



## Research Article

# Antibacterial Activity of *Stereospermum kunthianum* against Some Bacterial Isolates

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## ABSTRACT

Medicinal plants are known to possess bioactive compounds that can be used in the treatment of diseases. Despite the existence of synthetic antimicrobial agents, resistant strains of pathogenic microorganisms are continually appearing. Also, high cost of synthetic drugs and low potency of some synthetic drugs imposed the need for development of new drugs of herbal origin. This study was carried out to determine the antibacterial activities of *Stereospermum kunthianum* leaf and stem extracts against clinical and reference isolates of *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhi*. The phytochemicals and antibacterial activity of the plant extracts were carried out using standard laboratory techniques. The extracts were tested for antibacterial activities and screened for phytochemicals. Phytochemical screening revealed the presence of Tannins, Saponins, Flavonoids, Alkaloids, Phenol, Terpenoids and Steroids. The leaf extracts were more effective than the stem extract having zone of inhibition ranging between (26.12±0.17-10.25) for the leaf and (18.12±0.00-10.00±0.00) for the stem. The methanol crude extracts of the leaf exhibited the highest activities against both clinical and reference isolates with mean zone of inhibition ranging between (12.12±0.15-26.12±0.17) with MICs and MBCs ranging between 12.5-50mg/ml. These compounds could be responsible for the antibacterial activities.

**Keywords:** *Stereospermum kunthianum*; Phytochemicals; Potency; Antibacterial; Medicinal Plants

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## INTRODUCTION

Plant products or natural products can help prevent and treat disease by increasing antioxidant activity, inhibiting bacterial development, and modulating genetic pathways (Muhammad, 2016). Medicinal plants are gaining a lot of attention these days because of their unique properties as rich source of therapeutic phytochemicals that could lead to the development of new medications. The majority of phenolics and flavonoids have been shown to improve health and prevent cancer (Azawanida, 2015). The plant kingdom is a rich source of structural biodiversity and offers a variety of natural products. Plants have been utilized to produce

various types of medicines for thousands of years. More recently, the use of plants has different chemical compounds like secondary metabolites with many biochemical and bioactivity properties (Suja and Thajun, 2018).

*Stereospermum kunthianum* belongs to the family *Bignoniaceae* also referred to as pink jacaranda, in English. It is known locally as "Sansami" among the Hausa of northern Nigeria, umana among the Tiv of middle belt of Nigeria, "Ayada" among the Yoruba of south West Nigeria and "Alakiriti" among the Igbo of South East Nigeria (Nenge *et al.*, 2021).

*Stereospermum kunthianum* is a plant that is used in traditional medicine to cure gastritis, asthenia, and respiratory disorders, as well as wound healing. Bronchitis, pneumonia, cough, rheumatoid arthritis, and diarrhea are treated with a stem bark decoction or infusion (Sarr *et al.*, 2021).

Infectious diseases have repeatedly been identified as one of the most serious threats to human health around the world. Infectious diseases accounted for 61.7 percent (5.9 million) of the 9.6 million fatalities in Africa in 2013, according to the World Health Organization WHO (Harriet *et al.*, 2020). Pathogenic bacteria are capable of causing diseases in humans which include food poisoning, gastritis, ulcers, wound infection, UTI meningitis among others (Williams *et al.*, 2019). High cost of antibiotics has made treatment of diseases very difficult. Therefore, there is need to explore plant extracts which can act in the same biological pathways as pharmaceutical medicines and pose a lesser side effect. The aim of this work was to determine the antibacterial activity of *Stereospermum kunthianum* against some bacterial isolates.

## MATERIALS AND METHOD

### Identification of Plant Material

The leaves and stem bark of *Stereospermum kunthianum* were collected from Sumaila, LGA, Kano State. The plant was identified by a botanist at Biological Sciences Department Kaduna State University Kaduna with voucher number 2114. The plant leaves and stem barks were washed thoroughly with water and cut into pieces and dried under shade at room temperature for three weeks. The dried plant parts were pounded using mortar and pestle under aseptic condition then stored in a clean polythene bag for extraction purpose.

### Extraction of the Plant Extracts

Each powdered plant part was subjected to extraction using methanol and distilled water. A total of 150g of the powdered leaves and stem bark was dissolved in 750ml of methanol and placed in an orbital shaker for 24 hours. The extracts obtained was filtered with no 1 Whatman filter paper and was allowed to evaporate. The same procedure was adapted using distilled water. The crude extracts were stored in a sterile airtight container until required.

### Phytochemical Analysis

Phytochemical analyses of the plant extracts were carried out using standard laboratory techniques for qualitative determination of the following bioactive compounds: alkaloids, glycosides, saponins, steroids phenols, tannins flavonoids, diterpenes using the method of Ogbeba *et al* (2017).

### Bacterial Isolates Collection

A total of 75 isolates (25 each) *Escherichia coli*, *Salmonella Typhi* and *Staphylococcus aureus* were collected from Yusuf Dan Tsoho Memorial Hospital Tudun Wada Kaduna. The isolates were sourced from urine, stool and wound samples of patients. Similarly, the Reference isolates of (*S. aureus* ATCC 6538, *E. coli* ATCC 43888, and *S. Typhi* ATCC 48765) were collected from National Veterinary Research Institute (NVRI), VOM, Jos, Plateau State to serve as positive control.

### Reconfirmation of Bacterial Isolates

All media listed were prepared according to manufacturer's instruction. *Escherichia coli* isolates were sub cultured on Eosin methylene blue Agar the colonies appeared as a green metallic sheen, *Salmonella Typhi* were subcultured on *Salmonella shigella* agar (SSA) the colonies appeared as transparent with black centered and *Staphylococcus aureus* were sub cultured on mannitol salt agar the colonies appeared as golden yellow.

### Antibacterial Susceptibility Test

#### Preparation of Plant extracts

The concentrations of the plant extracts were prepared using the method of Abubakar and Haque (2020). One gram (1g) each of water and methanol crude extracts of leaf and stem of *S. kunthianum* were weighed and 10ml each of 10% dimethyl sulfoxide (DMSO) was added to get 100mg/ml solutions of each concentration. Utilizing two-fold serial dilution, concentrations of 50mg/ml, 25mg/ml and 12.5mg/ml were prepared from each stock solution. Similarly (0.5g) of ciprofloxacin was measured and dissolved in 10ml of 10% DMSO to obtain 50mg/ml of antibiotic concentration.

### **Preparation and Standardization of Inocula**

Nutrient broth/Agar were prepared according to manufacturer's instructions. The bacterial isolates were subcultured on Nutrient agar, incubate for 24 hours then re-grown in Nutrient broth. McFarland's standard method was adopted to standardize the organism to  $1.5 \times 10^8$  cfu/ml. (Cheesbrough, 2009).

### **Antimicrobial Susceptibility Test**

The agar well diffusion method of Cheesbrough (2009) was used. Mueller Hinton agar was prepared as indicated by maker's directions, the media was cooled and afterward filled in sterile petridishes, the plates were allowed to solidify and then it was surface inoculated with the prepared standard inoculum containing each of the isolated organism. A sterile 6mm diameter cork borer was used to make holes on the surface of inoculated Mueller Hinton agar plates. Aliquots of different concentrations of the extracts was introduced into each well. The inoculated plates were kept at room temperature for 2 hours to permit the extract to diffuse into the agar. The plates were incubated at 37°C for one day. (The plates were prepared in duplicates). The antibacterial activity was determined by observing growth zones of inhibition. Diameter of zone of inhibition was measured using transparent meter rule and values were recorded in meter. Separates plate were prepared containing the same organisms in which standard antibiotic (Ciprofloxacin) was used as a control.

### **Minimum Inhibitory Concentration (MIC)**

To determine the minimum inhibitory concentration, different concentration 100 mg/ml, 50 mg/ml, 25 mg/ml and 12.5 mg/ml each of the extract was made by two-fold serial dilutions. One hundred (100µl) each of a standardized inoculum of the test bacterium was added to mixtures of different concentrations of extracts with Mueller Hinton broth (Andrew, 2019).

Every tube was incubated for 24 hours at 37°C. The test tubes were examined for turbidity, indicating growth of bacteria. The MIC was recorded as the least concentrates of extract which hindered the growth of the organisms.

### **Minimum Bactericidal Concentration (MBC)**

To affirm the MIC and determine Minimum Bactericidal Concentration (MBC), a loopful of each

tube that showed no growth during MIC was removed and sub cultured on agar surfaces of MHA. After incubation at 37°C for 24 hours, the minimum concentrates of extract in which development was completely stopped was noted as the minimal bacteriacidal concentrates (Andrew, 2019).

### **Data Analysis**

The p values were expressed as mean  $\pm$  standard deviation (SD). Analysis of variance (ANOVA) was used to determine the significant differences among isolates against control p value  $\leq 0.05$  was considered as significant.

### **RESULTS**

Phytochemical screening of methanol and aqueous crude extracts of the leaf and stem bark of *S. kunthianum* were determined as indicated in (Table 1). It revealed the presences of Saponins, Tannins, Steroids, Flavonoid, Glycoside, Phenol and Terpenoids while Alkaloids, Quinone and glycoside were absent in the different plant parts.

The result of the antibacterial activity of the methanol and aqueous extracts against the clinical and reference isolates were presented in table (Table 2). All the isolates were susceptible to the extracts at 100mg/ml with mean zone of inhibition ranging between  $19.00 \pm 0.00 - 26.12 \pm 0.17$ mg/ml. The methanol leaf extracts was more effective against both clinical and reference isolates of *S. aureus* with mean zone of inhibition ranging between  $13.37 \pm 0.17 - 26.12 \pm 0.17$ , followed by *S. Typhi*  $15.00 \pm 0.00 - 23.12 \pm 0.17$ . However, *E. coli* was less susceptible to the extract with mean zone of inhibition with mean zone of inhibition ranging between  $13.00 \pm 0.00 - 22.25 \pm 0.35$ . Means with the same letter(s) are not significantly different at p value  $\leq 0.05$ .

The minimum inhibitory concentrations (MIC) for the leaf and stem plant part on both clinical and reference isolate at different concentrations 100mg/ml 50mg/ml, 25mg/ml and 12.5mg/ml for (crude methanol and aqueous extract) indicated that the MIC's ranged between 12.5-50mg/ml (Table 3). Likewise, the Minimum Bactericidal Concentration (MBC') (Table 4.5a) ranged between (25mg/ml to 50mg/ml) depending on the bioactivity of the extract on the test organism at the highest concentration of the extracts.

**DISCUSSION**

In this research, the phytochemicals detected in the leaf and stem bark of *S. kunthianum* were similar to the compounds detected by many researchers (Nenge *et al.*, 2021; Sarr *et al.*, 2021; Jain *et al.*, 2019 and Aliyu *et al.*, 2009) These phytochemical constituents are the secondary metabolites synthesized by plants and are known to play a crucial role in plant defense mechanisms. However, the finding from the current research is not consistent with the work of Aliyu (2009). Who extracted steroids, coumarins alkaloids and carbohydrate from the leaf of *S. kunthianum*. The difference in the detected phytochemicals could be attributed to variations in the extraction method and the choice of solvent for extraction.

In this study the significant high activities of methanolic extracts of both the leaf and stem bark against all isolate of bacteria compared to the aqueous extract could be associated with difference in the polarity, it has been observed that the polarity of solvent plays a crucial role in extraction of bioactive compounds, as evidenced by the methanolic extracts. The difference in the two solvents may be attributed to the type and nature of bonding present in the solvent in relation to the phytochemicals present in the plant parts. This finding aligns with the work of Hassim *et al.*, (2014). Who explained that methanol due to its high polarity, is commonly used as an extraction solvent and can yield high phytochemicals.

From this study the high activity of the leaf extracts compared to the stem could be because the leaf contains more phytochemicals than the stem. This is comparable with the report of Nwinyi *et al.*, (2021)

who also reported the presence of four phytochemicals in the stem bark of *S. kunthianum*.

The antibacterial activity of *S. kunthianum* extracts could be attributed to the cellular structure of the bacterial isolates. From this study *S. aureus* being Gram positive bacteria with no lipopolysaccharide and tend to allow the passage of phytochemicals into the cell was found to exhibit the highest zone of inhibition than *E. coli* and *Salmonella typhi*. However, *E. coli* and *S. typhi* being Gram negative bacteria were less susceptible and they possess lipopolysaccharide that might minimize the permeability of phytochemicals into their cell as observed in this study. These finding lines up with the findings of Aliyu *et al* (2009). Who investigates the extracts of *S. kunthianum* against *E. coli*, *S. typhi*, *S. aureus*, *Klebsiella spp* *Pseudomonas aeruginosa*. *S.aureus* displayed the highest zone of inhibition. Ruiz and Bertani (2018) reported that lipopolysaccharide creates a permeability barrier at the cell surface and is the main contributor to the innate resistance that Gram negative bacteria display against many antimicrobials.

In the present study, the antibacterial activity was found to be concentration dependent as the higher the concentration the higher the activity. The result of the MIC and MBC revealed that the phytochemicals present in the plant have bactericidal potential on the test organisms. Aliyu *et al.*, (2009) reported lower MICs and MBCs of (8.30, 10.41mg/ml. 2.09, 2.09mg/ml and 4.17, 8.34mg/ml) for *E. coli*, *S. aureus* and *Salmonella spp* respectively. The differences in the MICs and MBCs could be as a result of differences in the strain of the test organisms, geographical variation or were the plant was grown.

**Table 1: Phytochemical Constituents of Aqueous and Methanol Crude Extracts of *S kunthianum***

Phytochemical Group	Aqueous Extract		Methanolic Extract	
	Leaf	Stembark	Leaf	Stembark
Tannin	+	-	+	-
Saponin	+	+	+	+
Flavonoid	+	+	+	+
Alkanoid	-	-	+	-
Quinones	-	-	-	-
Phenol	+	+	+	+
Terpenoid	+	+	+	+
Steroid	+	-	+	+
Glycoside	-	-	-	-

**Key:** += Positive (Detected); - = Negative (Not detected)

Table 2: Antibacterial Activity of Leaf and Stem Crude Extracts of *S. kunthianum* against Bacterial Isolates

Plant part	Solvent	Conc. (mg/ml)	Mean and standard error of zone of inhibition					
			<i>E. coli</i> Ref	<i>E. coli</i> clin.	<i>S. typhi</i> Ref	<i>S. typhi</i> clin.	<i>S. aureus</i> Ref	<i>S. aureus</i> clin.
Leaf	Methanol	100	19.00±0.00 <sup>a</sup>	22.25±0.35 <sup>a</sup>	20.00±0.00 <sup>a</sup>	23.12±0.17 <sup>a</sup>	23.25±0.35 <sup>a</sup>	26.12±0.17 <sup>a</sup>
		50	16.13±0.17 <sup>b</sup>	18.87±0.53 <sup>b</sup>	18.00±0.35 <sup>a</sup>	20.0±0.00 <sup>a</sup>	20.62±0.53 <sup>a</sup>	23.25±0.35 <sup>a</sup>
		25	13.00±0.00 <sup>c</sup>	15.12±0.17 <sup>c</sup>	15.00±0.35 <sup>b</sup>	16.12±0.17 <sup>b</sup>	18.25±0.35 <sup>b</sup>	20.50±0.00 <sup>a</sup>
		12.5	0.00±0.00 <sup>d</sup>	00.00±0.00 <sup>d</sup>	0.00±0.00 <sup>d</sup>	00.00±0.00 <sup>d</sup>	13.37±0.17 <sup>d</sup>	15.12±0.17 <sup>c</sup>
	Aqueous	100	15.37±0.35 <sup>b</sup>	18.0±0.00 <sup>a</sup>	15.25±0.35 <sup>a</sup>	16.75±0.70 <sup>a</sup>	17.12±0.17 <sup>a</sup>	18.00±25.76 <sup>a</sup>
		50	13.75±0.17 <sup>b</sup>	14.25±0.35 <sup>b</sup>	12.25±0.35 <sup>b</sup>	15.62±0.88 <sup>b</sup>	14.12±0.17 <sup>b</sup>	14.50±0.035 <sup>b</sup>
		25	0.00±0.00 <sup>d</sup>	9.88±0.53 <sup>c</sup>	0.00±0.00 <sup>d</sup>	11.50±0.35 <sup>c</sup>	0.00±0.00 <sup>d</sup>	00.00±0.00 <sup>d</sup>
		12.5	0.00±0.00 <sup>d</sup>	00.00±0.00 <sup>d</sup>	0.00±0.00 <sup>d</sup>	00.00±0.00 <sup>d</sup>	0.00±0.00 <sup>d</sup>	00.00±0.00 <sup>d</sup>
Stem bark	Methanol	100	13.75±0.17 <sup>a</sup>	20.12±0.25 <sup>a</sup>	13.75±0.17 <sup>a</sup>	20.12±0.25 <sup>a</sup>	15.00±0.00 <sup>a</sup>	22.12±0.26 <sup>a</sup>
		50	12.00±0.00 <sup>b</sup>	15.75±0.16 <sup>b</sup>	12.35±0.16 <sup>b</sup>	16.62±0.16 <sup>a</sup>	13.00±0.00 <sup>b</sup>	18.25±0.15 <sup>a</sup>
		25	0.00±0.00 <sup>d</sup>	12.12±0.15 <sup>c</sup>	0.00±0.00 <sup>d</sup>	12.37±0.035 <sup>c</sup>	0.00±0.00 <sup>d</sup>	15.12±0.014 <sup>b</sup>
		12.5	0.00±0.00 <sup>d</sup>	00.00±0.00 <sup>d</sup>	0.00±0.00 <sup>d</sup>	00.00±0.00 <sup>d</sup>	0.00±0.00 <sup>d</sup>	00.00±0.00 <sup>d</sup>
	Aqueous	100	11.50±0.25 <sup>a</sup>	15.37±0.16 <sup>a</sup>	11.75±0.35 <sup>a</sup>	14.62±0.17 <sup>a</sup>	13.50±0.17 <sup>a</sup>	15.62±0.35 <sup>a</sup>
		50	10.75±0.17 <sup>b</sup>	13.12±0.12 <sup>b</sup>	10.00±0.00 <sup>b</sup>	10.62±0.15 <sup>b</sup>	12.00 ±0.00 <sup>b</sup>	13.12±0.17 <sup>b</sup>
		25	0.00±0.00 <sup>b</sup>	00.00±0.00 <sup>d</sup>	0.00±0.00 <sup>d</sup>	00.00±0.00 <sup>d</sup>	0.00±0.00 <sup>d</sup>	00.00±0.00 <sup>d</sup>
		12.5	0.00±0.00 <sup>d</sup>	00.00±0.00 <sup>d</sup>	0.00±0.00 <sup>d</sup>	00.00±0.00 <sup>d</sup>	0.00±0.00 <sup>d</sup>	00.00±0.00 <sup>d</sup>
Control	Ciproflaxacin	50mgg/ ml	23.00±0.00 <sup>a</sup>	28.00±0.00 <sup>a</sup>	30.00±0.00 <sup>a</sup>	35.00±0.00 <sup>a</sup>	35.00±0.00 <sup>a</sup>	40.00±0.00 <sup>a</sup>

**Key:** Means with the same letters in a column are not significantly different at P≤ 0.05 level of significant.

Conc: Concentration; *E. coli*: *Escherichia coli*; Ref: Reference; *S. Typhi*: Salmonella Typhi; Clin: Clinical; *S. aureus*: *Staphylococcus aureus*

**Table 3: Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of Aqueous and Methanol Extracts Against Bacterial Isolates**

Test organisms													
Plant Part	Solvents	<i>E. coli</i> Clin		<i>E. coli</i> Ref.		<i>S typhi</i> Clin		<i>S typhi</i> Ref		<i>S aureus</i> Clin		<i>S aureus</i> Ref	
		MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Leaf	Aqueous	25	50	25	50	25	50	25	50	12.5	25	12.5	25
	Methanol	25	50	25	50	25	50	25	50	12.5	25	12.5	25
Stem	Aqueous	25	50	25	50	25	50	25	50	12.5	25	25	25
	Methanol	25	50	25	50	25	50	25	50	12.5	25	25	25

**Key**

MIC= Minimum Inhibitory Concentration

MBC= Minimum Bactericidal Concentration

## CONCLUSION

In this research the antibacterial activities of *Stereospermum kunthianum* plant could be as a result of the presence of phytochemicals present in the plant. Phytochemical compounds detected from the leaf and stem bark of *S. kunthianum* revealed the presence of saponins, tannins, flavonoids, alkaloids, phenols, terpenoids and steroids. All the plant parts showed a good antibacterial activity against the test organisms but the leaf extract was more effective than the stem. The results of these studies provided more basis for the use of these for the treatment of pathogenic microorganisms used in this study. Therefore, the safety of *Stereospermum kunthianum* extracts should be thoroughly evaluated such as acute and chronic toxicity studies should be conducted to determine the extracts' safety profiles and to identify potential adverse effects

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