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Research Article

Comparative Phytochemical and Antibacterial Evaluation of Methanol and Ethanol Extracts of *Senna occidentalis* Leaves against Uropathogenic Bacteria

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ABSTRACT
Urinary tract infections (UTIs) remain among the most common bacterial infections worldwide and are increasingly complicated by multidrug resistance among uropathogens. This study evaluated the phytochemical composition and antibacterial activities of methanol and ethanol leaf extracts of *Senna occidentalis* against clinical UTI-associated isolates, including *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Proteus vulgaris* and *Proteus mirabilis*. Antibacterial efficacy was assessed using the agar well diffusion method, while minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) were determined by the agar dilution method. Phytochemical screening was conducted to identify bioactive constituents. Both extracts exhibited notable antibacterial effects, with the methanol extract demonstrating superior inhibition zones (16–24 mm) compared to the ethanol extract (12–18 mm). The MIC values for methanol extract ranged from 6.25 to 25 mg/mL and 12.5 to 25 mg/mL for ethanol extract. Minimum bactericidal concentration values were lower for the methanol extract (12.5–25 mg/mL) than for the ethanol extract (25–50 mg/mL). Phytochemical analysis revealed tannins, alkaloids, flavonoids, saponins, steroids, phenols and terpenoids, with flavonoids absent in the ethanol extract. These secondary metabolites are well-documented for antimicrobial mechanisms, including protein precipitation, enzyme inhibition and membrane disruption. The findings highlight the strong antibacterial potential of *S. occidentalis*, particularly its methanol extract and support its traditional use in the treatment of urinary tract infections. Further investigations are needed to isolate, characterize and evaluate the bioactive compounds *in vivo* for potential development of novel plant-derived therapeutics.

Keywords: Antibacterial activity; Multidrug resistance; Phytochemicals; *Senna occidentalis*; Urinary tract infections

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INTRODUCTION

Urinary tract infections (UTIs) are among the most prevalent bacterial infections worldwide, affecting an estimated 150 million people annually and accounting for significant morbidity, healthcare costs and antibiotic use (Murray *et al*., 2022). Although UTIs occur in all age groups, they are disproportionately common in women, older adults and immune-compromised individuals (Leocadio, 2020). The global burden of UTIs has risen markedly over the past three decades, with the highest increases observed in low and middle-income countries, where self-medication and irrational

antibiotic use exacerbate treatment challenges (Auta *et al*., 2019; World Health Organization, 2022).

Uropathogenic *Escherichia coli* (UPEC) is the leading causative agent of both uncomplicated and complicated UTIs, accounting for nearly 80% of cases. Other important pathogens include *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Flores-Mireles *et al*., 2015; Kot, 2019). The pathogenicity of these organisms is driven by a range of virulence determinants adhesins, toxins, siderophores and biofilm-forming capabilities that enhance colonization, persistence and recurrence of infection (Flores-Mireles *et al*., 2015).

The rise of antimicrobial resistance (AMR) has further complicated the management of urinary tract infections. Multidrug-resistant (MDR) UPEC and other uropathogens are increasingly resistant to first-line drugs such as β-lactams, fluoroquinolones and trimethoprim-sulfamethoxazole, while extended-spectrum β-lactamase (ESBL)-producing *Klebsiella* and carbapenem-resistant Enterobacterales represent major therapeutic threats (World Health Organization, 2025). A recent global systematic review reported resistance rates exceeding 50% in community-acquired UTI isolates across Africa and Asia (Barry *et al*., 2017; Sugianli *et al.*, 2021). The consequences of MDR-UTIs include longer hospital stays, higher treatment costs and increased risks of complications such as pyelonephritis and sepsis (Madrazo *et al*., 2023).

Given the diminishing efficacy of conventional antibiotics, there is an urgent need to identify alternative or adjunct therapies. Medicinal plants represent a promising avenue, as they are rich in secondary metabolites including alkaloids, flavonoids, tannins, terpenoids and saponins that exhibit diverse antimicrobial mechanisms. These mechanisms include disruption of microbial cell membranes, inhibition of essential enzymes, interference with quorum sensing and suppression of biofilm formation (Cowan, 1999; Dzotam *et al*., 2016). Unlike single-target antibiotics, phytochemicals often exert multi-target effects, reducing the likelihood of rapid resistance development (Khameneh *et al*., 2021).

Among ethnomedicinal plants, *Senna occidentalis* L**.** (family Fabaceae), commonly known as coffee senna, has been widely used in traditional medicine across Africa, Asia and South America. Traditionally, its leaves, roots and seeds have been employed to treat fever, liver disorders, skin diseases and various microbial infections (Yadav *et al*., 2010; Amponsah *et al*., 2016). Phytochemical analyses have revealed the presence of anthraquinones, flavonoids, alkaloids, tannins, saponins and terpenoids, many of which are associated with antimicrobial, anti-inflammatory and antioxidant activities (Oyewole *et al*., 2023).

Recent experimental studies provide scientific support for these traditional uses. For example, methanol extract of *Senna occidentalis* have shown significant inhibitory activity against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa in vitro* (Angelin *et al*., 2021). *In silico* studies further suggest that specific phytochemicals from *S. occidentalis* may target bacterial quorum-sensing regulators, thereby reducing virulence (Imon *et al*., 2023). However, despite this growing body of evidence, there remains a notable gap regarding the comparative antibacterial efficacy of methanol and ethanol extracts of *S. occidentalis* specifically against clinically isolated uropathogens. This study addresses this gap by evaluating their phytochemical profiles and antibacterial activities against urinary tract pathogens.

MATERIALS AND METHODS

Study Area

This study was conducted at General Hospital Dutsin-Ma, Katsina State, Nigeria, a secondary healthcare facility that serves as a referral center for surrounding communities. Dutsin-Ma lies in a semi-arid region of northern Nigeria, characterized by seasonal rainfall and a predominantly agrarian economy. The geographical and environmental context of this location contributes to the high prevalence of urinary tract infections (UTIs) and frequent reliance on empirical antibiotic therapy (Auta *et al*., 2019).

Plant Material Collection and Authentication

Fresh leaves of *Senna occidentalis* were collected in March, 2025 from the premises of Isa Kaita College of Education, Dutsin-Ma, Katsina State, Nigeria. The plant was authenticated by a taxonomist in the Department of Plant Science and Biotechnology, Federal University Dutsin-Ma and a voucher specimen (No. FUDMA/PSB/00046) was deposited in

the institutional herbarium. Plant authentication followed international standards for botanical verification (Bridson and Forman, 2023).

Preparation of Plant Extracts

The collected leaves were thoroughly washed under running tap water to remove debris and shade-dried at room temperature (28 ± 2 °C) to preserve thermolabile phytoconstituents. The dried samples were pulverized using a sterilized industrial grinder into fine powder and stored in airtight containers. For extraction, 300 g of powdered leaves were soaked separately in methanol and ethanol (3:2, v/v with sterile distilled water) for 4 days at room temperature with intermittent agitation. Filtration was performed using Whatman No. 1 filter paper and the filtrates were concentrated under reduced pressure at 40 °C using a rotary evaporator (Heidolph, Germany). The resulting extracts were further dried in a desiccator and weighed to determine the yield. Extract residues were stored at 4 °C until use. This protocol aligned with current recommendations for phytopharmacological research to ensure reproducibility and bioactivity retention (Abubakar and Haque, 2020).

Collection and Confirmation of Clinical Isolates

Clinical isolates of *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Proteus vulgaris* and *Proteus mirabilis* were obtained from the Microbiology Laboratory of General Hospital, Dutsin-Ma, Katsina State, Nigeria. These pathogens were selected based on their established role as predominant uropathogens (Flores-Mireles *et al*., 2015). The isolates were confirmed using a combination of cultural, morphological and biochemical tests including Gram staining, catalase, oxidase, indole, citrate utilization, urease, Voges-Proskauer, methyl red and triple sugar iron tests (Cheesbrough, 2006; Clinical and Laboratory Standards Institute, 2024). Pure colonies were preserved on nutrient agar slants at 4 °C for subsequent assays.

Standardization of Inoculum

The bacterial inocula were standardized according to the 0.5 McFarland turbidity standard, equivalent to 1.5×10^8 Cfu/mL. The standard was prepared by mixing 0.05 mL of 1% barium chloride with 9.95 mL of 1% sulfuric acid, producing a suspension with absorbance between 0.08–0.10 at 625 nm (Clinical and Laboratory Standards Institute, 2024). Standardization ensures reproducibility of antimicrobial assays (Andrews, 2001).

Antibacterial Susceptibility Testing

The antibacterial activities of methanol and ethanol extracts were evaluated using the agar well diffusion method, following Clinical and Laboratory Standards Institute (Clinical and Laboratory Standards Institute, 2024) guidelines. Mueller–Hinton agar plates were inoculated with 0.1 mL of standardized bacterial suspensions and spread evenly. Wells of 6 mm diameter were aseptically bored into the agar and filled with 100 µL of each extract solution (50 mg/mL). Plates were pre-incubated at room temperature for 1 h to allow diffusion and subsequently incubated at 37 °C for 24 h. Zones of inhibition were measured in millimeters using a meter rule. Standard antibiotics (streptomycin, ceftriaxone and ampicillin, 1 mg/mL each) served as positive controls, while 10% dimethyl sulfoxide (DMSO) was used as a negative control. Each assay was performed in triplicate. This methodology remains widely adopted for primary antimicrobial screening of plant extracts (Balouiri *et al*., 2016; Oyewole *et al*., 2023).

Determination of Minimum Inhibitory Concentrations (MICs)

The minimum inhibitory concentrations were determined using the agar dilution method. Two-fold serial dilutions of each extract were prepared to obtain concentrations ranging from 0.78–50 mg/mL. Each concentration was incorporated into molten nutrient agar, poured into Petri dishes and allowed to solidify. Standardized bacterial inocula were streaked onto the surface and incubated at 37 °C for 24–48 h. Minimum inhibitory concentrations were defined as the lowest concentration that completely inhibited visible bacterial growth (Oyewole *et al*., 2023).

Determination of Minimum Bactericidal Concentrations (MBCs)

The minimum bactericidal concentrations (MBC) were determined by sub-culturing aliquots from plates showing no visible growth at MIC onto fresh extract-free nutrient agar. Plates were incubated at 37 °C for 24–48 h. The MBC was recorded as the lowest concentration showing no bacterial growth. This step distinguishes bactericidal from bacteriostatic effects (Abidoye *et al*., 2020).

Qualitative Phytochemical Screening

Qualitative phytochemical screening of methanol and ethanol extracts was conducted to detect bioactive constituents using standard methods (Harborne, 1998; Trease and Evans, 2009). The following tests were performed:

Test for Tannins

Exactly, 1.0 g of each crude extract was dissolved in 10 mL of sterile distilled water and filtered through Whatman No. 1 filter paper. Two drops of 10% ferric chloride ($FeCl_3$) solution were added to the filtrate. Development of a blue-black coloration indicated the presence of hydrolyzable tannins.

Test for Alkaloids

About 0.5 g of the crude extract was dissolved in 5 mL of 1% hydrochloric acid (HCl) on a steam bath and filtered. One milliliter (1 mL) of the filtrate was treated with a few drops of Dragendorff's reagent. The appearance of an orange-brown precipitate or turbidity was taken as a positive indication of alkaloids.

Test for Flavonoids

Exactly, 0.5 g of the extract was dissolved in 5 mL of ethyl acetate and heated on a steam bath for 3 minutes for flavonoid detection. The mixture was filtered and 4 mL of the filtrate was combined with 1 mL of dilute ammonia solution. The formation of an intense yellow coloration, which became clearer upon standing, confirmed the presence of flavonoids.

Test for Saponins (Frothing Test)

Two grams (2.0 g) of the crude extract were boiled in 20 mL of distilled water in a water bath for 5 minutes and filtered. Exactly, 10 mL of the filtrate was diluted with 5 mL of distilled water in a test tube and shaken vigorously for 30 seconds. The persistence of a stable froth for at least 15 minutes indicated the presence of saponins. To further confirm, three drops of olive oil were added to the froth, shaken vigorously and the formation of an emulsion was regarded as a positive test.

Test for Steroids

Exactly, 0.5 g of the extract was dissolved in 3 mL of chloroform and filtered. To the filtrate, 2 mL of concentrated sulfuric acid (H_2SO_4, 98%) was carefully added to form a lower layer. The appearance of a reddish-brown coloration at the interface indicated the presence of steroids.

Test for Terpenoids (Salkowski's Test)

Exactly, 0.2 g of the crude extract was dissolved in 2 mL of chloroform. To this solution, 3 mL of concentrated sulfuric acid (H_2SO_4) was added carefully along the side of the test tube to form two distinct layers. The development of a reddish-brown coloration at the interphase was considered a positive test for terpenoids.

Test for Phenols

Two milliliters (2 mL) of the crude extract were treated with a few drops of freshly prepared 3% ferric chloride ($FeCl_3$) solution. The formation of a deep bluish-green coloration confirmed the presence of phenolic compounds.

Data Analysis

All experiments were conducted in triplicates. Results were expressed as mean ± standard deviation (SD). Statistical analyses were performed using IBM SPSS version 26.0 (IBM Corp., Armonk, NY, USA). Comparisons of mean inhibition zones, MIC and MBC values were analyzed using one-way analysis of variance (ANOVA), with significance set at $p < 0.05$.

RESULTS

Antibacterial Activity of *Senna occidentalis* Leaf Extracts

Methanol and ethanol extracts of *Senna occidentalis* leaves demonstrated dose-dependent antibacterial activity against all tested uropathogens. At 50 mg/mL, methanol extract exhibited larger inhibition zones (16–24 mm) compared with ethanol extract (12–18 mm) (Table 1). Among the tested bacteria, *Proteus vulgaris* was the most susceptible to the methanol extract (24 ± 2.0 mm), while *Staphylococcus aureus* displayed the least susceptibility (16 ± 1.0 mm) (Table 1). By contrast, ethanol extract yielded modest activity, with inhibition zones ranging from 12 ± 2.0 mm (*S. aureus*) to 18 ± 2.0 mm (*P. mirabilis*). Positive control antibiotics (streptomycin, ampicillin and ceftriaxone) produced inhibition zones ranging from 16 to 32 mm. Ceftriaxone exhibited the highest activity (up to 32 ± 2.0 mm against *P. mirabilis*), whereas ampicillin was ineffective against *Staphylococcus aureus* and *Proteus vulgaris*.

Minimum Inhibitory Concentrations (MIC)

The methanol extract displayed lower MIC values (6.25–25 mg/mL) compared with the ethanol extract (12.5–25 mg/mL). The lowest MIC (6.25 mg/mL) was

observed for *Escherichia coli* and *Proteus vulgaris* treated with the methanol extract, indicating strong antibacterial potency. In contrast, ethanol extract exhibited relatively higher MIC, with *Stahphylococcus aureus* and *Klebsiella pneumoniae* inhibited only at 25 mg/mL (Table 2).

Minimum Bactericidal Concentrations (MBC)

Methanol extract exhibited bactericidal activity at lower concentrations (12.5–25 mg/mL) compared with ethanol extract (25–50 mg/mL). The lowest MBC value was 12.5 mg/mL for *E. coli* and *P. vulgaris* treated with methanol extract, highlighting their heightened sensitivity. *Klebsiella pneumoniae* was the least susceptible, requiring 50 mg/mL for bactericidal effect in both extracts (Table 2).

Phytochemical Composition of Extracts

Phytochemical screening revealed the presence of alkaloids, tannins, saponins, steroids, terpenoids and phenols in both methanol and ethanol extracts. Flavonoids, however, were detected only in the methanol extract (Table 3). This difference in phytochemical profile likely accounts for the superior antibacterial activity of the methanol extract, as flavonoids are known for their antioxidant, membrane-disruptive and enzyme-inhibitory properties (Ndongo, 2017).

Table 1: Antibacterial activities of methanol and ethanol extracts of *Senna occidentalis*

Bacteria isolates	Zones of inhibition (mm)				
	Ethanol extract (50 mg/mL)	Methanol extract (50 mg/mL)	STP (1mg/mL)	AMP (1mg/mL)	CFX (1mg/mL)
Escherichia coli	14±2.00	22±2.00	24±2.00	20±2.00	32±2.00
Staphylococcus aureus	12±2.00	16±1.00	16±2.00	0	21±1.00
Proteus vulgaris	14±1.00	24±2.00	26±2.00	0	30±2.00
Proteus mirabilis	18±2.00	20±1.00	21±2.52	18±2.02	32±2.00
Klebsiella pneumoniae	14±2.00	16±2.00	24±1.00	20±1.00	20±2.00

Key: STP: Streptomycin; AMP: Ampicillin; CFX: Ceftriaxone, 0=Not sensitive

Table 2. Minimum inhibitory concentrations and minimum bactericidal concentrations of *Senna occidentalis* leaf extracts against uropathogens

	Ethanol extract (mg/mL)		Methanol extract (mg/mL)	
Bacterial Isolates	**MIC**	**MBC**	**MIC**	**MBC**
Escherichia coli	25	50	6.25	12.5
Staphylococcus aureus	25	50	12.5	25
Proteus vulgaris	25	50	6.25	12.5
Proteus mirabilis	12.5	25	12.5	25
Klebsiella pneumoniae	25	50	25	50

Table 3. Phytochemical composition of methanol and ethanol leaf extracts of *Senna occidentalis*

Phytochemical test	Ethanol extract	Methanol extract
Alkaloids	Detected	Detected
Tannins	Detected	Detected
Saponins	Detected	Detected
Flavonoids	Not Detected	Detected
Steroids	Detected	Detected
Phenols	Detected	Detected
Terpenoids	Detected	Detected

DISCUSSION

The present study demonstrated that both methanol and ethanol leaf extracts of *Senna occidentalis* possess substantial antibacterial activity against major uropathogens with the methanol extract showing superior efficacy. These findings provide scientific validation for the ethnomedicinal use of *S. occidentalis* in treating infections and highlight its potential as a source of novel antimicrobial agents for urinary tract infections (UTIs).

The methanol extract produced larger inhibition zones (16–24 mm) and lower minimum inhibitory

concentrations (6.25–25 mg/mL) compared with ethanol extract (12–18 mm inhibition zones; MICs 12.5–25 mg/mL). This suggests that methanol was more efficient at extracting bioactive compounds. Methanol, a polar solvent, often penetrates plant matrices more effectively, dissolving a broader spectrum of secondary metabolites than ethanol (Abubakar and Haque, 2020). Similar solvent-dependent variations have been reported in other medicinal plants, where methanol extract exhibited stronger antibacterial activities due to higher yields of phenolics and flavonoids (Mhamdi *et al*., 2025). These findings corroborated earlier reports on *S. occidentalis* antibacterial potential by Angelin *et al*. (2021) who documented inhibitory activity against *Escherichia coli* and *Staphylococcus aureus*, while Imon *et al*. (2023) reported dose-dependent inhibition of MDR on *Pseudomonas aeruginosa*.

Interestingly, the MIC values observed in this study (6.25–25 mg/mL for methanol extract) were lower than those reported in some earlier works, suggesting possible differences in extraction protocols, bacterial strain susceptibility or regional variations in phytochemical content (Oyewole *et al*., 2023). These variations highlight the importance of standardizing extraction and testing methods to allow cross-study comparisons and reproducibility. Also, *Proteus vulgaris* and *E. coli* were the most susceptible to methanol extract, with MIC values as low as 6.25 mg/mL. This aligned with previous studies where *S. occidentalis* extracts inhibited Gram-negative bacteria more effectively than Gram-positive species (Angelin *et al*., 2021; Imon *et al*., 2023). However, *Klebsiella pneumoniae* demonstrated the least susceptibility, requiring higher concentration (MBC: 50 mg/mL). This resistance may be attributed to its thick polysaccharide capsule and extended-spectrum β-lactamase (ESBL) activity, which enhance survival against antimicrobial agents (Abdul Raouf *et al*., 2022).

In comparison with standard antibiotics, the extracts displayed moderate yet significant antibacterial activity. While streptomycin and ceftriaxone produced larger inhibition zones, methanol extract showed activity within the accepted threshold for meaningful antimicrobial efficacy (Suffredini *et al*., 2006). Importantly, the extracts were active against pathogens that exhibited reduced sensitivity to ampicillin, suggesting potential as complementary or alternative therapeutics in the face of antibiotic resistance.

Phytochemical screening revealed alkaloids, tannins, saponins, steroids, terpenoids and phenols in both methanol and ethanol extracts with flavonoids present only in the methanol extract. This difference may explain the enhanced potency of methanol extract, as flavonoids are well documented for their antimicrobial, antioxidant and anti-inflammatory activities (Ndongo, 2017). Flavonoids exert antibacterial activity by inhibiting nucleic acid synthesis, disrupting cytoplasmic membranes and modulating energy metabolism (Zhenyou *et al*., 2022). Tannins act through protein precipitation and enzyme inhibition, impairing bacterial adhesion and growth (Shimada, 2006; Dzotam *et al*., 2016).

Saponins form complexes with sterols in microbial membranes, increasing permeability and causing cell lysis (Francis *et al*., 2002). Alkaloids interfere with DNA replication and protein synthesis, contributing to broad-spectrum antimicrobial activity (Thawabteh *et al*., 2019). Terpenoids and phenolics disrupt cell wall integrity and inhibit virulence factors such as quorum sensing and biofilm formation (Huang *et al*., 2022). The synergy of these phytochemicals likely accounts for the broad-spectrum antibacterial effects observed in this study. Previous reports have suggested that whole extracts may act more effectively than isolated compounds due to additive or synergistic interactions among metabolites (Rasoanaivo *et al*., 2011).

CONCLUSION

This study demonstrates that *Senna occidentalis* leaf extracts, particularly the methanol fraction, exhibit significant antibacterial activity against common uropathogens with inhibitory and bactericidal effects attributable to a rich phytochemical repertoire. These findings provide scientific support for the ethnomedicinal use of *S. occidentalis* and underscore its potential for developing novel plant-based therapeutics to combat MDR-UTIs. Further research should focus on bioactive compound isolation, mechanistic elucidation and *in vivo* validation to advance its clinical applicability.

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