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Full Length Research Paper

Phytochemical and antimicrobial studies on stem bark extract of *Sterculia setigera,* Del.

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Antibacterial activity using broth and disc diffusion methods, and phytochemical screening was carried out on crude stem bark extracts of *Sterculia setigera,* a plant used ethnomedically by various communities in Nigeria. These extracts showed low antibacterial activity against *Staphylococcus aureus, Proteus mirabilis* and *Klebsiella pneumonia.* Phytochemical screening revealed the presence of alkaloids, phenolics/tannins in acetone and methanol extracts, and triterpenoid in boiling petroleum ether extract.

Key words: *Steculia setigera*, extracts, antibacterial activity, alkaloids, triterpenoid, phenolics/tannins.

INTRODUCTION

The plant, *Sterculia setigera* Del. (Family: Sterculiaceae) is known by different indigenous cultural communities in Nigeria: Hausa– "Kukuki"; Nupe– "Kokongiga"; Fulani– "bo'boli"; Kanuri– "Sugubo"; Yoruba– "Ose-awere", "eso funfun"; Etulo– "Idafu"; Idoma– "Ompla", "Upula"; Igede– "Upuru"; Igala– "Ufia"; Tiv– "Kume-ndul", "Kumenduur" (Adjanahoun et al., 1991; Igoli et al., 2002, 2003, 2005; Tor-Anyiin et al., 2003; Keay, 1989; Keay et al., 1964; Agishi, 2004). It is a savannah tree, widespread in savannah areas of tropical Africa. The seeds are with yellow aril and the tree is found in open savannah woodlands, often characterised by stony hills (Adjanahoun et al., 1991; Keay et al., 1964; Agishi, 2004).

This plant is used in trado-medicine by various indigenous communities. For instance, the Yorubas of Nigeria use a black soap prepared from black powder obtained from burnt mixture of the fruits and seeds in dermatosis (Adjanahoun et al., 1991). In Sudan, dried bark hot water extract is used for jaundice (Almagboul et al., 1995; El-Kamali and El-Khalifa, 1999) and dried stem bark for treating wounds (El-Kheir and Salih, 1980). Stem bark decoction is used to treat diarrhea (Almagboul et al., 1995) by the Igedes, its bark as a mixture is macerated and used against dysentery by some tribes in central Nigeria (Igoli et al., 2002, 2003).

The acceptance of traditional medicine as an alternative and complementary form of healthcare and the development of microbial resistance to the available antibiotics has led to a renewed interest in investigating the antimicrobial activity of medicinal plants. Bacterial infections are an important and increasing cause of mortality in Africa (Iwu et al., 1999) and are listed in the first position among the common microorganisms responsible for opportunistic diseases associated with acquired immune deficiency syndrome (AIDS) (Kone et al., 2004).

Screening of plant extracts for antimicrobial activity has shown that higher plants represent a potential source of new anti-infective compounds (Press, 1996). Only a small fraction of the known plant species have been evaluated for the presence of antimicrobial compounds, and thus, it is necessary to increase the efforts in collecting and screening plants for the development of novel and environmentally safe antimicrobial agents (Stein et al., 2005).

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Abbreviations: PE, Petroleum ether; **AC,** acetone; **ME,** methanol; **PEE,** petroleum ether extract; **ACE,** acetone extract; **MEE,** methanol extract.

Considering the health problems associated with bacteria infections and the potentials of higher plants as reservoir of potentially useful antimicrobial agents, it has become natural to screen plants used in phytotherapy against pathogenic agents. Such screenings will substantiate folkloric claims as well as contribute to the search for newer and safer chemotherapeutic agents.

Some of the ailments treated with this plant are bacterial related diseases. This plant is yet to be systematically investigated. Thus we reported here, the first in a series of investigations, antibacterial activity studies on the stem bark extracts of this plant from Benue State, Nigeria.

MATERIALS AND METHODS

The stem bark of *S. setigera* (2.5 kg) was collected in 2003 from Engineering Complex area of the University of Agriculture, Makurdi and was air-dried for three weeks. The plant was identified by the Department of Forestry and Wildlife, University of Agriculture, Makurdi, where specimen sample has been deposited. It was powdered using pestle and mortar and excess fibers were removed. The powdered material was stored in polythene bags at room temperature until needed.

Extraction and purification

Sixty grams (60 g) of dried and pulverised, fibre-free stem bark of *S. setigera* was extracted exhaustively via maceration with 3 x 300 cm^3 petroleum ether (40 - 60°C), acetone and methanol, each at room temperature for 24 h. Excess solvent was removed *in vacuo* to give crude extracts as PEE, ACE and MEE from petroleum ether, acetone and methanol, respectively.

Phytochemical screening

Crude petroleum ether extract (PEE) was screened phytochemically using standard procedures (Harborne, 1984; Sofowora, 1984).

The disc diffusion method

The paper disc diffusion assay technique (Akpuaka et al., 2003 and Nuhu et al., 2000) was done.

Preparation of the medium

The nutrient agar medium was prepared by dissolving 7.0 g of agar in 250 ml of distilled water in a conical flask and heated to dissolve. The solution was sterilized in an autoclave at 121°C for 15 min, cooled and poured into Petri dishes to set.

Preparation of culture medium and inoculation

Cultures of *Staphylococcus aureus, Proteus mirabilis* and *Klebsiella pneumoniae* (which were supplied by the bacterial research division of National Veterinary Research Institute, Vom (NVRI) were obtained from TOSEMA Diagnostic Laboratories. Pure isolates were obtained by sub-culturing unto fresh nutrient agar plates. The bacteria were separately used to inoculate the Petri dishes. The plates were incubated at 37 ± 2°C for 24 h.

Assay of extracts

Two different concentration of each extract were obtained by suspending 250 mg of each extract in 6.0 ml of absolute ethanol and the volume was made up to 10.0 ml using sterile distilled water to give a concentration of 25 mg/ml (25,000 µg/ml). These also served as stock solutions.

The second concentrations were obtained by diluting 2.0 ml each of the stock solutions with 2.0 ml of sterile distilled water giving a concentration of 12.5 mg/ml (12,500 µg/ml). Sterile 6 mm Whatman's filter paper discs were soaked in the extracts and placed on the plates and incubated for 24 h at 37 ± 2°C. The plates were examined for clear zones of activity. The zones of inhibition were measured using transparent plastic meter ruler.

Broth diffusion method

Equal volumes (1.0 ml) each of the extract solutions (25 and 12.5 mg/ml) were mixed with same volume (1.0 ml) of an overnight broth culture of *S. aureus* and *P. mirabilis* to give solutions of final concentrations of 12.5 and 6.25 mg/ml, respectively, in a test tube. These were then incubated at 37 ± 2°C for 24 h and observed for turbidity. All the tubes were then sub-cultured unto blood agar plates and again incubated at 37 ± 2°C for 24 h. The plates were observed for the presence of bacterial growth microscopically.

RESULTS AND DISCUSSION

The results of the antibacterial screening are as shown in Tables 1 and 2. For broth diffusion method, there was turbidity in the petroleum ether extract (PEE) at a concentration of 6.25 mg/ml for *S. aureus* and *P. mirabilis* (Table 1). Also, at a concentration of 12.5 mg/ml, there was turbidity in methanol 1 (ME 1), acetone (AC) and PE extracts for *P. mirabilis*. At the concentration of 6.25 mg/ml, extracts ACE, MEE 2 and MEE 1 did not show any turbidity for both organisms; at 12.5 mg/ml, all the extracts showed no turbidity for *S. aureus* and only ME 2 for *P. mirabilis*. These results indicated that the extracts are either bacteriostatic or bactericidal or both, where there was no turbidity.

However, the overnight broth sub-culture results showed growth in extracts PEE, ACE, MEE 1 and MEE 2 for both organisms at the lower concentration; and growth for MEE 1, ACE and PEE for *P. mirabilis* (a Gram negative pathogen) at the higher concentration. There was no growth observed in ME 2 extract for both organisms at the concentration of 12.5 mg/ml, and only for *S. aureus* (a Gram positive pathogen) for ME 1 and AC extracts at the same concentration. The absence of growth is a confirmation of the fact that MEE 2 is bactericidal to both *S. aureus* and *P. mirabilis* at a concentration of 12.5 mg/ml. Extracts ME 1 and AC were bactericidal at 12.5 mg/ml for only *S. aureus*.

The results of the disc diffusion method are as shown in Table 2. These results indicate that only ME 1 and ME 2 extracts showed some level of antibacterial activity on all the tested organisms: *S. aureus, P. mirabilis* and *K. pneumoniae* and this activity was only at the higher con-

Table 1. Turbidity and overnight growth culture of extracts at concentrations of 6.25 and 12.5 mg/ml.

Extract	Concentration (mg/ml)	Organism						Streptomycin (30 µg/ml)	
		S. aureus		*P. mirabilis*		*K. pneumoniae*			
		Turbidity	Growth	Turbidity	Growth	Turbidity	Growth	Turbidity	Growth
PEE	6.25	T	G	T	G	NT	NT	-	-
ACE	6.25	-	G	-	G	NT	NT	-	-
MEE 2	6.25	-	G	-	G	NT	NT	-	-
MEE 1	6.25	-	G	-	G	NT	NT	-	-
MEE 2	12.5	-	-	-	-	NT	NT	-	-
MEE 1	12.5	-	-	T	G	NT	NT	-	-
ACE	12.5	-	-	T	G	NT	NT	-	-
PEE	12.5	T	G	T	G	NT	NT	-	-

T = Turbidity seen; - = no turbidity or no growth; G = growth occurred; NT = not tested.

Table 2. Antibacterial activity and zone of inhibition (mm) of extracts of *S. setigera* by disc diffusion.

Extract	Concentration	Organism			
		S. aureus	*P. mirabilis*	*K. pneumoniae*	*S. aureus*
MEE 2	25 mg/ml	10	8	10	15
	12.5 mg/ml	-	-	-	-
MEE 1	25 mg/ml	8	6	20	-
	12.5 mg/ml	-	-	-	-
ACE	25 mg/ml	-	-	15	-
	12.5 mg/ml	-	-	-	-
PEE	25 mg/ml	-	-	-	-
	12.5 mg/ml	-	-	-	-
Streptomycin	25 µg/ml	30	NT	NT	NT
Levoxin	30 µg/ml	NT	30	35	30

NT = Not tested/done; - = no inhibition.

centration of 25 mg/ml. *Klebsiella pneumoniae* showed higher sensitivity to these extracts than the other organisms. There was a good level of inhibition of this organism by the acetone (AC) extract at a concentration of 25 mg/ml.

When compared to the control/standard antibacterials used in this study (25 µg/ml for streptomycin and 30 µg/ml for levoxin), the level of antibacterial activity of these extracts was low, considering the high concentration required to elicit any activity.

Phytochemical analysis of these extracts (Table 3) is indicative that MEE and ACE contain alkaloids, while PEE, a triterpenoid-lupeol(20). Alkaloids have been implicated in the inhibition activities of many bacterial species (Akpuaka et al., 2003; Nuhu et al., 2000; Tor-Anyiin, 2009; Alinnor, 2007, 2008; Hassan et al., 2004). The low antibacterial activity shown by these extracts may therefore be attributed to the presence of these alkaloid(s). Although the PEE is known to contain lupeol (a triterpenoid), and triterpenoids are known to possess a wide range spectrum of biological activities including bactericidal (Patoca, 2003), this result shows that lupeol probably has no such biological activity on the tested microorganisms.

Also, Table 2 shows the effect of storage/shelf-life on the biological activity of these extracts. MEE 1 and MEE 2 were obtained in 2006 and 2008, respectively. MEE 2 has higher antibacterial activity (higher zone of inhibition) on *S. aureus* and *P. mirabilis* than MEE 1. However, with *K. pneumonia*, it appears the older/longer the storage, the greater or higher the antibacterial activity/zone of inhibition. Similarly, MEE 2 was the only extract that

Table 3. Phytochemical screening of PE, ME and AC extracts.

Test	Reagent	Extract		
		PEE	**AC**E	**ME**E
Alkaloid	Dragendorf's	-	+	+
	Mayer's	-	+	+
Tannins/phenolics	5% $FeCl_{3aq}$	-	+	+
	10% KOH_{aq}	-	+	+
Saponins	$NaHCO_{3aq}$	-	-	-
	Emulsion (olive oil)	-	-	-
	Foaming test	-	-	-
Phloba tannins	HCl_{aq}	-	-	-
Flavonoids	Mg-Conc. HCl	-	-	-
	Conc. H_2SO_4	-	-	-
Free/combined anthraquinones	Borntrager's	-	-	-
	Conc. H_2SO_4-Bz-NH_3	-	-	-
Cardiac glycosides	Kedde's	-	-	-
	Keller-kilianis	-	-	-
	Salkowski's	+	-	-
Sterols/triterpenes	Liebermann-Burchards	+	-	-
Reducing sugars	Fehling's I & II	-	-	-

showed neither turbidity nor growth for both *S. aureus* and *P. mirabilis* at a concentration of 12.5 mg/ml in the broth diffusion method (Table 1).

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