NJBAS

Available online at http://www.ajol.info/browse-journals
Nigerian Journal of Basic and Applied Science (2009), 17(2):235-239

ISSN 0794-5698

Phytochemical and Antibacterial Properties of Leaf Extract of *Stereospermum kunthianum* (Bignoniaceae)

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ABSTRACT: Phytochemical screening and studies on antibacterial activity of leave extract of *Stereospermum kunthianum* -a plant widely used by traditional medical practitioners in Northern Nigeria, were carried out on *Escherichia coli*, *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella* spp, *Salmonella* spp and *Aeromonas hydrophila* using agar well diffusion technique. Preliminary phytochemical screening revealed the presence of sterols, coumarins, higher fatty acids and the absence of flavones aglycone and alkaloids in the leave extract. The activity of the extract was more potent at 30 $mgml^{-1}$ with zone of growth inhibition against *S. aureus* (35 mm), *Klebsiella* spp (28 mm) and *A. hydrophila* (28 mm). The least activity was on *P. aeruginosa* (9 mm). The minimum inhibitory concentration (MIC) of the extract was 2.09 $mgml^{-1}$ on *Klebsiella* spp and *S. aureus* while 4.17 $mgml^{-1}$ on *Salmonella* spp and *A. hydrophila*. Similarly, the minimal bactericidal concentration (MBC) was 2.09 $mgml^{-1}$ on *S. aureus* and 4.17 $mgml^{-1}$ on *Klebsiella* spp. However, higher MIC and MBC of 16.67 $mgml^{-1}$ were observed on *P. aeruginosa*. The spectra of activities displayed by the extract can be attributed to the presence of these phytochemicals which signifies the potential of *S. Kunthianum* as a source of therapeutic agents. This therefore, supports the traditional medicinal use of *S. kunthianum* in Zaria, Nigeria.

KEYWORDS: *Stereospermum kunthianum,* phytochemical, antibacterial activity, Bignoniaceae

INTRODUCTION

The use of plants for medicinal purposes is an age old tradition in Africa (Karou, *et al.,* 2006). Today, more than 70% of the people in Africa refer to traditional healers concerning health issues (Kamanzy *et al*., 2002). The World Health Organization (WHO) encourages the inclusion of herbal medicines of proven safety and efficacy in the healthcare programs of developing countries because of the great potential they hold in combating various diseases (Amos *et al.,* 2001). Plant extracts represent a continuous effort to find new compounds with the potential to act against multi-resistant bacteria. Approximately 20% of the plants found in the world have been submitted to pharmacological or biological test, and a substantial number of new antibiotics introduced on the market are obtained from natural or semi-synthetic resources (Mothana and Lindequist, 2005). Thus, it is anticipated that phytochemicals with adequate antibacterial efficacy will be used for the treatment of bacterial infections.

Stereospermum kunthianum is a deciduous shrub or tree found in the dry areas of deciduous forest, woodland, bush, rocky outcrops, termite mound and margins of evergreen forests. The plant is known locally as *Sansami* (Hausa) and is widely used by traditional medical practitioners in Northern Nigeria for the treatment of various human diseases. The pods are chewed with salt to treat coughs and are used in treatment of ulcers, leprosy, skin eruptions and venereal diseases, while the stem bark decoction or infusion is used to cure bronchitis, pneumonia, cough, rheumatic arthritis and dysentery (Gill, 1992). The twigs are chewed to clean teeth and to treat toothache. The roots and leaves have been found useful in treating venereal diseases, respiratory ailments, gastritis (Gill, 1992) and a disease called "*Rana*" with symptoms of haematuria (Dalziel, 1955). Previously, the antiplasmodial activities of naphthaquinones and one anthraquinone from the lipophilic root bark extract was reported (Onegi *et al.,* 2002). Antidiarrhoeal (Ching *et al.,* 2008),

analgesic activities of stem bark (Ching *et al.,* 2009), as well as the anthelmintic activity of ethanol leave extract has been reported (Monglo *et al.,* 2006).The present study therefore, reports the phytochemical screening and antibacterial activity of the lipophilic leaves extract of *Stereospermum kunthianum* with a view to understanding the molecular basis of some of its therapeutic uses in traditional medicine, which had not been investigated hitherto.

MATERIALS AND METHODS

Collection and identification of plant material

Whole fresh plant bearing fruits and leaves growing wild was collected in Zaria in December, 2005. The plant was authenticated at the herbarium unit of the Department of Biological Sciences, Ahmadu Bello University, Zaria, where a voucher number 1381 was deposited. Leaves were air dried at room temperature for 21 days to a constant weight. The dried leaves were pulverized to coarse powder using mortar and pestle and sieved with 20 mesh (British standard). The powdered leaves were stored for further studies.

Extraction

One hundred and sixty five grams (165 g) of the powdered leaves were soxhlet extracted with petroleum ether (60-80^{o}C) until the draining solvent was clear (Chhabra, *et al.,* 1982). The solvent was removed under reduced pressure below 50^{o}C to give a crude extract. The crude extract was further dried in vacuum desiccator over anhydrous copper sulphate to give a dry solid of the extract (6.64 g). This was used for phytochemical screening and bioassay.

Phytochemical screening

Phytochemical screening of the extract was carried out to identify the constituents, using standard phytochemical methods as described by Sofowora (1993) and Trease and Evans (1996).

Evaluation of antimicrobial activity

Clinical isolates of *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Salmonella* spp*, Aeromonas hydrophila* and *Klebsiella* spp were obtained from the Department of Microbiology, Ahmadu Bello University, Zaria. The susceptibilities of the test organisms to the plant extract were assayed as described by Karou *et al.* (2006). Briefly, the test organisms from growth on nutrient agar incubated at 37^{o}C for 18hr were suspended in saline solution (0.85% NaCl) and adjusted to match a turbidity of 0.5 (10^{8} cells/ml) McFarland standard. The standardized suspension was used to inoculate the surfaces of Mueller Hinton agar plates (90 mm in diameter) using sterile cotton swab. Six millimetre (6mm) diameter wells were punched using cork borer in agar and filled with the desired concentrations (30 $mgml^{-1}$, 20 $mgml^{-1}$ and 10 mg/ml^{-1}) of the extracts. Commercial antibiotics (augmentin (30 µg) and gentamicin (10 µg) Abtek Code NO. GC 20/p) were used as reference standards to determine the sensitivity of the isolates. Discs were directly placed onto the bacterial culture. The plates were allowed to stand for 2 h at room temperature for extract to diffuse into the agar and then incubated at 37^{o}C over night. Antibacterial activities were evaluated by measuring inhibition zone diameters. The entire test was conducted in duplicate.

Determination of MIC and MBC

Minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of the plant extract were determined using agar dilution technique as described by Musa *et al.* (2000). The required extract concentrations were prepared in 20 ml sterile melted Mueller-Hinton agar each and poured aseptically into sterile Petri dishes (90 mm) in duplicates and allowed to solidify. Sterile filter paper discs (6 mm diameter) were aseptically placed firmly on the agar in triplicate per organism. Using a micropipette, 20 µl of the standardized inoculums was then placed on the paper discs aseptically and incubated at 37^{o}C for 18 h. The lowest concentration that inhibited growth around the edges of the filter paper discs was taken as the MIC. Filter paper discs in the determination of MIC that showed no visible growth around their edges were sub cultured into Mueller-Hinton broth (supplemented with 3% Tween 80 and 0.5% egg lecithin) and incubated at 37^{o}C for 48 h. Concentrations at which there was no growth or turbidity were taken as the MBCs of the plant extract.

RESULTS AND DISCUSSION

Preliminary phytochemical screening of lipophilic leaves extract of *S. kunthiamun* revealed the presence of sterols, coumarins, higher fatty acids

and the absence of flavones, aglycone and alkaloids (Table 1). Antibacterial activity indicated by the zone of growth inhibition ranged from 6-35 mm. *S. aureus* had the largest zone of inhibition (35 mm), *Klebsiella* spp (28 mm) and *A. hydrophila* (28 mm) at concentration of 30 $mgml^{-1}$, while *Pseudomonas aeruginosa* had the smallest zone of inhibition (9 mm) (Table 2). The MIC and MBC showed that *Klebsiella* spp and *S. aureus* had the lowest MIC (2.09 $mgml^{-1}$), while *Salmonella* spp and *A. hydrophila* had MIC value of 4.17 $mgml^{-1}$. *Pseudomonas aeruginosa* and *E. coli* had the highest MIC values of 16.67 and 8.34 $mgml^{-1}$ respectively. Higher MBC of 16.67 $mgml^{-1}$ was observed against *P. aeruginosa*, (Table 3).

Table 1: Phytochemical profile of the leaf extract of *S. kunthianum*

Constituent	Occurrence
Sterols/ triterpenes	+
Flavone aglycone	-
Coumarins	+
Alkaloids	-
Higher fatty acids	+
Glycosides	-
Carbohydrates	+

+ = present, - = absent

Table 2: Diameter of zones of inhibition (mm) by *S. kunthianum* leaf extract against the bacteria

Test organism	Zones of inhibition(mm)/concentration ($mgml^{-1}$)		
	30 $mgml^{-1}$	20 $mgml^{-1}$	10 mg/ml^{-1}
Escherichia coli	23	17	12
Staphylococcus aureus	35	28	22
Klebsiella spp	28	21	16
Salmonella spp	25	24	18
Aeromonas hydrophila	28	24	19
Pseudomonas aeruginosa	9	6	6

Values greater than 6mm indicate some activity

Table 3: MIC and MBC of *S. kunthianum* leaf extract against the test organisms ($mgml^{-1}$)

Test organism	MIC ($mgml^{-1}$)	MBC ($mgml^{-1}$)
Escherichia coli	8.30	10.41
Staphylococcus aureus	2.09	2.09
Klebsiella spp	2.09	4.17
Salmonella spp	4.17	8.34
Aeromonas hydrophila	4.17	6.26
Pseudomonas aeruginosa	16.67	16.67

Antibacterial activity of the lipophilic leave extract of *S. kunthianum* has demonstrated significant antibacterial activity against most of the test organisms. The extract was more potent on gram-positive *Staphylococcus aureus* with maximum zone of growth inhibition of 35 mm, at 30 mg/ml. Although gram-negative bacteria tend to have higher intrinsic resistance to most antimicrobial agents (Ndukwe *et al*., 2005), In spite of this, impressive activity against gram-negative bacteria was observed (*E. coli* 23 mm, *S. typhi* 25 mm, *K. spp*. 28 mm and *A. hydrophila* 28 mm). Low MIC and MBC values (2.09 mg/ml) demonstrated by the extract especially on *K. spp*. and *S. aureus* is an indication that the lipophilic phytoconstituents have bactericidal potential. Similarly, the MIC (4.17 mg/ml) and MBC (6.26 mg/ml) exhibited against *Aeromonas hydrophila* -a gram negative bacillus capable of causing septicemia in fish and amphibians as well as gastroenteritis, cellulitis, meningitis, bacteraemia, soft tissue infections, peritonitis, broncho-pulmonary infections, in humans with compromised immune systems (Janda 1991; Chang *et al*. 1997), is yet another indication of the therapeutic potentials of the lipophilic extract against such diseases.

Owing to their popular use as remedies for many infectious diseases, plants with secondary metabolites such as terpenoids, flavonoids, coumarins and steroids have been found *in vitro* to have antimicrobial properties (Sibanda and Okoh, 2007; Cowan, 2002). Terpenoids for instance, are synthesized from acetate units which share origins with fatty acids. Their tremendous activities against bacteria have been reported (Ahmed *et al*., 1993; Barre *et al*., 1997; Amaral *et al*., 1998). It is perhaps not surprising that higher fatty acids were found present in the lipophilic extract (Table 1). Coumarins have been found to exhibits great diversity with respect to growth inhibition activity against both Gram positive and negative bacteria (Kayser and Kolodziej, 1999). Thus, the spectra of activity displayed by the crude lipophilic extract can be explained by the combined effects of terpenoids, coumarins and steroids or due to synergism. The purified components may have even more potency with respect to inhibition of microbes. Further work on the phytoconstituents isolation and purification of the bioactive components are recommended.

In conclusion, the significant activity exhibited by the lipophilic extract against clinical isolates of S. *aureus* (35 mm), *E. coli* (23 mm) *Klebsiella* spp and *A. hydrophila* (28 mm) and *Salmonella* spp (25 mm) that are associated with various infectious diseases, have provided scientific justification in this research for the ethno medicinal uses of the plant in Zaria, Kaduna State, Nigeria.

ACKOWLEDGEMENT

We wish to acknowledge the contribution of Ahmadu Bello University, Zaria, for providing the facilities for conducting the research.

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