.8 ml (stock concentration) in sterile nutrient broth by mixing 9 ml of the seed oil with 5 ml of the sterile nutrient broth (OXOID). One (1) ml of 0.02% Tween 80 (SIGMA) was added to enhance the solubility of the oil. This mixture was shaken at 200 rpm using a mechanical shaker (HEIDOLPH UNIMAX 2010). The stock concentrate was doubly diluted to give concentrations ranging from 1 ml to 0.125 ml respectively. The diluted concentrates were also shaken at 200 rpm prior to the addition of 0.1 ml of the standardized microbial inocula. Differing dilutions of Tween 80 in nutrient broth which ranged from 1ml (v/v) to 0.125 ml (v/v) were prepared and inoculated with 0.1 ml of the standardized microbial test culture. This served as a positive control. An initial concentration of 40 mg/ml was prepared for both reference antibiotics; gentamicin and nystatin. The concentrate was doubly diluted to 1.25 mg/ml. The tubes were seeded with 0.1 ml of the microbial broth cultures. All the tubes were incubated at ambient temperatures (26 ± 2ºC) for 16 h for bacteria and 48 h for the fungal cultures. The least concentration of the seed oil, Tween 80 and antibiotics which inhibited the visible growth of the inoculum was considered as the minimum inhibitory concentration.

Phytochemistry and nutritional analyses of K. senegalensis seed oil

Procedures as stated by [Ref. 23-25] were employed in the determination of both the qualitative and quantitative tests for the presence of alkaloids, tannins, glycosides, saponins, flavonoids, oxalates, phytate and the nutritional composition of the seed oil.[23-25]

Results

The antimicrobial activity of *K. senegalensis* seed oil and the antibiotics against the pure microbial isolates are shown in Table 1. For the seed oil, the highest inhibitory zone; 15.67 mm ± 0.33, was elaborated by B. subtilis exposed to 5 ml of the seed oil (Table 1). The least zone of inhibition elicited against the seed oil; 2.67 mm ± 0.33 was displayed by *E. coli* (Table 1). All the fungal cultures were resistant to the varied quantities of the seed oil (Table 1). The inhibitory zones elaborated by the bacterial isolates against gentamicin ranged from 10.00 mm ± 0.15 for *S. aureus* to 20.00 mm ± 0.58 for *B. subtilis* (Table 1). The zones of inhibition elicited by the fungal cultures exposed to Nystatin varied from 10.00 ± 0.15 for *C. albicans* to 20.00 ± 0.58 for *P. notatum* (Table 1).

Table 1: Antimicrobial (zone of inhibition (mm)) activity of *K. senegalensis* seed oil

Test isolate	1 ml	5 ml	CN (30 µg)	Nys (50 mg/ml)
P. aeruginosa	6.00±0.58	12.00±0.58	14.00 ± 1.33	NA
S. aureus	NMZI	9.00±0.58	10.00± 0.15	NA
B. subtilis	8.00±0.58	15.67±0.33	20.00 ± 0.58	NA
E. coli	NMZI	2.67±0.33	11.00 ± 0.45	NA
C. albicans	NMZI	NMZI	NA	10.00 ± 0.0
P. notatum	NMZI	NMZI	NA	20.00 ± 0.0
A. niger	NMZI	NMZI	NA	15.00± 0.16

n=3; Values are mean ±SEM; CN= Gentamicin, Nys; Nystatin, NMZI;No Measurable Zone of Inhibition, NA; Not Applicable.

The minimum inhibitory concentration (MIC) of the *K. senegalensis* seed oil, Tween 80 and antibiotics against the test microbial isolates are presented in Table 2. The MIC values for the seed oil ranged from >1.8 ml for *C. albicans*, *P. notatum* and *A. niger* to 0.5 ml for *P. aeruginosa* and *B. subtilis* respectively (Table 2). All the microbial cultures were resistant to varying concentrations of Tween 80 (Table 2). The MIC readings displayed by the respective bacterial isolates against varied concentrations of gentamicin ranged from 10 mg/ml for *E. coli* to 2.5 mg/ml for *P. aeruginosa* (Table 2).The MIC values of the diluted concentrations of Nystatin against the fungal cultures varied from 20 mg/ml for *C. albicans* and *A. niger* to 10 mg/ml for *P. notatum* (Table 2).

Table 2: Minimum Inhibitory Concentration (MIC)) of *K. senegalensis* seed oil, Tween 80 and antibiotics

Test isolates	MIC^{oil}	$MIC^{Tween\ 80}$	MIC^{CN}	MIC^{Nys}
P. aeruginosa	0.5[a]	>1[a]	2.5[b]	ND
S. aureus	1	>1	5	ND
B. subtilis	0.5	>1	5	ND
E. coli	1	>1	10	ND
C. albicans	>1.8	>1	ND	20[b]
P. notatum	>1.8	>1	ND	10
A. niger	>1.8	>1	ND	20

ND; Not Determined, CN; Gentamicin, Nys; Nystatin, a; Values are in ml, b; Values are in mg/ml.

Differing amounts of alkaloids, tannins, flavonoids, saponins, phytates and oxalates were detected in K. senegalensis seed oil (Table 3). Glycosides were absent in the seed oil (Table 3). The seed oil had comparatively high quantity of oxalates (8.56 mg/100) whilst the flavonoids concentration; 1.02 mg/ 100, was the least amongst the phytochemicals present in the seed oil (Table 3).

Table 3: Qualitative and quantitative analysis of *K. senegalensis* seed oil.

Parameters	Qualitative analysis	Quantitative analysis (mg/100)
Alkaloids	+	1.48
Tannins	+	1.25
Flavonoids	+	1.02
Saponins	+	2.54
Glycosides	-	-
Phytates	+	1.82
Oxalates	+	8.56

+ ; present - ; absent

Amongst the micro-nutrients present in *K. senegalensis* seed oil, manganese had the least concentration (0.05 mg/ml) while potassium had the highest amount (3.33 mg/ml) (Table 4).

Table 4: Nutritive values (mg/kg) of *K. senegalensis* seed oil.

Parameters	Values (mg/ml)
Sodium	2.38
Potassium	3.33
Calcium	1.42
Magnesium	1.96

Iron	0.51
Zinc	0.17
Lead	ND
Manganese	0.05
Chromium	ND

ND: Not detected

Discussion

K. senegalensis seed oil showed broad spectrum antibacterial activity against the test bacterial isolates (Table 1 and 2). This phenomenon is similar to an earlier report by [Ref. 26] which stated the antibacterial activity of the seed oil on *S. aureus* and *E. coli*.[26] The presence of several phytochemical compounds in differing amounts (Table 3) might be responsible for this activity. There was an observed relationship between the amount of the undiluted *K. senegalensis* seed oil and the resultant antibacterial activity (Table 1). This trend is in agreement with a report by [Ref. 27] which indicated that the expressed antibacterial activity of *Leuconia leucocephala* seed oil was dependent on the concentration and volume of the oil. *K. senegalensis* seed oil did not exhibit any antifungal activity against the test fungal cultures (Table 1 and Table 2).[27] This trend might be attributed to the inability of the antimicrobial compounds present in the seed oil to permeate into the exposed actively growing eukaryotic fungal cells. This observation was in disagreement with a report by [Ref. 9, 29] which both indicated the cytoxicity of several doses of *K. senegalensis* seed oil to exposed Artemia salina larva and antifungal activity of solvent extracts derived from Swietenia mahogany.[9, 29] Comparatively the antimicrobial activity of the seed oil was lesser than that of the control antibiotics; gentamicin and nystatin (Table 1 and 2). This phenomenon was similar to a report by [Ref. 28] which stated that the antimicrobial activity of *Garcina kola* oil was lesser than that of the antibiotics; gentamicin and ketoconazole used in the study.[28] He also reported that the difference in activity is because these are pure synthetic drugs compared to the oil that contains minor dissolved secondary metabolites. Several phytochemical compounds such asoxalates, phytates, saponins, tannins, alkaloids, flavonoids and tannins were present in the seed oil (Table 3). However, glycoside was absent in the seed oil (Table 3). The presence of saponins and tannins and the absence of glycosides in *K. senegalensis* plants have also been reported.[30] The authors stated that the biological actions are primarily due to these components in a very complicated concert of synergistic or antagonistic activities. Mixtures of such chemicals show abroad spectrum of biological effects and pharmacological properties.[31] Stated that phytochemicals have been considered as crucial nutritional components without official recommendations of how much is to be taken with ability to prevent chronic diseases such as cancer, cardio-vascular diseases, diabetes and ageing. A lot of these researches have come up with the fact that some of these plant chemicals which biologically function as anti-nutritional or antioxidants have potentials in helping to reduce the risk of several deadly diseases in man.[32] Varying concentrations of sodium, potassium, calcium, magnesium, iron, zinc and manganese were detected in the *K. senegalensis* seed oil (Table 4). The heavy metals; lead and chromium were absent in the analyzed seed oil (Table 4). Aside from the potassium value, the sodium reading was higher the other mineral parameters. This trend contrasted with a report by [Ref. 33] which stated the low sodium content of *Buchholzia coriacea* dried seeds.[33] The zinc content of the seed oil was very low (Table 4). Zinc has been reported to be involved in the normal functioning of the immune system and is a component of over 50 enzymes in the body.

Conclusion

K. senegalensis seed oil exhibited broad spectrum antibacterial activity against the test bacterial isolates. Several relevant phytochemical constituents such as saponins, alkaloids and oxalates that can be used as components of new antimicrobial agents were also present in different amounts. There is a need to conduct further studies aimed at determining the percentage yield of antimicrobial compounds and the antibacterial activity of the seed oil on multiple drug resistant bacteria.

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