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Antibacterial activity and phytochemical profile of *Senna occidentalis* (L.) leaf extracts

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Abstract
Senna occidentalis is a medicinal plant traditionally used for the treatment of various ailments. This study evaluated the phytochemical constituents and in vitro antibacterial activity of methanol and ethanol leaf extracts of *S. occidentalis* against clinical isolates of *Staphylococcus aureus* and *Escherichia coli*. The leaves were collected, authenticated, air-dried, pulverized, and extracted using methanol and ethanol. Phytochemical screening was conducted using standard qualitative methods, while antibacterial activity was evaluated using the agar well diffusion method, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC). The extracts contained alkaloids, flavonoids, steroids, glycosides, tannins, and anthraquinones in varying intensities. Both extracts demonstrated antibacterial activity against the test organisms. The methanol and ethanol extracts produced inhibition zones ranging from 7–17 mm and 4–19 mm, respectively. The MIC was 12.5 mg/mL for both extracts against both organisms. The MBCs of the methanol extract were 50 mg/mL for *E. coli* and 100 mg/mL for *S. aureus*, while those of the ethanol extract were 25 mg/mL and 50 mg/mL, respectively. The findings demonstrate the antibacterial potential of *S. occidentalis* leaf extracts, support their traditional medicinal use and suggests its potential as a source for developing plant-based antimicrobial agents to combat drug-resistant infections.

Keywords: Antimicrobial activity; *Escherichia* ***coli;*** *Senna occidentalis*; *Staphylococcus aureus*

INTRODUCTION

The global rise in antimicrobial resistance (AMR) poses a severe threat to public health and the effective management of infectious diseases, rendering many conventional antibiotics increasingly ineffective. The World Health Organization (WHO) has declared AMR one of the top ten global public health threats in recent years [1]. This crisis has been described as a "silent pandemic" due to the accelerating spread of resistant pathogens and their profound impact on morbidity, mortality and healthcare systems globally [2]. As antimicrobial resistance erodes the efficacy of existing antibiotics, there is an urgent need for innovative strategies and therapeutic alternatives to safeguard modern medicine [3,4]. Because of this escalating challenge, the search for novel antimicrobial agents has intensified, with natural products particularly from medicinal plants offering a viable alternative. Plant-derived compounds

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often possess a rich diversity of bioactive constituents (alkaloids, flavonoids, terpenes, saponins and so forth) and may exhibit lower levels of resistance development compared to synthetic drugs, thereby providing promising leads in antimicrobial drug discovery [5,6]. In many low- income settings, medicinal plants also present more accessible and affordable therapeutic options, aligning with traditional healthcare systems and local ethnomedicinal practices. *Senna occidentalis* (L.) (synonym: *Cassia occidentalis*), commonly known as coffee senna or "Tafasan Doki" in Northern Nigeria, is a shrub belonging to the family Fabaceae.

In Nigeria, the plant is known by various local names, including "Ewe Oriesi" in Yoruba, "Akidi agbara" in Igbo, and "Sanga-Sanga" in Hausa [7,8]. It is widely employed in traditional medicine across Africa and Asia for the management of a variety of ailments including wounds, skin diseases, fever, hepatitis and typhoid [9,10,11]. Typical ethnomedicinal preparations involve leaf pastes or infusions (roots, stems or leaves) administered topically or internally according to local customs. Pharmacological studies have begun to validate many of the traditional uses of *S. occidentalis*. The therapeutic potential of *S. occidentalis* is attributed largely to its rich secondary-metabolite profile: alkaloids, flavonoids, anthraquinones, tannins and saponins have been identified and may contribute to its antimicrobial and other biological effects [12,13]. Given the documented traditional uses of *S. occidentalis*, and the urgent need for new antimicrobial agents in the era of rising AMR, the present study was designed to investigate the phytochemical composition and evaluate the in vitro antibacterial efficacy of methanol and ethanol leaf extracts of *S. occidentalis*. The antibacterial activity was assessed against two clinically significant bacterial pathogens: *S. aureus* a Gram-positive bacterium frequently implicated in skin infections, pneumonia and sepsis and *E. coli* a Gram-negative pathogen commonly responsible for urinary tract infections, diarrhoeal disease and bloodstream infections.

EXPERIMENTAL METHODS

Collection and identification of plant material. Fresh leaves of *Senna occidentalis* were collected from the vicinity of Gombe State University, Nigeria, in November 2023. The plant specimen was authenticated (Voucher Specimen Number: GSUH508) at the Department of Plant Science, Gombe State University.

Preparation of plant extracts. The leaves were air-dried at room temperature and pulverized into a fine powder using a mortar and pestle. Fifty grams (50 g) of the powdered material was macerated in 250 mL of either methanol or ethanol with occasional shaking for 6 hours, followed by standing for 24 hours. The mixture was filtered using Whatman No. 1 filter paper. The filtrate was concentrated, and the crude extract was stored at 4°C until use. The percentage yield was calculated.

Phytochemical screening. The methanol and ethanol extracts were subjected to qualitative phytochemical analysis using standard procedures to detect the presence of alkaloids, flavonoids, steroids, saponins, glycosides, tannins, and anthraquinones [14,15].

Ethical consideration. Ethical approval was obtained from the ethics and publication committee of Gombe State University to permits the collection of bacterial isolates.

Bacterial isolates and confirmation. Bacterial isolates of *S. aureus* and *E. coli* were obtained from the Microbiology Laboratory, Gombe State University. The bacterial isolates were subjected to Gram staining and biochemical tests for confirmatory identification. For Gram staining, a thin smear of each isolate was prepared on a clean glass slide, air-dried, heat-fixed, and sequentially

stained with crystal violet, Gram's iodine, decolorizer, and safranin. The stained smears were examined microscopically under the oil immersion objective (×100). *Staphylococcus aureus* was identified as Gram-positive cocci occurring predominantly in clusters, while *Escherichia coli* was identified as Gram-negative bacilli.

The isolates were further characterized based on colony morphology and biochemical reactions. The catalase test was performed by placing a portion of a fresh colony on a clean glass slide and adding a drop of 3% hydrogen peroxide. Immediate bubble formation was recorded as a positive reaction. The coagulase test was performed for suspected *S. aureus* isolates using plasma; visible clot formation was interpreted as a positive reaction. For *E. coli* identification, the indole test was performed by inoculating the test organism into tryptone broth and incubating at 37°C for 24 hours, after which Kovac's reagent was added. Development of a red ring at the surface indicated a positive result. Motility was determined by observing the growth pattern of the organism in semi-solid medium, with diffuse growth away from the inoculation line indicating motility. Urease activity was determined using urea-containing medium, with a change in colour indicating a positive reaction. The results of the Gram stain, colony morphology, and biochemical tests were compared with standard characteristics of the respective organisms for final identification [16].

Antibacterial susceptibility testing. The antibacterial activity was evaluated using the agar well diffusion method [17,18]. Briefly, Fresh bacterial cultures were prepared from 18–24-hour-old cultures, and colonies were suspended in sterile normal saline and adjusted to a turbidity equivalent to 0.5 McFarland standard, corresponding to approximately 1.5×10^8 CFU/mL. The standardized inoculum was used to inoculate the surface of the Mueller-Hinton Agar (MHA) plates. Wells of 6 mm diameter were aseptically punched, and 100 µl of each extract at concentrations of 100, 50, 25, and 12.5 mg/mL dissolved in Dimethyl Sulfoxide, DMSO was added to the respective wells. DMSO served as the negative control. The plates were incubated at 37°C for 24 hours, and the zones of inhibition were measured in millimeters (mm).

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). The Minimum Inhibitory Concentration (MIC) was determined using the broth dilution method [19,20]. The bacterial inoculum was standardized to 0.5 McFarland turbidity, corresponding to approximately 1.5×10^8 CFU/mL. Serial dilutions of the extract (400 to 6.25 mg/mL) were prepared in Mueller-Hinton broth and inoculated with the test organism. Each dilution was inoculated with the standardized bacterial suspension and incubated at 37°C for 18–24 hours. The MIC was recorded as the lowest concentration of the extract that showed no visible growth or turbidity compared with the uninoculated control. The MIC was defined as the lowest concentration with no visible growth. The Minimum Bactericidal Concentration (MBC) was determined by sub-culturing from the clear tubes onto nutrient agar; the MBC was the lowest concentration that killed 99.9% of the inoculum.

RESULTS

Phytochemical screening. The qualitative phytochemical analysis revealed the presence of various bioactive compounds (Table 1). Alkaloids, glycosides, and tannins were prominently present. Notable differences were observed between the two solvents; for instance, tannins were more intense (+++) in the methanol extract compared to the ethanol extract (+), while anthraquinones were absent in the ethanol extract.

Antibacterial activity. Both extracts exhibited antibacterial activity against *S. aureus* and *E. coli* in a concentration-dependent manner (Table 2). The methanol extract showed a broader range of inhibition (7-17 mm) compared to ethanol (4-19 mm). The positive control showed expected zones of inhibition (16-19 mm).

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). The MIC for both extracts against both bacterial isolates was consistently 12.5 mg/mL. The MBC values indicated that the ethanol extract was more bactericidal against *E. coli* (MBC = 25 mg/mL) than the methanol extract (MBC = 50 mg/mL). For *S. aureus*, both the ethanol and methanol extracts exhibited an MBC of 50 mg/mL (Table 3).

Table 1: Phytochemical constituents of *Senna occidentalis* leaf extracts.

Phytochemical constituent	Solvent used	
	Methanol	Ethanol
Alkaloids	+	+
Flavonoids	+	+
Steroids	+	+
Saponins	-	-
Glycosides	+	+
Tannins	+	+
Anthraquinones	+	-

+: Present; - : Absent

Table 2: Zone of inhibition of methanol and ethanol extract of *S. occidentalis*

Isolates	Concentrations and zone of inhibition (mm) for Methanol				
	100 mg/mL	50 mg/mL	25 mg/mL	12.5 mg/mL	Ciprofloxacin (5 µg/disc)
E. coli	16	12	17	12	19
S. aureus	13	7	9	12	16
	Concentrations and zone of inhibition (mm) for Ethanol				
	100 mg/mL	50 mg/mL	25 mg/mL	12.5 mg/mL	Ciprofloxacin (5 µg/disc)
E. coli	13	6	5	4	16
S. aureus	15	11	6	11	19

Table 3: MIC and MBC (mg/mL) of *Senna occidentalis* extracts

Isolates	Methanol (mg/mL)		Ethanol Extract (mg/mL)	
	MIC	MBC	MIC	MBC
E. coli	12.5	50	12.5	25
S. aureus	12.5	50	12.5	50

DISCUSSION

The qualitative phytochemical screening revealed differences in the phytochemical profiles of the methanol and ethanol extracts. Both extracts contained alkaloids, flavonoids, steroids, glycosides, and tannins, while anthraquinones were detected only in the methanol extract and saponins were absent in both extracts. Tannins showed greater intensity in the methanol extract (+++) than in the ethanol extract (+). These differences suggest that the choice of extraction solvent may influence the types and relative abundance of phytochemical constituents recovered from *S. occidentalis* leaves, as reported in previous studies [21,22]. However, the present study compared only methanol and ethanol extracts; therefore, broader conclusions regarding the effect of solvent polarity on phytochemical recovery cannot be drawn from the present findings alone.

Qualitative phytochemical screening revealed marked variations in metabolite profiles between the extracts. Methanol, a highly polar solvent, yielded a phytochemical spectrum rich in tannins and anthraquinones, phytomarkers commonly associated with *Senna* species [23]. Tannins are well-documented antimicrobial agents, acting primarily through protein precipitation, enzyme inhibition, and disruption of microbial cell wall integrity [24]. The presence of tannins in the methanol extract therefore likely contributed to its strong inhibitory (bacteriostatic) effect, as reflected in the growth suppression observed during susceptibility testing. In contrast, ethanol extraction yielded a higher abundance of flavonoids, compounds known for their potent membrane-disruptive and metabolic-inhibitory antibacterial mechanisms [21].

A comparison of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values showed that, although the MIC was identical (12.5 mg/mL) for both extracts against both bacterial strains, the MBC values differed between the extracts.

The ethanol extract showed lower MBC values (*E. coli*: 25 mg/mL; *S. aureus*: 50 mg/mL) compared to the methanol extract (*E. coli*: 50 mg/mL; *S. aureus*: 50 mg/mL). This indicates that while the total inhibitory activity of the phytochemical "cocktail" in each extract is equivalent at the MIC threshold, the specific compounds in the ethanol extract may be more effective at causing irreversible, lethal damage to the bacterial cells. This observation may reflect the combined contribution of multiple phytochemical constituents present in the extracts, including flavonoids, alkaloids, glycosides, tannins, steroids, and anthraquinones, rather than the activity of any single metabolite class. The observed difference in MBC values therefore suggests that the overall phytochemical composition of the extracts may have contributed to their bactericidal effects. However, the relative contribution of individual constituents cannot be established from the qualitative phytochemical screening conducted in the present study.

The bactericidal action of the ethanol extract against *E. coli*, a Gram-negative organism with a robust outer membrane barrier, is particularly noteworthy. Gram-negative bacteria often have reduced susceptibility to plant-derived antimicrobial agents; thus, the enhanced activity observed here underscores the therapeutic promise of ethanol-soluble phytochemicals in *S. occidentalis*. The presence of alkaloids and glycosides in both extracts further suggests a shared baseline antimicrobial contribution from these metabolite classes, which have been associated with DNA intercalation and membrane impairment [27,28]. Neither extract tested positive for saponins, a metabolite class commonly implicated in plant-mediated antimicrobial activity. Future studies employing chromatographic purification and molecular docking approaches are warranted to isolate active constituents and further elucidate their antimicrobial mechanisms.

The zones of inhibition observed in the present study were used to demonstrate the antibacterial activity of the extracts.

Conclusion. This study confirms the antibacterial potential of *S. occidentalis*. The methanol extract showed potent bacteriostatic activity with broad-spectrum inhibition, In contrast, the ethanol extract emerges as a more effective bactericidal agent. The present study demonstrated that both methanol and ethanol extracts of *S. occidentalis* leaves possess notable antibacterial activity against *S. aureus* and *E. coli*, corroborating previous reports on the antimicrobial potential of the genus *Senna*.

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