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PHYTOCHEMICAL SCREENING AND EVALUATION OF STEM BARK EXTRACT OF *KHAYA SENEGALENSIS* (MELIACEAE) ON METHICILLIN RESISTANT *STAPHYLOCCOCUS AREUS*

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ABSTRACT

Khaya senegalensis is an herbal medicinal plant, used locally in Nigeria and South Africa for the treatment of cough and sexually transmitted diseases. This antibacterial activity prompted the phytochemical and antibacterial investigation of this herbal plant. The stem bark of *K. senegalensis* was subjected to phytochemical studies using standard experimental procedures testing for secondary metabolites. The crude extract was evaluated for its antimicrobial activity using methicillin resistant strains of *Staphyloccocus aureus* MRSA. The result of the study revealed the presence of alkaloids, saponins, tannins and flavonoids in the plant extract. The extract exhibited significant antibacterial and MRSA activities against the tested organisms. The study therefore provides natural source for drugs used for the treatment of methicillin resistant strains (MRSA) infections.

Keywords: *K. senegalensis*, stem bark extract, MRSA, bacterial infections.

INTRODUCTI ION

Natural Products are an important source of new structures leading to drugs discovery in all major disease areas. In order to find new drugs in plants, it is necessary to screen plant extracts for biological activities, with the aim of obtaining novel compounds. Once novel compounds are suspected, they are generally isolated in order to have material available for further biological and toxicological testing (Newman and Cragg, 2007). The demands of traditional medicine from the public and the growing economic importance of traditional medicine have led to the increased interest on the part of academic communities and the government. The need to study a local medicinal plant *Khaya senegalensis* cannot be over emphasized.

Khaya senegalensis belongs to the family Meliaceae, and is commonly called African Mahogany and it is endemic in many African countries. *K. senegalensis* is a deciduous evergreen tree, 15-30 m high, up to 1 m in diameter, with a clean bole to 8-16 m. The plant is used in ethnomedicine for the treatment of various disease conditions such as rheumatoid arthritis, diarrhea and cough (Dalziel, 1948; Brian and Stanfield, 1966; Egwim *et al*., 2002). It has also been used as an anthelmintic, emetic, emmenagogue and in jaundice treatment (Gill, 1992). The effect of the extract on the rat kidney has also been reported (Joseph *et al*., 2003). The aqueous extract of stem bark has been reported to reduce anemia (Sanni *et al*., 2005), and inflammation (Lompo *et al*., 1998). Some limonoids have been isolated from the stems, barks, leaves and flowers of *K. senegalensis* (Nakatani *et al*., 2001; Adesida *et al*., 1971). They include phragmalin limonoids named khayanolides D and E, khayanosides, 2, 6-dihydrofissinolide and two mexicanolides named khayanone and 2-hydro- xyseneganolide. Traditional herbalists in many parts of Africa (especially northern part of Nigeria) have achieved success with the use of this plant for the treatment of tuberculosis and bacterial infections.

Methicillin resistant strains have assumed increasing importance both as a cause of nosocomial and community acquired infections (Jevons, 1961; Knox, 1961). Infections caused by MRSA resulted in increased lengths of hospital stay, health care cost, morbidity and mortality (Niclaes *et al.*, 1999). The infusion of the plant extract is used by herbal healers for the treatment of cough and wounds and boils. This biological activity necessitated the scientific validation and justification of its use as herbal remedy. This study was therefore aimed at searching for natural products that could be used to combat the effect of MRSA infections among the natives since these herbal medicinal products are cheap and readily available in the tropical forest.

MATERIALS AND METHODS

Plant material

The plant (stem bark) was collected around January 2008 in Otukpo Local Government, Benue State, Nigeria. Botanical identification and authentication was done by a staff of the Department of Pharmacognosy, University of

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Benin. [Voucher specimen was deposited, no. AF 2008KS].

Extraction and preparation

The stem bark of *K. senegalensis* was dried and reduced to a fine powder with the aid of a mechanical blender. The powdered sample (600g) was extracted with 700ml of distilled water by maceration for 48 hours. The residue (54.28g) was stored in a refrigerator at -4^{o}C until use.

Phytochemical Screening

The crude plant sample was subjected to phytochemical screening testing for alkaloids, saponins, tannins, flavonoid and irridoids using standard experimental procedures of Trease and Evans (2002).

Biological assay

MRSA organisms isolated from clinical specimens obtained from patients visiting the University of Teaching Hospital, Benin City, were used for the study.

Drugs and Microbial Media

The antimicrobial agents used include amoxicillin (Smithkline Becham Pharmaceuticals UK), ciprofloxacin (Ranbaxy Pharmaceuticals, India), nutrient broth and Mannitol Salt agar (MSA) Oxoid, England.

Susceptibility Testing

The agar diffusion method was used to determine the antimicrobial activity of the extract (NCCLS, 1999; Barry and Thornsberry, 1985; Cruickshank *et al*., 1975). The susceptibility of isolates to methicillin using the E-test strips (AB Biodisk) was carried out by the disk diffusion method. Each isolate was grown in the nutrient broth for 18 hours. 2ml of the culture was aseptically used to seed molten mannitol salt agar which has been cooled to 45^{o}C, mixed gently and poured into sterile Petri dishes and allowed to solidify. The extract dissolved in dist. water was tested at 40mg/ml concentration. 2ml was delivered into wells (8mm diameter) bored into the surface of the already seeded MSA plates. Standard antibiotics concentration of ciprofloxacin (10mg/l) and amoxicillin (25mg/l) were assayed along using the agar well diffusion technique. The plates were incubated at 37^{o}C for 24 hours and zones of inhibition measured in mm diameter and recorded.

Determination of Minimum Inhibitory Concentration (MIC)

The modified agar well diffusion technique (Okeke *et al*., 2001) was used to determine the MIC of the extract. Two fold serial dilutions were prepared by first reconstituting in sterile distilled water, diluting to achieve a decreasing concentration range of 50 – 0.78 mg/ml. 0.1ml of each dilution was introduced into MSA plates seeded with standard inoculum (approx. 10^5 cfu/ml) of the test bacterial cells. All test plates were incubated at 37^{o}C for 24 hours. The test concentration of extract showing a clear zone of inhibition was taken as the MIC.

Statistical Analysis

The Student's *t*-test was used for the analysis of data (Dixon and Massey, 1969).

RESULTS AND DISCUSSION

The phytochemical testing (Table 1) showed that the plant contained flavonoids, saponins tannins and alkaloids in agreement with the result of Gbile (1986). The results of the antibacterial activity of the extract (inhibition zone diameter in mm) at 40mg/ml concentration, ciprofloxacin 10mg/ml and amoxicillin 25mg/ml against ten strains of are given in table 2. The dissolution solvent used for the extract did not show any activity at the volume used. The crude extract showed significant activity against all MRSA isolates whereas the standard antibiotics showed absence of activity against one and two isolates for ciprofloxacin and amoxicillin respectively.

Table 1. Phytochemical components of aqueous extract of *K. senegalensis.*

Phytochemical compositions	Presence of components
Alkaloids	+
Tannins	+++
Saponins	+++
Flavonoids	++++

+; presence of low amount of component
+++; presence of moderate amount of component
++++; indicates presence of high amount of component
-; indicates absence of components

The result further showed that the extract exhibited similar activity (zone diameter) as amoxicillin against the bacterial strains. The MIC of the extract (Table 3) is between 1.56mg/ml to 6.25mg/ml against all isolates used in this evaluation.

The observation of zones of inhibition against MRSA isolates indicates the presence of antibacterial activity which confirmed its ethnomedicinal use as anti-infective agent in bacterial infections. Further study on the prefractionation and isolation of the bioactive chemical constituents when carried out will lead to new compound which could be added to the potential list of antibacterial agents against MRSA. The work reveals for the first time marked activity of *K. senegalensis* against different isolates of MRSA at the concentration tested.

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Table 2. *In vitro* antibacterial activity of aqueous extract and standard antimicrobial agents.

Micro organisms	Extract (40mg/mL)	CP (10 mg/mL)	AMX (25 mg/mL)	DW (0.2mL)
a	18.5 ± 0.70	29.0 ± 0.00	-	-
b	15.5 ± 0.70	28.5 ± 4.9	25.5 ± 74	-
c	19.5 ± 0.70	27.0 ± 0.01	22.0 ± 0.02	-
d	19.0 ± 1.40	30.5 ± 0.70	16.5 ± 2.10	-
e	17.0 ± 1.40	27.0 ± 1.40	22.0 ± 0.00	-
f	20.5 ± 3.50	24.5 ± 0.71	19.5 ± 3.51	-
g	20.0 ± 1.40	31.5 ± 0.72	17.5 ± 0.72	-
h	20.0 ± 0.05	26.0 ± 0.01	14.5 ± 0.71	-
I	21.5 ± 4.90	-	-	-
J	17.5 ± 0.70	25.5 ± 0.70	20.0 ± 0.01	-

Key: a – j, isolates of MRSA; CP, Ciprofloxacin; AMX, Amoxicillin; DW, distilled water; #, mean of two replicates determinations; -, absence of zone of inhibition

Table 3. Minimum Inhibitory concentration of MIC aqueous extract of *K.senegalensis.*

Organisms	MIC (mg/mL)
a	3.13
b	6.25
c	3.13
d	3.13
e	3.13
f	1.56
g	3.13
h	3.13
I	1.56
J	3.13

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