African Journal of
Medicine, Surgery and Public Health Research

ISSN: 1595-8000

Index: Harvard, Boston, Sydney University, Dimensions, Lens, ResearchGet Scilit, Semantic, Google Scholar, Base etc

https://doi.org/10.58578/AJMSPHR.v2i3.7038

Antimicrobial Activity of *Senna siamea* Fresh Leaf Extracts (Ethanolic and Aqueous Solution) on *Shigella* Species and *Pseudomonas aeruginosa*

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Article Info:

Submitted:	Revised:	Accepted:	Published:
Jun 24, 2025	Jul 19, 2025	Jul 31, 2025	Aug 5, 2025

Abstract

The rising threat of antibiotic-resistant bacteria has intensified the search for alternative antimicrobial agents, particularly those derived from plants. Numerous plant species have long been used in traditional medicine, with leaf extracts showing promising antimicrobial potential. This study investigates the antimicrobial activity of ethanol and aqueous leaf extracts of *Senna siamea* against *Shigella* spp. and *Pseudomonas aeruginosa*. Fresh leaves of *S. siamea* were collected, authenticated, and subjected to solvent extraction using ethanol and distilled water. The antimicrobial effects of the extracts were evaluated using the agar well diffusion method, while the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were determined through standard microbiological procedures. The ethanol extract exhibited marked inhibitory effects against *P. aeruginosa* at all tested concentrations, whereas the aqueous extract showed greater efficacy against *Shigella* spp. at 100% concentration. Both extracts demonstrated bactericidal properties at a concentration of 1 mg/mL. These findings indicate that *S. siamea* contains bioactive compounds with significant antimicrobial activity, affirming its traditional use in treating infectious diseases. The results support the potential of *S. siamea* as a natural alternative for managing bacterial infections, particularly those involving resistant strains. Further research involving phytochemical profiling, toxicological analysis, and clinical evaluation is recommended to isolate the active compounds and assess their pharmaceutical applications.

Keywords: *Senna siamea*; Antimicrobial Activity; Ethanol and Aqueous Extracts; *Shigella* spp.; *Pseudomonas aeruginosa*

INTRODUCTION

In recent times, there has been growing interest in the development of natural medicines to complement synthetic antimicrobial drugs, which are becoming less effective due to rising resistance. Many plant leaves and their extracts have shown antimicrobial activity against various pathogens. Traditionally, hundreds of plant species have been used for medicinal purposes [1]. According to the World Health Organization, about 80% of the global population depends on traditional medicine, much of which involves the use of plant-based extracts [1, 2].

Medicinal plants' phytochemical components are essential to their therapeutic efficacy. Many pharmacological actions, such as pain relief, antimicrobial action, anti-inflammatory effects, antiparasitic functions, hormone regulation, and ulcer healing, are attributed to these natural substances, which include terpenoids, flavonoids, alkaloids, steroids, and phenolics.

Recent research has emphasized the development of antimicrobial agents to counter the harmful effects of microorganisms. Bacterial infections arise when pathogenic bacteria enter the body through inhalation, ingestion, or skin breaches and bypass the body's defence systems. If the immune response fails to contain them, infection symptoms develop. However, antimicrobial resistance (AMR) has become a major global challenge, reducing the effectiveness of existing antibiotics. The rise of drug-resistant bacteria threatens modern treatment approaches, with the problem being especially severe in developing countries

Senna siamea, a member of the Fabaceae sub-family (Caesalpinioideae) within the Leguminosae family, is commonly referred to as the kassod tree or yellow cassia [3]. Locally, it is known as "Malga" in Hausa, "Asunon Oyibo" in Yoruba, and "Akidi Nmuo" in Igbo. Originally from tropical Asia, this plant has been widely cultivated across tropical Africa. Various parts of *S. siamea,* including its leaves, stems, roots, flowers, and seeds, are used for medicinal purposes, particularly for treating malaria and typhoid endemic diseases with significant morbidity and mortality rates.

The leaves of *Senna siamea* are widely used in traditional medicine, especially across Africa and Asia, for preparing herbal remedies. In Burkina Faso, for example, a decoction made by boiling fresh or dried leaves with water and lemon juice is consumed or used in body baths to reduce fever. *S. siamea* has been traditionally used to manage a variety of conditions including constipation, diabetes, insomnia, hypertension, asthma, typhoid fever, and as a diuretic [4]. Its leaves and bark are also employed for malaria treatment [5]. Ethnomedicinal uses extend to treating jaundice, abdominal and menstrual pain, high blood sugar, digestive and urinary disorders, herpes, rhinitis, intestinal worms, and childhood convulsions. The leaves, in particular, are highly valued for their effectiveness against infectious diseases

Bacterial infections continue to pose major public health threats due to their wide disease spectrum in both humans and animals. Notably, *Shigella* and *Pseudomonas* species are significant contributors to global morbidity and mortality. *Shigella* is mainly responsible for shigellosis, a severe diarrheal illness, while *Pseudomonas aeruginosa* is an opportunistic pathogen that causes serious infections in immunocompromised individuals [6].

Mainstream medicine is increasingly embracing the use of antimicrobial and other plant-derived drugs, especially as traditional antibiotics lose effectiveness and new, particularly viral, diseases prove challenging to treat. Local communities often grapple with various illnesses and infections such as diarrhea, typhoid, fever, cough, headaches, and ulcers, many of which are caused by microorganisms. Addressing these health issues is crucial, particularly considering the medicinal properties of *Senna siamea*.

Senna siamea is a promising source of antimicrobial agents, with the potential for phytochemicals of significant antimicrobial efficacy to be utilized in treating infections. The clinical effectiveness of many existing antibiotics is increasingly compromised by the emergence of multidrug-resistant pathogens. This study could benefit individuals, medical professionals, policymakers, and researchers, as there is growing interest in folk medicine to discover more effective treatments for microbial infections. The findings from this research are expected to provide a foundation for further investigations, offering baseline epidemiological data to support future studies and the development of improved antimicrobial therapies and aimed to evaluate the antimicrobial effects of aqueous and ethanol extracts of fresh *Senna siamea* leaves against *Shigella* and *Pseudomonas* species.

MATERIALS AND METHODS

Study area

Wukari Metropolis, situated in Taraba State and the region is intersected by the River Donga and River Benue [7, 8]. Wukari is located at latitude 7.53'43'N and longitude 9.47'59'E, with an estimated population ranging from 5,000 to 10,000. The area, covering 4,308 km², is mapped on plate 86F of the *Times Comprehensive Atlas of the World* [9,10].The Jukun people form the predominant ethnic group in Wukari with common spoken languages include Jukun, Hausa, Fulani, and Tiv. Beyond agriculture, commerce and civil service also play vital roles in the local economy the vegetation reflects a Savannah landscape, characterized by grasslands interspersed with scattered trees [7]. Major economic activities in the region include farming, fishing, and livestock rearing [11].

Collection of Plant Sample

The mature leaves of *Senna siamea* were obtained along Roger Road Marmara Area Wukari, Taraba State. The plant was authenticated by a botanist from the Department of Biological Sciences, Federal University Wukari Nigeria. It was rinsed with tap water to remove dust and other unwanted particles [12]

Test Organism

The bacterial strains used in this study were *Shigella* and *Pseudomonas* species. Stock cultures of these organisms were sourced from the National Veterinary Research Institute (NVRI) Vom, Plateau State, Nigeria. Gram staining was performed on the stock cultures, and additional biochemical tests were conducted to confirm the identity of the test organisms.

Preparation of Plant for Extraction

Fresh leaves were collected, washed with tap water, and air-dried for approximately two weeks. The dried leaves were then pulverized into a fine powder using a pestle and mortar in a controlled laboratory environment, as described by Samuel *et al.* [12]. For aqueous and ethanol extractions, 50 g of the powdered leaves sample was measured into separate conical flasks containing 450 ml of distilled water and 350 ml of ethanol, respectively. The mixtures were vigorously shaken initially and left to stand for three days. Afterward, they were filtered through muslin cloth, and the filtrates were collected into sterile crucibles. The filtrates were transferred to sterile reaction tubes and subjected to continuous heating in a water bath at 78°C for ethanol extraction and 105°C for distilled water extraction. The residues were then stored at room temperature.

Standardization of Extracts

Under aseptic conditions, the extracts were reconstituted by dissolving 1 g of each extract in 10 ml of dimethyl sulfoxide (DMSO), resulting in a concentration of 100 mg/ml.

Preparation of Media

Mueller Hinton agar was prepared using ready-to-use agar powder, following the manufacturer's instructions. To prepare the agar, 35 g of the powder was dissolved in 100 ml of distilled water in a conical flask and gently heated until the agar dissolved. The mixture was then autoclaved at 121°C for 15 minutes. After sterilization, the media was allowed to cool to 45°C, at which point approximately 25 ml was poured into sterile Petri dishes and allowed to solidify.

Standardization of the inoculums

The inoculum was standardized using the 0.5 McFarland standard, with barium sulfate ($BaSO_4$) serving as the reference for preparing the broth culture of the organisms. In prepare the solution, 1 g of barium chloride ($BaCl_2$) was dissolved in 99 ml of distilled water. Then, 1 ml of tetraoxosulphate (VI) acid was added to 99 ml of distilled water. Finally, 0.5 ml of 1% sulfuric acid (H_2SO_4) was added to the $BaCl_2$ solution (99.5 ml) to form barium sulfate ($BaSO_4$).

Antimicrobial Screening/Test

The sensitivity test was conducted using the agar well diffusion method as described by Brown *et al.* [8]. The appropriate volume of molten agar was poured into sterile Petri dishes and allowed to solidify. The Petri dishes were then inverted and incubated overnight to check for contamination. Sterile cotton swabs were dipped into the standardized inoculum and used to evenly swab the surface of the agar plates. A 6 mm cork borer was used to create equidistant wells on the agar. Extracts of different solvents and concentrations were added to the wells. A ciprofloxacin disc was placed in the center of the plate as the control. The plates were allowed to stand for 1 hour to allow the extracts to diffuse into the agar, and were incubated at 37°C for 24 hours. After incubation, the plates were examined for zones of inhibition around the wells.

Determination of Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) was determined using the method of Brown *et al.* [12]. Two-fold serial dilutions of the leaf extract were prepared, and 2 ml of each concentration was added to 18 ml of pre-sterilized molten nutrient agar at 40°C to achieve final concentrations of 50, 100, and 200 mg/ml. The mixture was poured into sterile petri dishes and allowed to solidify. After the surface dried, 18-hour-old bacterial isolates were streaked onto the medium. Ciprofloxacin was used as a positive control. The plates were incubated at 37°C for 24 hours, after which they were checked for bacterial growth. The MIC was defined as the lowest concentration of the extract that completely inhibited bacterial growth.

Statistical Analysis

Consider adding basic statistical analysis (e.g., bar chart or confidence intervals) to validate differences in prevalence rates.

RESULTS

Plant derived drugs come into use in the modern medicine through the uses of plant material as indigenous cure in folklore or traditional systems of medicine. Various plants have been employed as a result of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the flora. Medicinal plants constitute an efficient beginning for both traditional and advanced medical specialty [12]. Herbal medicines already form the basis of therapeutic use in the developing countries, but of recent, there has been an increase in the use of herbal medicines in the developed world too, due to easy accessibility and price effectiveness of this medicine [12; 13].

Ethanol extract showed no inhibition against *Shigella spp.* at all tested concentrations but exhibited a consistent inhibition (16mm) against *Pseudomonas aeruginosa* across 100%, 50%, and 25% concentrations. Aqueous extract displayed a dose-dependent inhibition against *Shigella spp.*, with zones of inhibition of 22mm (100%), 11mm (50%), and no inhibition at 25%. It inhibited *Pseudomonas aeruginosa* at 100% concentration (30mm) but showed no inhibition at lower concentrations. Positive controls exhibited significantly higher zones of inhibition, confirming the effectiveness of standard antibiotics. Both ethanol and aqueous extracts exhibited inhibitory effects at 1g/ml for *Shigella spp.* and *Pseudomonas aeruginosa*, while lower concentrations (0.5g/ml and 0.25g/ml) showed no inhibition. The MBC for both ethanol and aqueous extracts was determined to be 1g/ml for both *Shigella spp.* and *Pseudomonas aeruginosa*, indicating that a concentration of 1g/ml is required to kill these bacteria.

Table 1: Antimicrobial activity of ethanol leaf extract of fresh *Senna siamea* showing the zone of inhibition (mm)

	Concentrations (g/ml)			
Organisms	100%	50%	25%	Positive control
Shigella spp	-	-	-	30mm
Pseudomonas aeruginosa	16mm	16mm	16mm	35mm

Key: mg/ml = milligram per mile**, (-) = N**o Inhibition

Table 2: Antimicrobial activity of aqueous leaf extract of fresh *Senna siamea* showing the zone of inhibition (mm)

	Concentrations (g/ml)			
Organisms	100%	50%	25%	Positive control
Shigella spp	22mm	11mm	-	37mm
Pseudomonas aeruginosa	30mm	-	-	30mm

Key: mg/ml = milligram per mile**, (-) = N**o Inhibition

Table 3: Minimum inhibitory concentration (MIC) of ethanol leaf extract of fresh *Senna siamea* showing the zone of inhibition (mm)

	Concentrations (g/ml)		
Organisms	1g	0.5g	0.25g
Shigella Species	+	-	-
Pseudomonas aeruginosa	+	-	-

Key: mg/ml = milligram per mile**, (-) = N**o Inhibition, (+) = Inhibition

Table 4: Minimum inhibitory concentration (MIC) of aqueous leaf extract of fresh *Senna siamea* showing the zone of inhibition (mm)

	Concentrations (g/ml)		
Organisms	1g	0.5g	0.25g
Shigella Species	+	-	-
Pseudomonas aeruginosa	+	-	-

Key: mg/ml = milligram per mile**, (-) = N**o Inhibition, (+) = Inhibition

Table 5: Minimum bactericidal concentration (MBC) of both ethanol and aqueous leaf extract of Fresh *Senna siamea* showing the zone of inhibition (mm)

	Concentrations (g/ml)	
Organisms	Ethanol	Aqueous
Shigella Species	1g	1g
Pseudomonas aeruginosa	1g	1g

Key: mg/ml = milligram per mile**, (-) = N**o Inhibition, (+) = Inhibition

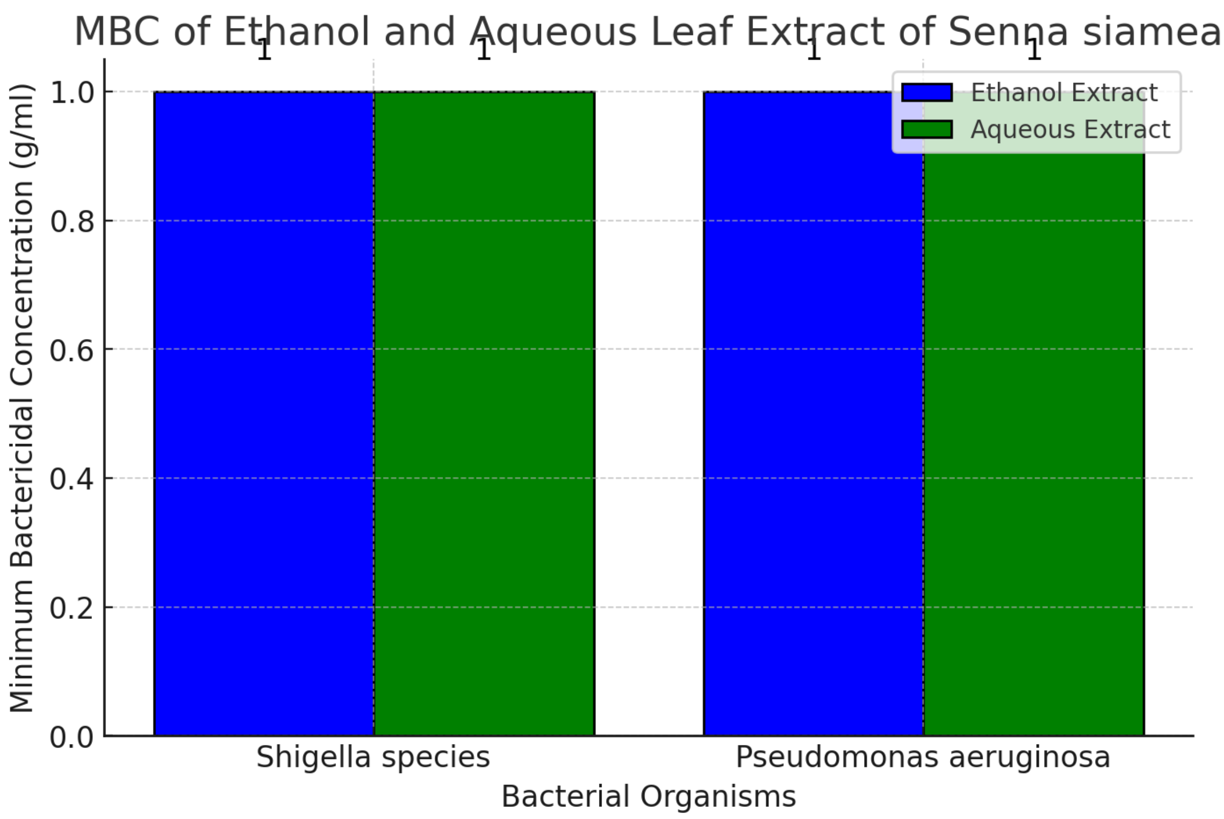

Figure 1: Minimum bactericidal concentration (MBC) of both ethanol and aqueous leaf extract

DISCUSSION

The antimicrobial activity of ethanol and aqueous extracts of *Senna siamea* leaves was evaluated against *Shigella spp*. and *Pseudomonas aeruginosa*. The findings indicate that *Senna siamea* exhibits antibacterial properties, supporting previous studies on the medicinal potential of Senna species [12, 13]. The inhibitory effects observed are likely attributed to the presence of bioactive compounds such as flavonoids, tannins, alkaloids, and saponins, which have been previously reported in *Senna* species [14].

Comparing ethanol and aqueous extracts, ethanol demonstrated better antimicrobial activity against Pseudomonas aeruginosa at all tested concentrations (Table 4.1 and 4.2). This trend aligns with studies by Sofowora [15] and Cowan [16], which reported that ethanol is a better solvent for extracting bioactive compounds due to its ability to dissolve both polar and non-polar constituents.

However, the aqueous extract showed stronger inhibition against *Shigella* spp. at 100% concentration (22mm) compared to ethanol, which showed no inhibition against *Shigella* spp. This may be due to the solubility of certain antimicrobial compounds in water, which specifically target *Shigella* spp. [17].

The Minimum Inhibitory Concentration (MIC) results (Tables 4.3 and 4.4) showed that the lowest effective concentration of both ethanol and aqueous extracts was 1g/ml, as inhibition was observed at this concentration for both test organisms. Lower concentrations (0.5g/ml and 0.25g/ml) showed no inhibition, suggesting that higher concentrations are required for efficacy. This aligns with the findings of Ahmad *et al.* [18], who reported that MIC values for medicinal plant extracts vary based on the plant species, extraction method, and test organism.

Minimum Bactericidal Concentration (MBC) results (Table 4.6) confirmed that 1g/ml of both ethanol and aqueous extracts was required to completely kill *Shigella* spp. and *Pseudomonas aeruginosa*. The fact that MIC and MBC values were the same suggests a bactericidal effect, rather than a bacteriostatic one, as the extracts effectively eliminated the bacteria rather than just inhibiting their growth [19].

The antimicrobial properties of *Senna siamea* are consistent with previous reports on Senna species. Goyal *et al.* [20] found that *Senna alata* extracts exhibited strong antibacterial effects against *Escherichia coli* and *Staphylococcus aureus*, supporting the broad-spectrum potential of Senna-derived compounds. Tona *et al.* [21] reported that other *Senna* species contain anthraquinones and flavonoids, which contribute to antimicrobial activity. Ogunjobi *et al.* [22] also observed significant inhibition of *Pseudomonas aeruginosa* using ethanol extracts of medicinal plants, reinforcing the current findings.

The results highlight the potential of *Senna siamea* as a natural antimicrobial agent, supporting its traditional use in herbal medicine. Given the increasing resistance of bacteria to conventional antibiotics, the development of plant-based alternatives could provide a sustainable solution [12]. The ability of ethanol and aqueous extracts to inhibit *Pseudomonas aeruginosa* and *Shigella* spp. suggests their potential for treating gastrointestinal and wound infections.

Senna siamea leaf extracts demonstrated significant antibacterial properties, particularly against Pseudomonas aeruginosa and Shigella spp. Ethanol extracts were more effective than aqueous extracts, highlighting the importance of solvent selection in extracting bioactive compounds. The MIC and MBC *results suggest that Senna siamea exhibits bactericidal activity, which is promising for therapeutic applications. Future research should focus on identifying and purifying the specific bioactive compounds responsible for antimicrobial activity to enhance their medicinal use.*

CONCLUSION

This study demonstrated the antimicrobial potential of *Senna siamea* leaf extracts against *Pseudomonas aeruginosa* and *Shigella* spp., confirming its traditional medicinal uses. The ethanol extract exhibited stronger antibacterial activity than the aqueous extract, particularly against *Pseudomonas aeruginosa*. However, the aqueous extract was more effective against *Shigella* spp. at higher concentrations. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) results indicate that both extracts have bactericidal effects, making them promising candidates for natural antimicrobial agents. Given the increasing resistance of bacterial pathogens to conventional antibiotics, plant-derived antimicrobials offer a valuable alternative for infection control. The bioactive compounds in *Senna siamea* responsible for this antimicrobial activity should be further studied, isolated, and characterized to explore their full therapeutic potential. Future research should also assess the toxicological effects and clinical efficacy of these extracts to validate their safe application in pharmaceutical and medical industries. These findings support the integration of herbal medicine into modern healthcare, especially in regions where antibiotic resistance poses a significant public health challenge.

Recommendation

Based on the findings of this study, the following recommendations are made:

1. The bioactive compounds responsible for the antimicrobial activity of *Senna siamea* leaf extracts should be isolated, characterized, and identified through advanced analytical techniques such as GC-MS, HPLC, and NMR spectroscopy.

2. Extensive toxicological assessments should be conducted to determine the safety profile of these extracts for human and animal use. The extracts should be subjected to in vivo studies to evaluate their pharmacokinetics, dosage optimization, and therapeutic efficacy.

3. Comparative studies should be performed to evaluate the effectiveness of *Senna siamea* extracts against standard antibiotics, particularly for treating antibiotic-resistant *Pseudomonas aeruginosa* and *Shigella* spp. infections.

4. The antimicrobial potential of *Senna siamea* suggests its application in the development of herbal-based pharmaceutical products such as ointments, capsules, or antiseptic solutions.

5. Large-scale cultivation and sustainable harvesting of *Senna siamea* should be promoted to ensure a consistent supply of the plant for medicinal use. Policies should be developed to integrate traditional medicinal plants into primary healthcare systems.

6. Public health awareness programs should educate communities on the benefits of medicinal plants as alternative therapies. Clinical trials should be conducted to explore the practical application of *Senna siamea* in modern medicine, particularly for treating gastrointestinal and wound infections caused by antibiotic-resistant bacteria.

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