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Antibacterial Activity and Phytochemical Composition of *Anogeissus Leiocarpus* Stem Bark Extract Against Uropathogenic *Klebsiella Sp.* Isolated from Ahmad Sani Yarima Bakura Specialist Hospital, Gusau

Ishaq, S. A.[1*], Sa'ad[1], A and Abubakar, N[1]

[1]Department of Applied biology, Faculty of Applied Sciences, Federal University of Technology, Babura, Jigawa State.

ABSTRACT: The stem bark of *Anogeissus leiocarpus* has attracted scientific interest due to its demonstrated activity against a range of microorganisms, including Gram-positive and Gram-negative bacteria. However, limited studies have focused specifically on its antibacterial efficacy against uropathogenic *Klebsiella* species, particularly clinical isolates associated with UTIs. This study evaluated the antibacterial activity and phytochemical constituents of *Anogeissus leiocarpus* stem bark extracts against uropathogenic *Klebsiella* spp. isolated from urine of patients at Yarima Bakura Specialist Hospital, Gusau, Nigeria. Ethanol and aqueous extracts of the stem bark were prepared and subjected to standard phytochemical screening. Antibacterial activity was assessed using the agar well diffusion method.The phytochemical screening revealed the presence of alkaloids, flavonoids, saponins, tannins, phenols, and triterpenes. The ethanol extract exhibited greater efficacy, producing a 25 mm zone of inhibition at 200 mg/mL, compared to 22 mm for the aqueous extract. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts were 25 µg/mL and 50 µg/mL, respectively, demonstrating strong antibacterial potential. Statistical analysis ($p = 0.0449$) confirmed the significantly higher activity of the ethanol extract. The findings indicate that *Anogeissus leiocarpus* stem bark especially the ethanol extract may serve as a promising natural therapeutic agent against antibiotic-resistant *Klebsiella* spp. implicated in urinary tract infections.

KEYWORDS: *Anogeissus leiocarpus*, *Klebsiella spp.*, antibacterial activity, phytochemicals.

INTRODUCTION

Urinary tract infections (UTIs) remain among the most common bacterial infections globally, affecting millions annually and contributing significantly to morbidity, healthcare costs, and antimicrobial resistance challenges. Among the bacterial agents implicated in UTIs, *Klebsiella* species particularly *Klebsiella pneumoniae* are increasingly recognized as major uropathogens due to their virulence factors, adaptability, and rising multidrug-resistance profiles (Ndlovu *et al.,* 2023). The emergence of extended-spectrum β-lactamase (ESBL)-producing *Klebsiella* strains has further complicated treatment options, as these organisms exhibit resistance to multiple classes of antibiotics commonly used for UTI management (Padmini *et al.,* 2017). This growing resistance highlights the need for new therapeutic alternatives, especially from natural sources that may provide effective, accessible, and affordable antimicrobial agents.

Medicinal plants have long served as vital sources of therapeutic compounds, particularly in African traditional medicine where plant-based remedies are widely used for treating infectious diseases (Attah *et al.,* 2021). *Anogeissus leiocarpus*, commonly known as African birch, is a widely distributed plant in West Africa and has been traditionally employed to treat various ailments including diarrhea, wounds, respiratory infections, and urinary complications (Dayok *et al.,* 2018). Phytochemical studies on different parts of the plant have reported the presence of bioactive compounds such as tannins, flavonoids, saponins, and phenolic compounds, many of which possess antimicrobial and antioxidant properties (Altemimi *et al.,* 2017).

The stem bark of *Anogeissus leiocarpus* has attracted scientific interest due to its demonstrated activity against a range of microorganisms, including Gram-positive and Gram-negative bacteria (Dahiru *et al.,* 2023). However, limited studies have focused specifically on its antibacterial efficacy against uropathogenic *Klebsiella* species, particularly clinical isolates associated with UTIs. Considering the increasing prevalence of antibiotic-resistant *Klebsiella* spp. in healthcare settings, investigating the antibacterial potential of *Anogeissus leiocarpus* stem bark extracts provides an important step toward discovering alternative treatment options.

This study therefore evaluates the phytochemical constituents and antibacterial activity of ethanol and aqueous stem bark extracts of *Anogeissus leiocarpus* against *Klebsiella* spp. isolated from patients with UTIs in Yariman Bakura Hospital, Gusau, Nigeria. By

determining the minimum inhibitory and bactericidal concentrations, this research aims to provide scientific support for the plant's traditional use and contribute to ongoing efforts to develop plant-based therapies for resistant uropathogens.

MATERIALS AND METHOD

Collection and Authentication of Plant Material

Anogeissus leiocarpus was collected in February, 2025, within Federal University Gusau campus. The plant was, thereafter, identified in the herbarium of Biological Sciences Department, Federal University Gusau with the Voucher no: FUG/BIO/HEB/161. The plant's bark was stripped from the plant and then cleaned to remove dust under tap water. It was air-dried for seven (7) days at room temperature, grinded into fine powder and prepared for use in a plastic container.

Extraction of Plant Materials

200 g of the grinded plant material was subjected to cold extraction using Ethanol and water for three days with constant shaking. Extracts obtained were filtered with Whatman No.1 filter paper and the filtrates were collected and concentrated to dryness using water bath. The concentrated extracts were dried in open air in the laboratory and stored under refrigeration until further use (Ahmad-Qasem *et al.,* 2016).

Phytochemical Screening

Extracts of *Anogeissus leiocarpus* were subjected to qualitative phytochemical screening for the detection of phytochemical constituents according to standard method reported by (Orlando *et al.,* 2019).

Test for Alkaloids: 2 ml of the extract was added with 2 drops of Wagner's reagent (1.27g of iodine and 2g of potassium iodide dissolved in 100 ml of distilled water). The formation of reddish-brown precipitate confirms the presence of alkaloids.
Test for Saponins: A portion of the extract was diluted with distilled water until the volume was made up to 20 ml. The solution was vigorously agitated and the formation of persistent froth indicates presence of saponins.
Test for Tannins: A fraction of the extract was added to 10% alcoholic $FeCl_3$ solution and the formation of blue or brown to dark green coloration indicates the presence of tannins.

Test for Glycosides: 5 ml of the extract was mixed with two (2) ml of glacial acetic acid (CH_3CO_2H) containing 1 drop of $FeCl_3$. The mixture was carefully added to a prepared 1 ml of concentrated H_2SO_4 to form a lower layer. The presence of a brown ring at the interface indicates presence of glycosides.

Test for Terpenoids: 2 ml of the extract was added with one (1) ml of chloroform followed by 2 drops of concentrated sulfuric acid. The immediate presence of reddish-brown layer indicates the presence of terpenoids.

Test for Flavonoids: 3 drops of sodium hydroxide solution (aqueous NaOH and HCl) were added to a small amount of the extract and observed for the formation of yellow to orange color.
Test for Sterols: 1 ml of the extract was dropped with 2 ml of acetic anhydride and chloroform, then carefully added with 2 drops of H_2SO_4. The formation of dark green to red color indicates presence of sterols.

Test for Triterpenes: The extract was treated with chloroform and concentrated sulfuric acid (typically 95% H_2SO_4) (Nathiya *et al.,* 2017). A reddish-brown color was observed, indicating the presence of triterpenes.

Test for Phenol Compounds: The extract was treated with 5% ferric chloride ($FeCl_3$) solution (Klangmanee and Athipornchai, 2019). A green, blue, or purple color was observed, indicating the presence of phenols.

Test for Resins: The extract was treated with a diluted hydrochloric acid (5% HCl) (Fouda *et al.,* 2016). The formation of a precipitate was observed, indicating the presence of resins.

Isolation and Identification of Bacterial Isolate: Urine samples were collected from patients suffering from urinary tract infection. The specimen was transported to microbiology laboratory, Federal University Gusau for identification of *klebsiella* specie. The urine samples were inoculated unto MacConkey agar, the growth of *klebsiella* was distinguished by its mucoid growth. Gram staining was performed and biochemical test was carried out to confirm the test isolate.

Source of the Media, Solvent and Reagents

All chemicals and reagents used for the analysis were of analytical grade (Amalar), manufactured by British Drug House Limited (BDH) in England, while the media were oxoid products.

Antibacterial Activity

Each of the extract 0.1 g was used to prepare a solution with 2ml of Dimethyl sulphoxide (DMSO) used as diluent. Agar well diffusion method was used for the screening of extracts. Medium used to grow the microorganisms was Mueller-Hinton agar. The

medium sterilization was done for 15 minutes at 121 ºC. This was subsequently transferred into sterile petri-dishes and allowed to set. Test microorganisms' standard inoculums (0.1 ml) was seeded and evenly spread over the surface of the medium with the aid of sterile swab. Wells were created by the use of a sterile cork borer (4 mm in diameter) on the medium already inoculated. 0.1 ml of the solution of each extract (Aqueous and Ethanol) (10mg/ml) was afterwards transferred into the well on the inoculated medium. Incubation of plates containing inoculated medium was done for 24 hours at 37ºC, after which zones of inhibition of microorganisms' growth were observed. The zones were measured in (mm) and recorded.

Minimum Inhibition Concentration (MIC)

The tube dilution method was used to determine the MICs of the extracts as described by Chikezi, (2017). Nutrient Broth was prepared in concentrations ranging from (200- 3.125µg/ml), 10 ml was dispensed into cleaned test tubes. This was followed by sterilization for 15 minutes at 121ºC, the Broth was then allowed to cool (Jain *et al.*, 2020).

The *Klebsiella spp.* isolate was inoculated, incubated at 37°C for 24 hours and observed for turbidity. The minimum inhibitory concentration (MIC) was determined as the highest dilution of the fraction that showed no visible growth.

Minimum Bactericidal Concentration (MBC)

The Minimum Bactericidal Concentration (MBC)was determined by sub culturing the tubes that showed no visible growth in the MIC determination on Nutrient agar plates and incubated at 37°C for 24 hours. The lowest concentration that yielded no growth on the plate was considered the MBC of the extract (AL-Saadi, 2016).

RESULT

Table 1: presents the isolated *Klebsiella* specie, confirmed the gram reaction. The isolates showed a mucoid pink color on the medium, pink color under the microscope indicating they are Gram-negative bacteria. The biochemical tests confirmed citrate+, indole-, methyl red+.

Table 1: Morphological and Biochemical characteristics of *Klebsiella spp.*

Isolate code	Appearance	Gram rxn	Shape	Ind	MR	Cit	Glu	Lac	Suc	Suspected Org
Kleb-1	Pink	-ve	Short rod	-	-	+	+	+	+	*Klebsiella spp.*

Key: Ind= Indole, MR= Methyl Red, Cat= Catalase, Glu: =Glucose, Lac= Lactose, Suc= Sucrose, spp. =Species, += positive, - = Negative

Table 2: The phytochemical screening of ethanol showed the presence of Alkaloids, Flavonoids, Glycosides, Phenol compounds, Saponins, Steroids, Tannins and Triterpenes. The aqueous extract contained Alkaloids, Flavonoids, Glycosides, Phenol compounds, Saponins, Steroids, Tannins and Triterpenes.

Table 2: Phytochemical Constituents of *Anogeissus leiocarpus* (Marke) stem bark Extracts

S/N	Phytochemicals	Aqueous	Ethanol
1	Alkaloid	+	+
2	Flavonoids	+	+
3	Glycosides	+	-
4	Phenol compounds	+	+
5	Resins	-	-
6	Saponins	+	+
7	Steroids	+	-
8	Tanins	+	+
9	Terpenoids	-	+
10	Triterpenes	+	+

Key: += positive, - = Negative

Table 3: The result indicated that the extract are potent antimicrobials against *Klebsiella* specie. The antibacterial activity was screened for the zone of inhibition. The result shows that ethanol extract is more effective at 200mg/ml, where it produces a zone

of inhibition of 25 mm, whereas 100 mg/ml produces a zone of 22 mm, 50 mg/ml produces a zone of 20 mm and 25 mg/ml produces 18 mm. Also, aqueous extract shows less effective against the test organism at 200 mg/ml produces a zone of inhibition of 22 mm, whereas at 100 mg/ml produces a zone of 20 mm, 50 mg/ml produces a zone of 20 mm and 25 mg/ml produces a zone of 17 mm.

Table 3: Antibacterial Activity of *Anogeissus leiocarpus* (Marke) Stem Bark Extracts

Concentration of Stem Bark Extracts of *Anogeissus leiocarpus* (mg/ml)								
Zone of Inhibition (mm) Produced by Stem Bark Extract								
Organism	**Extract**	**200**	**100**	**50**	**25**	**Control-1**	**Control-2**	**p-value**
Klebsiella spp.	Aqueous	22	20	20	17	30	0.00	0.0449
Klebsiella spp.	Ethanol	18	20	22	25	30	0.00	

The p- value is 0.0449 which is (<0.05). This means there is a statistically significant difference between the two extracts, with the ethanol showing greater antibacterial activities

Key: Control 1= Positive Control = Chloramphenicol (30 μg/ml).

Table 4: Shows the Minimum Inhibitory Concentration the stem bark extract against the test isolate. The MIC result ranging from 200 mg/ml to 3.125 mg/ml. Where 25 mg/ml was taken as the MIC.

Table 4: Minimum Inhibitory Concentration (MIC) of the Extracts

Isolate	**Aqueous (mg/ml)**	**Ethanol (mg/ml)**
Klebsiella spp	25	25

Table 5: Shows the MBC results ranging between 200 mg/ml. The results from the MBC shows that from 50 mg/ml has bactericidal effect on the test organism as shown in Table 6.

Control 2 = Negative Control = Dimethyl Sulfoxide (DMSO): No zone of Inhibition

Table 5: **Minimum Bactericidal Concentration MBC of the extracts**

Isolate	**Aqueous (mg/ml)**	**Ethanol (mg/ml)**
Klebsiella spp.	25	25

DISCUSSION

significant antibacterial activity against uropathogenic *Klebsiella* spp., with the ethanol extract exhibiting superior efficacy compared to the aqueous extract. The larger zone of inhibition recorded for the ethanol extract (25 mm) suggests that ethanol is a more efficient solvent for extracting the bioactive compounds responsible for antibacterial activity. This aligns with the study by Ziani *et al.* (2023) study which reported that ethanol often extracts higher concentrations of phenolics, flavonoids, and other antimicrobial constituents than water.

The MIC (25 µg/mL) and MBC (50 µg/mL) values obtained in this study further confirm the potency of the ethanol extract. Similar findings have been reported in Elsiddig *et al.* (2015) who reported ethanol extracts of *A. leiocarpus* showed strong antimicrobial effects against pathogens such as *Staphylococcus aureus*, *E. coli*, and *Pseudomonas aeruginosa*. The presence of tannins, alkaloids, flavonoids, and saponins in the extract is consistent with earlier investigations that attributed the antibacterial activity of *A. leiocarpus* to these phytochemicals (Alhassan *et al.*, 2016).

When compared with other medicinal plants tested against *Klebsiella* spp., such as *Azadirachta indica*, *Combretum micranthum*, and *Terminalia avicennioides*, the ethanol extract of *A. leiocarpus* demonstrates comparable or even higher antibacterial activity, particularly in terms of inhibition zones and MIC values (N'do *et al.*, 2024). This suggests that *A. leiocarpus* may contain unique or synergistic phytochemical combinations with strong antimicrobial properties.

The statistical significance (p = 0.0449) observed between the ethanol and aqueous extracts aligns with previous findings that solvent polarity greatly influences extraction efficiency and antimicrobial outcomes. Furthermore, the activity against a clinical isolate of *Klebsiella*, which is known for its resistance to multiple antibiotics, supports the potential of *A. leiocarpus* as a promising natural antimicrobial agent.

However, despite the encouraging results, it is important to note that plant extracts often contain complex mixtures of compounds. Isolation, purification, and characterization of the specific active constituents are necessary to better understand the mechanisms of action and enable development into standardized therapeutic agents. Additionally, in vivo studies and clinical trials are needed before recommending its clinical application.

CONCLUSION

This study demonstrates that *Anogeissus leiocarpus* stem bark extract, particularly the ethanol fraction, exhibits strong antibacterial activity against uropathogenic *Klebsiella* spp. The presence of multiple phytochemical constituents supports its traditional use in treating infections, and the low MIC and MBC values indicate potent inhibitory and bactericidal effects. The superior activity of the ethanol extract underscores the importance of solvent selection in phytochemical and antimicrobial research. Given the rising threat of antibiotic-resistant uropathogens, *Anogeissus leiocarpus* shows promise as a potential source of new antimicrobial agents.

CONFLICT OF INTEREST

The authors declared no conflict of interest exists.

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