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**Research Article**

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# Assessment of the In-vitro antimicrobial potential of *Khaya Senegalensis* ethanol leaf extract

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**ABSTRACT**

*Khaya senegalensis is an important multipurpose tree in its natural range in sub-Saharan Africa. It is particularly valued for timber, fuel wood and medicinal purposes as well as being a popular shade and amenity tree [1]. Khaya senegalensis ethanol leaf extract was examined for antimicrobial potential against some selected clinical isolates (Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae and Candida albicans). Qualitative phytochemistry of the ethanol leaf extract confirmed the presence of the following active chemical constituents; saponins, flavonoids, tannins, alkaloids, glycosides and carbohydrates. Dose dependent antimicrobial activity was observed with zones of inhibition ranging from 15.00-26.00mm for S. aureus, 10.00-23.50mm for E. coli, 3.20-16.00mm for K. pneumonia and 0.00mm (no inhibition of growth) for C. albicans. MIC for the clinical isolates is 25.00mg/ml, 12.50mg/ml, 12.50mg/ml and no effect respectively while MBC is 50.00mg/ml and 25.00mg/ml for S. aureus and E. coli respectively. The data obtained demonstrated varying activities against selected bacterial isolates and thus, confirming the use of the plant in ethnopharmacology.*

**Keywords:** *Khaya senegalensis*, Antimicrobial potential, Phytochemistry, Leaf extract and In- vitro.

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## INTRODUCTION

The use of plant and animal parts in medicine have since been widely documented in the records of ancient China, India and Egypt, and the practice was based on series of "trial and error", which could not be substantiated by proven scientific theories, however, these practices have produced results of proven efficacies compared to the conventional modern medicine [2].

In recent times, herbal medicines have become an integral part of the Primary Health Care system of many nations [3, 4]. A major part of the total population in developing countries still uses traditional folk medicine obtained from plant resources [5].

*Khaya senegalensis*, commonly known as mahogany (in English), belongs to the meliaceae family. It is a large tree native to sub-Saharan savannah area from Senegal to Uganda, and one of the most popular medicinal meliaciuous plants in African traditional remedies [6]. In Nigeria, the tree is called with many local names in different parts of the country; '*Madaci*' in Hausa, '*Dalehi-Kahi*' in Fulani. '*Oganwon*' in Yoruba and '*Ono*' in Igbo languages. In its natural habitat, the plant is a medium to large sized tree that grows up to 30 m [7].

Substances that kill microorganisms or inhibit their growth are known to as anti-microbial agents. They are widely employed to cure diseases caused by pathogenic microorganisms [8]. Due to increase in multi drug resistant pathogens in humans, immense interest in the search of new drugs or preparations from the natural sources including plants have been triggered over the years [9]. Thus, this study is aimed at finding out the phytochemical constituents

and antimicrobial potential of the ethanol leaf extract of *Khaya senegalensis* in order to justify its use in ethno pharmacology.

## EXPERIMENTAL SECTION

**Source of Plant Material, Collection and Identification.**
Plant sample of *Khaya senegalensi*s (fresh leaves) was collected February 2011, from the University of Maiduguri environs; it was later on identified and authenticated to be the leaves of *Khaya senegalensi*s by a Plant Taxonomist in the Department of Botany, University of Maiduguri, a voucher specimen number PCG 0027 was assigned and deposited in the Department of Pharmacognosy Herbarium, University of Maiduguri.

**Preparation of the Leaf Extract**
The fresh leaf of *Khaya senegalensi*s collected was air dried to a constant weight, pulverized in a mill (TYPE YC100L-4, China) and stored in an air tight container for further use. A 500g weight of the pulverized leaf of *Khaya senegalensi*s was de-fattened using petroleum ether for 24 hours; the marc was dried and soaked in 300ml of ethanol for 24hours at room temperature with occasional mechanical shaking. The mixture was filtered and the marc was re-extracted with 300ml of ethanol. This procedure was repeated three times and the combined filtrate obtained was concentrated using Rotary evaporator (R201D, U.S.A.) and the extract subsequently air dried. The weight of the ethanol leaf extract of *Khaya senegalensi*s obtained was 20.5g (4.1% Yield).

**Source of the Microorganisms**
Clinical isolates of the test organisms (*Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae* and *Candida albicans*) were obtained from the Department of Microbiology, University of Maiduguri Teaching Hospital (UMTH).

**Phytochemical analyses of the ethanol leaf extract of *Khaya senegalensi*s**
The ethanol leaf extract of *Khaya senegalensi*s was subjected to preliminary phytochemical analyses to confirm the presence/absence of the various classes of active chemical constituents such as saponins, flavonoids, tannins, alkaloids, starch, glycosides, carbohydrates and terpenes in accordance with Evans [10] and Sofowora [11].

**Assessment of the In-vitro antimicrobial potential of *Khaya Senegalensis* ethanol leaf extract**
**Sterilization of the equipments and disinfection**
All work surfaces were mopped with a moist hand towel and was disinfected with Dettol® (chlorhexidine and cetrimmide) so as to reduce microbial load on working surface.

**Dry heat sterilization**
A hot air oven was used to sterilize the conical flasks, forceps, wire loop, pipettes, and beakers at 160˚C for 2 hours.

**Moist heat sterilization**
Moisture insensitive equipments and materials used for microbiological processes were sterilized in an autoclave (YX-280B, China) at 121°C for15 minutes.

**Preparation of Media**
The medium (Nutrient Agar and Nutrient broth) were prepared according to manufacturer's instruction.

**Zone of inhibition**
The procedure employed by Wakirwa *et al*., [12] was adopted. One gram weight of the extract was dissolved in 10ml sterile distilled water in order to obtain a stock concentration of 100mg/ml. The stock was then diluted serially to the following concentrations; 50mg/ml, 25mg/ml, 12.5mg/ml and 6.25mg/ml.

A standard cork borer (8mm in diameter) was used to bore three wells for each inoculated plate (inoculated using the agar diffusion method) and the agar removed from the well. 0.1ml of the test extract was then introduced into the well created at the center of each of the plate. The plates were incubated at 37˚C for 24 hours and observed for the zone of inhibition of growth while the fungal plates were incubated at $30^{o}$C for 1-7days. A transparent ruler was used to measure the zone of inhibition and the results recorded in millimeters. The screening was done in triplicate and the mean and standard deviation determined.

**Minimum inhibitory concentration (MIC) determination procedure;**
Broth dilution method as described by Ibekwe *et al.*, [13] was employed in the determination of MIC of the ethanol leaf extract of *Kyaha senegalensis.*

A set of 9 Bijou bottles were arranged serially and filled with 5ml of nutrient broth. The first bottle contained the double strength nutrient broth. A 1.0g ethanol leaf extract of *Kyaha senegalensis* was dissolved in 5ml distilled water and added to the first bottle (i.e. the double strength nutrient broth), it was mixed thoroughly and 5ml was subsequently withdrawn and poured into the second bottle. This procedure was continued up to the $7^{th}$ bottle where 5ml was withdrawn and discarded. Bottle number 8 contains only nutrient broth i.e. the negative control to scrutinize the sterility of the media and bottle number 9 contains the organism (positive control). A loop full of the diluted overnight culture of a sensitive gram negative (*Escherichia coli* and *klebsiella pneumoniae*) and gram positive organism (*staphylococcus aureus*) were inoculated. All the bottles were incubated at 37°C for 24 hours in an electric incubator (DHG-9023A, China).

The bottles were observed for turbidity of growth after 24 hours. The lowest concentration which showed no turbidity in the bottle was recorded as the MIC.

**Minimum bactericidal concentration (MBC) determination procedure;**
The broth dilution method as described by Ibukun [14] was adopted. All the test bottles which showed no turbidity in the MIC assay were sub-cultured into a nutrient agar plate and incubated at 37°C for 24hours and observed for colony growth.

The MBC was the plate with the lowest concentration of extract without colony growth.

**Determination of activity index**
The activity index of the ethanol leaf extract of *Kyaha senegalensis* was calculated according to Arya *et al.,* [15].

Activity index (A.I.) = Mean of zone of inhibition of the extract
Zone of inhibition obtained for standard antibiotic drug

**Statistical Analysis**
Paired-Samples T-Test was used in the analysis to determine the level of significance of the various bacterial zones of inhibition observed. P-value < 0.05 was considered significant.

## RESULTS AND DISCUSSION

In concordance with the findings of Abalaka *et al*., [16] and Makut *et al*.,[7] active chemical constituents (saponnins, flavonoids, tannins, alkaloids glycosides and carbohydrates) were established to be present in this study. Some of these active constituents (secondary metabolites) have been reported to have activity against micro-organisms [17]. In particular, the tannin containing remedies are used as anthelmintics, antioxidants, antimicrobials, antiviral and for cancer treatment [18, 19, 20, 21]. Hence, the presence of these active chemical constituents are indicators that the leave extract of *Khaya senegalensis* in ethanol medium posses antimicrobial activity.

As shown in table 2, the ethanol leaf extract of *Khaya senegalensis* demonstrated a dose dependent antimicrobial activity against both Gram positive (*staphylococcus aureus*) and Gram negative (*Escherichia coli* and *klebsiella pneumoniae*) bacterial isolates but pronounced inhibition was observed with *Staphylococcus aureus* at the different concentrations of the extract utilized. However, according to the findings emanating from this study, antifungal activity is absent. This result is in conformity with the findings of Abalaka *et al*., [16] but however contradicts the findings of Makut *et al*., [7] who found out that the ethanol leaf extract of *Khaya senegalensis* had fungicidal effect. This disparity can be attributed to geographical difference in the location of the plant collection.

The positive control (Ciprofloxacin 50mg/ml and Fluconazole 50mg/ml for bacterial and fungal isolates respectively) showed significantly higher zone of inhibition compared to the extract studied at various concentrations employed (P-value < 0.05). The difference may be attributed to the fact that positive controls employed are pure compounds compared with the doses of the crude ethanol leaf extract of *Khaya senegalensis* used [22].

Minimum inhibitory concentration (MIC) refers to the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation (MIC is used by diagnostic laboratories mainly to confirm resistance, but most often as a research tool to determine the *in vitro* activity of new antimicrobials)[23], while minimum bactericidal concentration (MBC) is the lowest concentration of antimicrobial that will prevent the growth of an organism after subculture on to antibiotic-free media[23]. Based on the results of the MIC and MBC presented in Table 3, both Gram positive and negative bacteria utilized demonstrates significant degree of sensitivity

to the extract tested at various doses with the activity index (AI) signifying a better activity against Gram positive bacteria.

**Table 1: Qualitative preliminary phytochemical analysis of ethanol leaf extract of *Khaya senegalensis*.**

| Active chemical constituents | Presence/Absence |
|---|---|
| Saponins | + |
| Flavonoids | + |
| Tannins | + |
| Alkaloids | + |
| Starch | _ |
| Glycosides | + |
| Carbohydrate | + |
| Terpenes | _ |

*+=Present,-=Absent*

**Table 2: In vitro antimicrobial potential of ethanol leaf extract of *Khaya senegalensis* showing the zones of inhibition (mm) (n=3).**

| Organisms | Concentration (mg/ml) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Negative control | Positive control | 100 | 50 | 25 | 12.5 | 6.25 |
| *S. aureus* | - | 31.00±0.28 | 26.00±0.21* | 21.50±1.20 | 15.00±4.24 | 0.00±0.00* | 0.00±0.00* |
| *E. coli* | - | 32.00±0.82 | 23.50±0.35 | 18.60±0.14 | 14.00±0.28 | 10.00±0.55 | 0.00±0.00* |
| K. *pneumonia* | - | 23.00±0.49 | 16.00±0.24 | 8.30±0.49* | 5.40±0.26* | 3.20±0.07* | 0.00±0.00* |
| *C. albicans* | - | 15.00±1.25 | 0.00±0.00* | 0.00±0.00* | 0.00±0.00* | 0.00±0.00* | 0.00±0.00* |

** indicates a significant difference with positive control at p-value < 0.05. (T- Test) Negative control = Distilled water. Positive control = Ciprofloxacin and fluconazole for bacterial and fungal isolates respectively. S. aureus= staphylococcus aureus, E. coli = Escherichia coli, K. pneumoniae = Klebsiella pneumonia, C. albicans = Candida albicans.*

**Table 3: MIC, MBC and Activity index (A.I.)**

| Organism | MIC (mg/ml) | MBC (mg/ml) | Activity index (A.I.) |
|---|---|---|---|
| *S. aureus* | 25.00 | 25.00 | 0.67 |
| *E. coli* | 12.50 | 50.00 | 0.52 |
| *K. pneumonia* | 12.50 | - | 0.36 |
| *C. albicans* | No effect | No effect | No activity index |

*MIC = Minimum inhibitory concentration. MBC= Minimum bactericidal concentration. S. aureus= staphylococcus aureus, E. coli = Escherichia coli, K. pneumonia = Klebsiella pneumoniae C. albicans = Candida albicans.*

## CONCLUSION

Based on the data emanating from this study, it can be concluded that the ethanol leaf extract of *Khaya senegalensis* contain some active chemical constituents (saponnins, flavonoids, tannins, alkaloids glycosides and carbohydrates) that are implicated in concentration dependent growth inhibition of clinical isolates used. The fact that these extract demonstrated activity against certain bacteria confirms its use of in ethnopharmacology.

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