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Full Length Research Paper

In vitro assessment of antibacterial activity of bark extracts of *Khaya senegalensis*

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***In vitro* antibacterial activity of aqueous, ethanol and acetone extracts of *Khaya senegalensis* were tested against *Staphylococcus aureus, Streptococcus pyogenes, Salmonella typhi, Shigella dysenteriae, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* isolated from clinical specimen. The acetone extracts showed higher antibacterial activity compared to the aqueous and ethanol extracts. The minimum inhibitory concentration of acetone extracts was in the range of 6.5 to 12.5 mg/ml while the minimum bactericidal concentration ranges between 6.5 to 25.0 mg/ml. The antibacterial activity of the extracts was not significantly affected by heating at 100° C for 1 h but the activity was lost at pH 8.0.**

Key words: *In vitro*, antibacterial activity**,** *Khaya senegalensis*.

INTRODUCTION

The use of plants and herbal products as major components of therapeutic agents and sources of chemical diversity in drug discovery programs is no longer in doubt. Recently, there is growing interest in obtaining samples of plant materials and/or ethnobotanical uses of plants with a view to explore the possibilities for new commercial medicinal products. This rekindled interest in the healing power of plants has been attributed to the current global upsurge of bacterial resistance to antimicrobials and drugs (Ferreira et al., 2004). This has brought considerable progress in understanding the nature of valuable components in plants for the treatment of various diseases (Enoh and Abiodun, 2001). It is in view of this that we seek to determine the antibacterial potency of *Khaya senegalensis.* The ethnobotanical uses of the bark of this plant have been reported by Ijeoma et al. (1997) and its use as an antiparasitic agent has been reported by Atawodi et al. (2003). Among the *HØBA* people in Hong local Government Area of Adamawa State, Nigeria, *K. senegalensis* is used in managing some ailments of microbial origin namely diarrhea, and respiratory tract infections.

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MATERIALS AND METHODS

Collection of plant samples

The bark of *K. senegalensis* A. Juss were obtained from the wild at Gashala Hong Local Government Area of Adamawa State. The plants were identified and authenticated in the Department of Biological Sciences, Federal University of Technology, Yola, Nigeria.

Preparation of plant materials for extraction

Bark samples of the plants were cut into small pieces using a sharp knife and then dried at room temperature for 7 days. The dried samples were then crushed using mortar and pestle. The crushed plant materials were reduced to fine powder using an electric blender (National, 1999) and the phytochemical components extracted using water, ethanol and acetone as solvents following the methods described by Irobi and Daramola (1993) and De and Ifeoma (2001). The phytochemical screening was carried out following the methods of Sofowora (1982)

Test organisms

The following organisms *Staphylococcus aureus, Streptococcus pyogenes, Salmonella typhi, Shigella dysenteriae, Klebsiella pneu-*

moniae and *Pseudomonas aeruginosa* were clinical isolates from Microbiology laboratory, Federal Medical Centre, Yola.

Determination of β- lactamase activity of isolated organisms

The clinical bacterial isolates were screened for *β*- lactamase activity following the iodometric method of Foley and Perret (1962). Briefly, 0.1 ml of 25 mg/ml Penicillin G was dispensed into 12 test tubes. A heavy inoculum's of each of test organisms on Nutrient agar slants was emulsified in 12 separate test tubes containing 0.1 ml of 25 mg/ml penicillin G. A heavy growth of test organism was also added to a tube containing 0.1 ml of distilled water served as control. The test tubes were then incubated at room temperature (30^oC) for 90 min. After which 2 drops of 3% starch solution were added to each test tube and mixed by gentle swirling. One drop of iodine solution was added to each of the test tubes and the tubes were then allowed to stand for 5 - 10 min. Disappearance of blue color within 5 - 10 min confirms the presence of β- lactamase activity.

Antibacterial susceptibility testing

Nutrient agar plate surfaces were inoculated with 0.5 ml of a 24 h culture suspension of test organisms adjusted to10^8 cells/ml (Mac Farland tubidometric standard) and spread using a glass spreader. The plates were kept on the bench for 15 min to dry. Filter paper discs impregnated with each of the crude extracts and antibiotics and also the discs impregnated with acetone used as controls were placed on the plates equidistant to one another. The plates were then incubated at 37^oC for 18 - 24 h and then observed for antimicrobial activities against the test organisms' by measuring diameter of zone of inhibition. The tests were carried out for each of the extracts, antibiotics and acetone in duplicates (Hugo and Russel, 1983; WHO, 2003).

Minimum inhibitory concentration (MIC) determination

The acetone extracts of *K. senegalensis* which showed significant antibacterial activity were selected for determination of MIC. A stock solution of 100 mg/ml was prepared by dissolving 1000 mg of extract in 1 ml of acetone and then made up to 10 ml using distilled water. This was serially diluted to obtain various ranges of concentrations between 3.123 mg/ml to 50 mg/ml. 0.5 ml of each of the dilutions of different concentrations was transferred into sterile test tube containing 2.0 ml of nutrient broth. To the test tubes, 0.5 ml of test organism previously adjusted to a concentration of 10^8 cells per ml was then introduced. A set of test tubes containing broth only were used as controls. All the test tubes and control were then incubated at 37^oC for 18 - 24 h. After the period of incubation, the tube containing the least concentration of extract showing no visible sign of growth was considered as the minimum inhibitory concentration (De and Ifeoma, 2002).

Minimum bactericidal concentration (MBC) of the extracts against the test organisms

From the tubes showing no visible sign of growth in MIC determination, 0.1 ml was inoculated onto sterile nutrient agar plates by the spread plate method. The plates were then incubated at 37^oC for 24 h. The least concentration that did not show growth of test organisms was considered as the MBC.

Stability tests

pH and temperature stability of the extracts were also determined using the method of Sammuelson et al. (1985). 50 mg/ml concentration of the constituted extracts was treated at 60^oC and 100^oC separately in a water bath for 60 min. The antibacterial activity of each of the treated and non-treated samples was determined as described earlier. For determination of effect of pH, 50 mg/ml dilutions of the samples were adjusted to pH ranges between 6.0 and 8.0 using pH meter (Labtech digital pH meter) using 0.1 N HCl and NaOH. The antibacterial activity of each of treated and non-treated extracts were determined as described previously.

RESULTS

The percentage yield of aqueous, ethanol and acetone extracts of *K. senegalensis* were 10.0, 7.0 and 5.6% with a pH value of 5.4. The consistency of extracts was brown and semi solid and phytochemical analysis showed the presence of alkaloids. Four of the test organisms namely *S. aureus, S. pyogenes, K. pneumoniae* and *S. typhi* selected for this study were positive for β- lactamase. The acetone extracts of all the samples showed significant antibacterial activity at 25 mg/ml (diameter of zone 6 -1 6 mm) against the test organism. The aqueous extracts showed activity against all organisms at 100 mg/ml but not at concentrations ≤ 25 mg/ml (Table 1).

Results in Table 2 showed that test organisms exhibitted varying degrees of multi drug resistance to standard antibiotics disk used in this study. The test organisms used in this study were resistant to penicillin, amoxicillin, cotrimoxazole and tetracycline at 50 µg/ml but susceptible to ofloxacin at 30 µg/ml. When compared to the antibacterial activity of the extracts it was observed that the inhibitory activity of the ethanol and acetone extracts of *K. senegalensis* at 25 mg/ml and those of aqueous extracts at 100 mg/ml was greater that those of the standard antibiotics (penicillin, tetracycline, amoxicillin, cotrimoxazole, augmentin and gentamycin). Most interestingly these extracts exerted inhibitory properties on β-lactamase positive isolates.

The MIC value of acetone extracts of *K. senegalensis* was in the range 6.5mg/ml to 12.5 mg/ml. The least MIC value was for *S. dysentriae* (6.5 mg/ml) but 12.5 mg/ml for all other test organism. The MBC value of the extract was 25 mg/ml for *S. dysentriae*. The antibacterial activity of the extracts on test organisms was not significantly reduced when treated at 100^oC for 1 h as shown in Table 3. However, the antibacterial potency of the extracts was reduced at pH 7.0 and lost at pH 8.0 (Table 4)

DISCUSSION

The extracts of the test plants show varying degree of antimicrobial activity against gram positive (*S. aureus* and *S. pyogenes*) and gram negative organisms (*K. pneumoniae, S. typhi, S. dysenteriae* and *P. aeruginosa*). The inhibitory activity against β-lactamase positive bacteria (*S. aureus, S. pyogenes, K. pneumoniae* and *S. typhi*) is of interest because these organisms causative

Table 1. Results of antibacterial activity of crude extracts of *K. senegalensis*.

Extracts (mg/ml)	Diameter of zone (mm)					
	P.aeruginosa	***S. aureus***	***S. pyogenes***	***S. typhi***	***S. dysentriae***	***K. pneumoniae***
A100	9	14	12	10	13	9
A50	-	9	8	-	7	-
A25	-	-	-	-	-	-
E100	11	13	15	17	16	10
E50	9	10	12	13	12	-
E25	7	7	10	10	9	-
C100	15	14	17	15	14	13
C50	12	12	13	13	11	10
C25	9	10	10	10	9	8

A25, A50 and A100 = Aqueous extracts at 25, 50 and 100 mg/ml, respectively.
E25, E50 and E100 = Ethanol extracts at 25, 50 and 100 mg/ml, respectively.
C25, C50 and C100 = Acetone extracts at 25, 50 and 100 mg/ml, respectively.

Table 2. Antibiogram of *S. aureus, S. pyogenes, S. typhi, S. dysentraie, K. pneumoniae* and *P. aeruginosa.*

Antibiotics	Diameter of zone (mm)						
	Conc. (µg/ml)	***S. aureus***	***S. pyogenes***	***S. typhi***	***S. dysentraie***	***K. pneumoniae***	***P. aeruginosa***
Penicillin	50	±	±	±	±	±	±
Tetracycline	50	±	±	±	±	++	±
Amoxycillin	25	±	±	±	±	±	±
Cotrimoxazole	50	±	±	±	±	±	±
Nitrofurantoin	100	++	+	±	±	±	±
Gentamycin	10	+	+	±	±	±	±
Nalidixic acid	30	++	+	+	++	++	±
Ofloxacin	30	++	+++	++	++	+++	+++
Augmentin	30	+	±	+	±	±	±

± → Zone of inhibition ≤ 3.0 mm.
+ → Zone of inhibition 4 - 10 mm.
++ → Zone of inhibition 11 - 15 mm.
+++ → Zone of inhibition 16 - 20 mm.

Table 3. Effect of temperature on antibacterial activity of acetone extracts of *K. senegalensis* at 100mg/ml.

Organism	Diameter of zone		
	NT	**60°C**	**100°C**
S. aureus	++	+	+
S. pyogenes	++	+	+
S. typhi	++	++	+
S. dysentriae	++	++	+
K. pneumoniae	++	+	+
P. aeruginisa	+++	++	+

± → Zone of inhibition ≤ 3.0 mm
+ → Zone of inhibition 4 - 10 mm
++ → Zone of inhibition 11 - 15 mm
+++ → Zone of inhibition 16 - 20 mm
NT → Not treated extract

Table 4. Effect of pH on antibacterial activity of acetone extracts of *K. senegalensis* at 100 mg/ml.

Organism	Diameter of zone			
	NT	**6.0**	**7.0**	**8.0**
S. aureus	++	+	+	±
S. pyogenes	++	+	+	±
S. typhi	++	++	+	±
S. dysentriae	++	++	+	±
K. pneumoniae	++	+	+	±
P. aeruginisa	+++	++	+	±

NT → not treated extract
± → Zone of inhibition ≤ 3.0 mm
+ → Zone of inhibition 4- 10 mm
++ → Zone of inhibition 11-15 mm
+++ → Zone of inhibition 16-20 mm

agents of infectious death and morbidity in million of people around the globe annually (Baron et al., 1994; Nester et al., 1998). To keep out potential invaders, plants produce a wide range of selective antibacterial, antifungal and antiviral compounds either in a constitutive or an inducible manner (Cowan, 1999; Grayer and Harbone, 1994). The acetone extracts of *K. senegalensis* showed highest activity (diameter of zone 6 - 10 mm at 25 mg/ml concentration) on the test organisms while the aqueous extract showed selective inhibitory property on test organism. This may be due to better solubility of the active component in acetone compared to water and ethanol. Traditional healers claim that these extracts are also very effective in treatment of some infectious diseases and this is not surprising since it has been reported that apart from the antimicrobial property of phytochemicals, some phytochemicals stimulate the immune system to produce antibodies or cells to eliminate invading microorganisms (Humphrey and Mckeenna, 2001). If such components are present in these plant extracts, they could be used for the management of ailments caused by these pathogenic bacteria and give impressive results which could only be determined *in vivo*.

The antibacterial property of the extracts of *K. senegalensis* at 25 mg/ml on test organisms which are multi-resistant to commonly used antibiotics (penicillin, tetracycline, amoxicillin, cotrimoxazole, augmentin, and gentamycin at 50 µg/ml) and β- lactamase positive isolates like *S. aureus, S. pyogenes, K. pneumoniae* and *S. typhi* is quite interesting as it holds promise for effective management of ailments caused by these organisms. The MIC and MBC values of the extracts of *K. senegalensis* against the test organisms were in the range of 6.5 - 12.5 mg/ml and 6.5 - 25.0 mg/ml, respectively. This is important because it will give insight to dosage determination of *K. senegalensis* herbal formulation.

The stability of the antibacterial activity of the extracts of *K senegalensis* to heating at 100^{o}C for one hour further verifies the used of decoction of the plant by traditional healers in the ethnobatanical usage of the plant. Also, the stability of the antibacterial property at acidic pH is not surprising as Molan (1992) who reported that many natural antimicrobials including honey are active against animal pathogens at acidic pH.

Conclusion

This study reveals that the bark extracts of *K. senegalensis* may be used to manage some infectious diseases which call for further experiments on isolation and characterization of the active component as well as determining the toxicity of the components.

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