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Phytochemical Profile and Antibacterial Activity of Stem Bark Extracts of Stereospermum kunthianum (Pink Jacaranda)

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ABSTRACT:

The global rise of antimicrobial resistance necessitates the search for novel therapeutic agents from natural sources, particularly medicinal plants. "Stereospermum kunthianum" (Pink Jacaranda) has long been employed in traditional medicine for the treatment of infectious diseases; however, its antibacterial efficacy requires scientific validation. This study investigated the phytochemical constituents and antibacterial activity of stem bark extracts of S. kunthianum. The dried stem bark powder was sequentially extracted with hexane, ethyl acetate, acetone, and methanol using a Soxhlet apparatus. Phytochemical screening revealed the presence of tannins, flavonoids, alkaloids, saponins, terpenoids, phenols, steroids, cardiac glycosides, phlobatannins, and carbohydrates. Antibacterial activity was assessed against clinical isolates of Escherichia coli and Staphylococcus aureus, using standard bioassay methods. The extracts demonstrated variable antibacterial effects, with inhibition zones ranging from 5.0 to 17.0 mm. Ethyl acetate and methanol extracts exhibited the most pronounced activity, particularly against S. aureus and E. coli. Minimum inhibitory concentration (MIC) tests revealed that the methanol extract inhibited S. aureus and E. coli at 12.5 mg/mL, while the ethyl acetate extract inhibited S. aureus at 12.5 mg/mL and E. coli at 25 mg/mL. Although less potent than chloramphenicol (Control), the findings affirm the antibacterial potential of "S. kunthianum" stem bark and provide scientific support for its ethnomedicinal use. These results highlight its promise as a source of bioactive compounds for drug discovery, warranting further investigation into its safety, mechanism of action, and potential for development into effective antimicrobial agents.

KEYWORDS:

Phytochemicals, Antibacterial activity, "Stereospermum kunthianum", Plant extracts, Bioassay, Drug discovery.

1. Introduction

Natural herbs are as old as human history; nowadays, plant products are used for many purposes, such as drugs and cosmetics (1). Natural products have been used for centuries as anti-infective agents; several antibiotics in use today were derived from plants (2). Medicinal plants are known to possess bioactive compounds that can be used in the treatment of diseases (3). Plant products can help treat disease by increasing antioxidant activity, inhibiting

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bacterial development, and modulating genetic pathways (4). More recently, the use of plants has identified different chemical compounds with many biochemical and pharmacological properties (5). These phytochemical constituents are the secondary metabolites synthesized by plants and are known to play a crucial role in plant defence mechanisms (3). An investigation of plant-originated pure compounds used as drugs in countries with WHO-Traditional Medicine Centres indicated that out of the 122 compounds so far identified, 80% were applied for the same or similar ethno-medical purposes and were obtained from 94 plant species only (6).

The plant kingdom is a rich source of biodiversity and offers a variety of plants with medicinal properties (3). "*Stereospermum kunthianum*" belongs to the Bignoniaceae family and is referred to as Pink Jacaranda in English. In Nigeria, it is designated 'Sansami' by the Hausa (Northern Nigeria), 'Ayada' by the Yoruba (Southwestern, Nigeria), and 'Alakiriti' by the Igbo (Southeastern, Nigeria) (7). According to (5), "*Stereospermum kunthianum*" is a plant that has its different parts used in traditional medicine for the treatment of different ailments and conditions, such as wounds, ulcers, gastritis, bronchitis, and other pain and inflammation-related health conditions. It is a deciduous shrub or small tree widely spread across Africa, with some species distribution in Asia. The species is well spread all over the Sahel region (8).

The leaf extracts of "*Stereospermum kunthianum*" have a promising antibacterial effect on three selected organisms, *S. aureus, S. typhi,* and *B. subtilis*, but are ineffective against *P. aeruginosa*(2). Similarly, *E. coli, S. typhi,* and *S. aureus* were susceptible to both aqueous and methanol extracts of the plant's leaf and stem bark(3). The antibacterial activity of "*S. kunthianum*" extracts could be attributed to the cellular structure of the bacteria: Gram-positive bacteria are more susceptible to the extracts than Gram-negative bacteria(3). On the other hand, the antibacterial activity of the plant is lower than that of Levofloxacin and Clotrimazole (2). This could be because of the presence of other compounds or impurities that prevent or reduce the effects of antibacterial compounds in the plants. Isolation of pure antimicrobial compounds from these extracts may result in compounds with comparable activity (2).

Infectious diseases have repeatedly been identified as one of the most serious threats to human health around the world (3). Infectious diseases accounted for 61.7 percent (5.9million) of the 9.6 million fatalities in Africa in 2013, according to the World Health Organization WHO(9). Pathogenic bacteria are capable of causing diseases in humans, which include food poisoning, gastritis, ulcers, wound infection, etc(10). The high cost of antibiotics has made the treatment of diseases very difficult. Therefore, there is a need to explore plant extracts that can act in the same biological pathways as pharmaceutical medicines and pose fewer side effects (3). Based on the aforementioned information, the study aimed to determine the phytochemical constituents of the stem bark of "*Stereospermum kunthianum*" and evaluate its antibacterial activity.

2. MATERIALS AND METHODS

Collection and Preparation of the Sample

The sample of the stem bark of *S. kunthianum* was obtained from the Yelwa Campus of Abubakar Tafawa Balewa University, Bauchi, Nigeria. Identification was carried out using standard taxonomic keys and available literature. It was dried under shade and crushed using a wooden mortar, sieved to a fine powder, and stored until used.

Extraction of the Plant Extracts

200g of the powdered sample of the stem bark of *S. kunthianum* was placed in a Soxhlet extractor. Hexane, ethyl acetate, acetone, and methanol were used sequentially for the extraction. The solvents were evaporated under reduced pressure below 50°C to give the crude solvent extracts. These were further used for phytochemical screening and antimicrobial screening.

Phytochemical Screening

Standard laboratory techniques were carried out for qualitative determination of tannins, saponins, flavonoids, cardiac glycosides, phlobatannins, terpenoids, steroids, carbohydrates, and alkaloids (11).

Collection of Bacterial isolates

Clinical isolates of *Staphylococcus aureus* and *Escherichia coli* were obtained from the

Department of Microbiology, Abubakar Tafawa Balewa University, Bauchi State, Nigeria.

Preparation of Culture Media

The culture medium used was nutrient agar (NA) for supporting the growth of microorganisms. The assay media was prepared following the manufacturer's direction by suspending 28g of the agar and dissolving it in 1 Liter of distilled water (1000ml). This gave 11.2g of the nutrient agar powder, which was dissolved in 400ml of distilled water in a 500ml conical flask. The solution was allowed to dissolve properly for 10 minutes by swirling the mixture in the flask. The flask was covered with a foil paper and autoclaved for another 15minutes at 121°C for sterilization. The prepared nutrient agar was allowed to cool before pouring it into the sterilised plates. A blue flame of the hot plate was used to sterilise the rim of the conical flask after each pouring. The plates were then covered, and the agar was left to gel. The method of bioassay employed is the Agar-disk diffusion method, and the test organisms used were *Staphylococcus aureus* and *Escherichia coli* (12).

Antimicrobial Susceptibility Test

The agar well diffusion method of (12) was used. Mueller Hinton agar was prepared as indicated by the manufacturer's directions. The media was cooled and then filled into sterile petri dishes. The plates were allowed to solidify, and then they were surface inoculated with the prepared standard inoculum containing each of the isolated organisms. A sterile 6mm diameter cork borer was used to make holes in the surface of inoculated Mueller-Hinton agar plates. Aliquots of different concentrations of the extracts were 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℃ for one day. The agar well diffusion assay was performed in triplicate (n=3 independent plates/wells per extract and organism). Zones of inhibition were measured in mm using a transparent ruler. Data are presented as mean ± standard deviation (SD). Separate plates were prepared containing the same organisms, in which standard antibiotic (Chloramphenicol) was used as a control(3).

Determination of Minimum Inhibitory Concentrations

Different concentrations of 400 mg/mL, 200 mg/mL, 100 mg/mL, 50 mg/mL, 25 mg/mL, and 12.5 mg/mL of each extract were 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 (13). Each 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 lowest concentration of extract, which hindered the growth of the organisms. After incubation, the series of dilution vessels was observed for microbial growth, usually indicated by turbidity and a pellet of microorganisms at the bottom of the vessel. The last tube in the dilution series that does not demonstrate growth corresponds with the minimum inhibitory concentration (MIC) of the antimicrobial agent (13). Commercial antibiotics (chloramphenicol) and DMSO were used as positive and negative (Control) reference standards to determine the sensitivity of the isolates.

Statistical Analysis

Where reported, results are significant ($p \leq 0.05$) compared to controls, with dose-response trends (e.g., increasing inhibition with concentration). Descriptive statistics were calculated using Microsoft Excel. Mean and standard deviation (SD) were computed from triplicate measurements for zones of inhibition.

3. RESULTS

Phytochemical analysis of the crude extracts (hexane, acetone, ethyl acetate, and methanol) of the stem bark of *S. kunthianum* was determined as indicated in Tables 1 and 2. Tannins, flavonoids, carbohydrates, and phlobatannins were all present in acetone, ethyl acetate, and methanol extracts. Both terpenoids and saponins were present in acetone and methanol extracts, while cardiac glycosides were present in ethyl acetate and methanol extracts. Alkaloids were present in the methanol extract, and Steroids were absent in all four crude extracts. None of the phytochemicals were detected in the hexane extract.

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The results of the antibacterial activity of the crude extracts were presented (Table 3). The crude extracts showed variable antibacterial activity against S. aureus and E. coli (Table 3). The methanol extract exhibited the strongest inhibition, with mean zones of 17.00 ± 0.50 mm (S. aureus) and 14.00 ± 0.50 mm (E. coli). Ethyl acetate followed closely (14.00 ± 0.44 mm and 12.00 ± 0.20 mm, respectively). Acetone showed moderate activity, while hexane was weakly active only against S. aureus (5.60 ± 0.53 mm) and inactive against E. coli (0.00 ± 0.00 mm). All extracts were less potent than the positive control chloramphenicol.

The minimum inhibitory concentration (MIC) for the methanol extract was 12.5 mg/mL against both *S. aureus* and *E. coli*. The MIC for the ethyl-acetate extract was 12.5 mg/mL against *S. aureus* and 25 mg/mL against *E. coli* (Table 4). Comparative analysis across studies shows aqueous extracts are less potent than methanolic ones, possibly due to better solubility of actives in methanol.

Table 1: Colour, texture, yields, and percentage recoveries of the crude extracts of Stereospermum kunthianum.

Solvent extract	Colour and Texture	Weight of sample(g)	Extract yield(g)	Percentage Recovery(%)
Hexane	Golden yellow, oily liquid	200	2.50	1.25
Ethylacetate	Dark green, oily Liquid	190	1.50	0.79
Acetone	Dark brown, oily Liquid	170	1.20	0.71
Methanol	Dirty brown Granules	150	2.00	1.30

Notes: Percentage recovery = (weight of extract/ weight of powdered sample) x 100. Hexane was used initially at 200 g; subsequent solvents were used on the remaining residue (sequential extraction).

"Crude extract yields ranged from 0.71% to 1.30% (mean = 1.01 ± 0.31%, n=4)."

Table 2: Phytochemical screening of the stem bark extracts of S. kunthianum

Phytochemicals	Names of test	Solvent extracts			
		Hexane	Ethyl-acetate	Acetone	Methanol
Alkaloids	Wagner's test	-	-	-	+
	Dragend-off's Test	-	-	-	+
Steroids	Cone. HSO-	-	-	-	-
Saponins	Frothing test	-	-	+	+
Tannins	Ferric chloride	-	+	+	+
Flavonoids	Lead acetate	-	+	+	+
Cardiac glycosides	Fehling's solution A and B	-	+	-	+
Phlobatan-nins	Hcl	-	+	+	+
Carbohy-drates	Benedict's reagent	-	+	+	+
Terpenoid	Chloroform	-	-	+	+

Key: + denotes 'Positive' (Detected); - denotes 'Negative' (Not detected)

- **Summary from text:** No phytochemicals detected in hexane extract. Tannins, flavonoids, carbohydrates, and phlobatannins are present in acetone, ethyl acetate, and methanol. Steroids are absent in all extracts.

Table 3: Antibacterial activity of the stem bark extracts of S. kunthianum

Test microorganisms	Solvent extracts and susceptibility to the test Microorganisms and zone of inhibition (mm)				Control	
	Hexane	Ethyl-acetate	Acetone	Methanol	+	-
S. aureus	5.60 ±0.53	14.00 ±0.44	10.00 ±0.50	17.00 ±0.50	19.00 ±1.00	0.0 ±0.0
E. coli	0.00 ±0.00	12.00 ±0.20	8.00 ±0.20	14.00 ±0.50	17.50 ±0.87	0.0 ±0.0

Key: +ve indicates (Chloramphenicol); -ve indicates Dimethylsulfoxide (DMSO).

By microorganism (mean across 4 extracts):

- *S. aureus*: 11.65 mm (SD of means = ±4.95 mm, range 5.60–17.00 mm)
- *E. coli*: 8.50 mm (SD of means = ±6.19 mm, range 0.00–14.00 mm)

By extract (mean across 2 bacteria, compared to chloramphenicol mean = 18.25 mm):

Extract	Mean Zone (mm)	Difference from Control (mm)	Interpretation
Hexane	2.80	−15.45	Very weak/inactive vs E. coli
Acetone	9.00	−9.25	Moderate
Ethyl-acetate	13.00	−5.25	Good
Methanol	15.50	−2.75	Strongest among the extracts
Chloram-phenicol	18.25	–	Positive Control

Values represent mean zones of inhibition (mm) from triplicate assays. Methanol extract showed the highest activity (mean 15.50 mm across organisms), followed by ethyl acetate (13.00 mm). All extracts were less potent than chloramphenicol (mean 18.25 mm).

All plant extract values represent mean ± standard deviation (SD) from triplicate (n=3) independent assays using the agar well diffusion method (6 mm well diameter, 400, 200, 100, 50, 25, 12.5 mg/mL). Assays were performed under standardized conditions at 37°C for 24 h.

Table 4: Minimum inhibitory concentration (MIC) of solvent extracts of S. kunthianum

Test Organisms	Solvent Extracts	Minimum inhibitory concentration (mg/ml)					
		400	200	100	50	25	12.5
S. aureus	Ethyl-acetate	ND	ND	ND	+	+	0+
	Methanol	+	+	+	+	+	0+
E. coli	Ethyl-acetate	ND	ND	+	+	0+	-
	Methanol	+	+	+	+	+	0+

Key: + = Inhibition; 0+ = Minimum inhibition (i.e., MIC); - = No inhibition; ND = Not determined.

- **Summary from text**:
- Methanol extract MIC = 12.5 mg/mL against both S. aureus and E. coli.
- Ethyl acetate extract MIC = 12.5 mg/mL against S. aureus; 25 mg/mL against E. coli.

MIC values (mg/mL, n=4 combinations):

Organism	Extract	MIC (mg/mL)
S. aureus	**Ethyl-acetate**	**12.5**
S. aureus	**Methanol**	**12.5**
E. coli	**Ethyl-acetate**	**25.0**
E. coli	**Methanol**	**12.5**

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By organism (mean MIC):

- *S. aureus*: 12.50 mg/mL
- *E. coli*: 18.75 mg/mL

By extract (mean MIC):

- Methanol: 12.50 mg/mL
- Ethyl-acetate: 18.75 mg/mL

The methanol extract exhibited the lowest mean MIC (12.50 mg/mL) against both organisms, indicating better potency than ethyl acetate (mean MIC 18.75 mg/mL). Overall mean MIC across tested combinations was 15.63 ± 6.25 mg/mL.

4. DISCUSSION

Preliminary phytochemical screening of the stem bark extracts of *S. kunthianum* revealed the presence of alkaloids, saponins, tannins, flavonoids, terpenoids, phlobatannins, and carbohydrates. The antimicrobial activity exhibited by the extracts of the stem bark of *S. kunthianum* may be due to the presence of the secondary metabolites revealed in the phytochemical screening. Secondary metabolites possess pharmacological activities that are responsible for the use of plants in traditional phytomedicine to treat diseases caused by pathogenic microorganisms (2). The finding of this study agrees with the result obtained by (14) that phenolic compounds, including alkaloids, exhibit a wide range of antimicrobial activities.

Previous findings have shown that *S. kunthianum* has no alkaloids in aqueous extract (3). However, this study showed that alkaloids were present in the methanolic extract. This may be due to geographical locations and the season. The antibacterial activity of the methanolic extract of the stem bark of *S. kunthianum* showed a marked activity compared to other solvent extracts. The extract was more potent on gram-positive *S. aureus* with a maximum zone of growth inhibition of 17.

The phytochemical profile indicates a rich diversity of bioactive compounds, predominantly polar ones (tannins, flavonoids, saponins) extractable in aqueous or methanolic solvents. This supports the plant's ethnomedicinal use, as tannins and flavonoids are known to disrupt microbial cell membranes, chelate metals, or inhibit enzymes(9). Inconsistencies (e.g., alkaloids present in some studies but absent in others) may arise from variations in extraction solvents, plant collection sites, or seasonal factors affecting metabolite accumulation. Overall, the stem bark appears to be a promising source of natural antimicrobials, but standardization of extraction methods is needed for reproducibility.

The methanol extract exhibited the lowest mean MIC (12.50 mg/mL) against both organisms, indicating better potency than ethyl acetate (mean MIC 18.75 mg/mL). Overall mean MIC across tested combinations was 15.63 ± 6.25 mg/mL.

Compared to the Control (chloramphenicol), the extracts were less potent. Indicating the crude extract is not highly potent but could be useful as a complementary therapy. Zones of inhibition (5-17 mm) suggest broad-spectrum potential, particularly against *S. aureus* and *E. coli*, which are common pathogens in traditional treatment contexts (e.g., wounds, respiratory infections).

High MIC values suggest the need for compound isolation (e.g., via chromatography) to identify potent leads(2). The antimicrobial effects validate traditional uses but highlight moderate potency, potentially enhanced by fractionation or combination with other agents.

5. CONCLUSION

The stem bark extract of *S. kunthianum* shows dose-dependent antibacterial activity against pathogenic bacteria, though it is less potent than standard antibiotics. The presence of many bioactive compounds supports its traditional use in treating infections, but high MIC values indicate the need for isolating active fractions to enhance potency. This research supports traditional applications that the plant is potent in the cure of skin infections, respiratory infections, diarrhea, venereal disease, and other infectious diseases linked to bacterial pathogens. However, clinical translation requires standardized extraction, purified compounds identification, and rigorous trials for efficacy and safety.

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