

Assessment of the Phytochemical Profile and Antibacterial Potential of *Senna siamea* Leaf Extracts against *Staphylococcus aureus*

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Abstract

Staphylococcus aureus, a Gram-positive bacterium, is well known for its resistance to antimicrobial agents and its ability to cause a broad spectrum of infections, ranging from mild skin conditions to severe illnesses such as sepsis. *Senna siamea* (Thai cassia), a plant native to Southeast Asia, has long been used in traditional medicine. This study aimed to evaluate the phytochemical composition and antibacterial potential of aqueous and ethanolic extracts of *Senna siamea* leaves against *S. aureus*. Phytochemical screening revealed the presence of tannins, alkaloids, glycosides, flavonoids, saponins, terpenoids, and phenols. Antibacterial activity was assessed using both disc diffusion and agar well diffusion methods. The ethanolic extract exhibited greater antibacterial activity, with inhibition zones ranging from 6 mm to 17 mm across concentrations of 25, 50, 75, and 100 mg/mL, while the aqueous extract showed limited activity, with no inhibition at 25 and 50 mg/mL. Similar trends were observed with the disc diffusion method. The minimum inhibitory concentration (MIC) was determined to be 6.25 mg/mL for the ethanolic extract and 12.5 mg/mL for the aqueous extract; both had a minimum bactericidal concentration (MBC) of 50 mg/mL. These findings suggest that *Senna siamea* leaf extracts, particularly the ethanolic extract, may serve as promising candidates for developing alternative treatments against staphylococcal infections.

Keywords: Phytochemicals, Antibacterial activity, *Senna siamea*, Plant constituents, *Staphylococcus aureus*, Infection.

Introduction

Senna siamea (synonym: *Cassia siamea*), commonly known as *Siamese cassia* or kassod tree, is a medium-sized evergreen tree in the *Fabaceae* family, native to South and Southeast Asia and widely naturalized in Africa and Latin America (Azani *et al.*, 2017; Morris *et al.*, 2019). It is a well-known medicinal plant in traditional healing systems across Thailand, India, Sri Lanka, and other tropical regions, where its leaves, bark, and roots are used for the management of fever, constipation, hypertension, microbial infections, and diabetes (Ahmed *et al.*, 2016; Ekor, 2014).

Phytochemical investigations have identified a diverse array of bioactive compounds in *S. siamea*, including chromone alkaloids (barakol, cassiarin A–B), anthraquinones (chrysophanol, emodin), flavonoids, phenolic acids, terpenoids, and saponins, which contribute to its pharmacological activities (Chomnawang *et al.*, 2009; Ogunniran *et al.*, 2024). Barakol, in particular, has been studied for its sedative and anxiolytic effects, while compounds such as quercetin and kaempferol are associated with antioxidant and antimicrobial activities (Gebrehiwot *et al.*, 2024; Panthong *et al.*, 2003).

The increasing emergence of antimicrobial resistance, especially from *Staphylococcus aureus* and methicillin-resistant *S. aureus* (MRSA), has created an urgent need for novel antibacterial agents (Nas *et al.*, 2018; Prestinaci *et al.*, 2015). Several studies have reported that extracts of *S. siamea* exhibit broad-spectrum antibacterial activity, inhibiting both Gram-positive and Gram-negative bacteria, including *S. aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* (Ogunniran *et al.*, 2024; Gebrehiwot *et al.*, 2024). Such activities are often attributed to its phytochemical diversity, which allows multiple modes of antibacterial action (Cowan, 1999).

Although traditional knowledge and preliminary scientific evidence point toward the antibacterial potential of *S. siamea*, there is limited focused research on its efficacy against clinically relevant, drug-resistant *S. aureus* strains. Investigating this potential can not only validate ethnopharmacological claims but also lead to the discovery of novel antibacterial leads with unique chemical scaffolds (Newman & Cragg, 2020; Harvey *et al.*, 2015). Therefore, this study evaluates the antibacterial efficacy of *S. siamea* leaf and root extracts against *S. aureus*, determines their minimum inhibitory and bactericidal concentrations, and correlates activity with phytochemical profiles to identify potential bioactive constituents.

MATERIALS AND METHODS

Sample Collection

Senna siamea leaves were obtained from Mashi local government area of Katsina state. Following collection, the leaves were identified at the Department of Biology, Al-alam University, Katsina.

Sample Preparation

The leaves were cleaned and dried, resulting in a moisture-free sample suitable for further grinding after which they were successfully ground into a fine powder, enhancing their suitability for extraction. Sieving of the ground leaf powder yielded a uniform particle size suitable for extraction procedures (Ali *et al.*, 2017).

Test Organism

Four isolates of *S. aureus* were obtained from Microbiology laboratory of Umaru Yar'adua University, Katsina. Biochemical Characterizations involving indole, citrate utilization, catalase, coagulase and triple sugar iron test (TSI) were conducted based on standard protocols suggested by Cheesbrough (2010) and Holt *et al.* (1994).

Extractions

Separate extracts from *Senna siamea* leaves were extracted using water and ethanol. Each solvent (500 mL) was used to soak 50 grams of plant powder. After three days at room temperature with periodic shaking, the mixture was filtered. The ethanol extract was evaporated using a rotary evaporator, while the water extract was evaporated in a water bath until dried samples were obtained. These dried samples were dissolved in 10% DMSO to achieve a final concentration of 200mg/ml as stock solution and stored at 4°C until use (Ali *et al.*, 2017).

Phytochemical Screening

Based on the methods adopted by Sofowora (1993), qualitative detections of the phytochemicals from the extracts were made, including tannins, saponins, flavonoids, terpenoids, glycosides, and alkaloids, each with specific procedures and indicators.

Tannins

To detect tannins, 0.15ml of the original extract solution was mixed with 10ml of distilled water and heated for 10 minutes. After cooling, 3 drops of 1% ferric chloride were added. A change from yellow to green color and the appearance of dark green precipitate indicate a positive result (Sofowora, 1993).

Saponins

For saponins detection, 0.5ml of the original extract solution was dissolved in 0.1ml of Ethyl acetate, heated for 2 minutes, then mixed with distilled water and vigorously shaken. The presence of persistent foam lasting at least 15 minutes indicates a positive result (Sofowora, 1993).

Flavonoids

Shinoda Test: A portion of the extract was treated with concentrated hydrochloric acid, followed by the addition of magnesium ribbon. The appearance of a pink, red, or magenta coloration indicated the presence of flavonoids (Sofowora, 1993).

Terpenoids

Salkowski Test: A portion of the extract was treated with chloroform and concentrated sulfuric acid. The development of a red-brown coloration at the interface indicated the presence of terpenoids (Sofowora, 1993).

Glycosides

Legal Test: A portion of the extract was treated with glacial acetic acid and a few drops of ferric chloride solution. The formation of a blue-green or violet coloration indicated the presence of glycosides (Sofowora, 1993).

Preparation of Inoculum

Three loop full of the test organism was introduced into a test tube containing 5ml of normal saline. It was then adjusted until it matched the 0.5 McFarland standard.

Assessment of Antibacterial Activity of the Extracts:

Agar Well Diffusion Method

Following the modified protocol of Ahmed (2001), a bacterial suspension adjusted to a 0.5 McFarland standard was uniformly inoculated onto sterile Mueller-Hinton agar plates. Using a sterile cork borer (6 mm in diameter), five wells were made in each plate. Each well was filled with 0.1 mL of the extract at concentrations of 25 mg/mL, 50 mg/mL, 75 mg/mL, and 100 mg/mL under aseptic conditions. After allowing the extracts to diffuse at room temperature for 1 hour, the plates were incubated at 37 °C for 24 hours. Zones of inhibition were then measured and recorded (Ahmed, 2001).

Minimum Inhibitory Concentration (MIC) Determination

The MIC was determined using the broth dilution method as described by the Clinical and Laboratory Standards Institute (CLSI, 2021) with slight modifications. Serial two-fold dilutions of the extract were prepared by adding 2 mL of a 100 mg/mL stock solution to test tubes containing 2 mL of Mueller-Hinton Broth (MHB), yielding final concentrations ranging from 50 mg/mL to 3.125 mg/mL. Each tube was inoculated with a bacterial suspension adjusted to a 0.5 McFarland standard. The tubes were then incubated at 37 °C for 24 hours. After incubation, the tubes were observed for turbidity, indicating bacterial growth. The lowest concentration that showed no visible turbidity was recorded as the minimum inhibitory concentration (MIC).

Minimum Bactericidal Concentration (MBC) Determination

The MBC was determined according to the method described by CLSI (2021). From the MIC tubes that showed no visible bacterial growth, 0.1 mL of each suspension was aseptically plated onto sterile Mueller-Hinton agar and evenly spread. The plates were incubated at 37 °C for 24 hours. After incubation, the plates were examined for the presence of bacterial colonies. The lowest concentration of the extract at which no visible growth occurred was recorded as the minimum bactericidal concentration (MBC).

Table 1: Biochemical Characterization of the Test Organism (*Staphylococcus aureus*)

Biochemical Test	Result	Interpretation
Indole	–	Indole not produced
Citrate Utilization	+	Citrate utilized
Catalase	+	Catalase enzyme present
Coagulase	+	Coagulase enzyme produced
Confirmed Identity	–	<i>Staphylococcus aureus</i>

Key: + = Positive result; – = Negative result

Phytochemical Constituents of the *Senna siamea*

Table 2 shows the phytochemical status of the *Senna siamea* obtained from aqueous and ethanolic extraction techniques. Both the aqueous and ethanolic extracts show positive result in phenolic compounds, alkaloids and terpenoids. They also show negative in glycoside. However, they differ in terms of tannins, saponin and flavonoids. Aqueous extract shows negative result in tannin and saponin, but positive in flavonoids, opposite is the case with regard to ethanolic extract.

Table 2: Phytochemical Constituents of the *Senna siamea* Obtained from Aqueous and Ethanolic Extractions.

S/N	Compound	Aqueous extract	Ethanolic extract
1.	Tannin	-	+
2.	Saponin	-	+
3.	Flavonoids	+	-
4.	Terpenoids	+	+
5.	Glycosides	-	-
6.	Alkaloids	+	+
7.	Phenolic compound	+	+

Key: + indicates positive result/presence while - indicates negative result/absence..



Figure 1: Tannin obtained from the *S. siamea* plant



Figure 2: Flavonoid obtained from the *S. siamea*

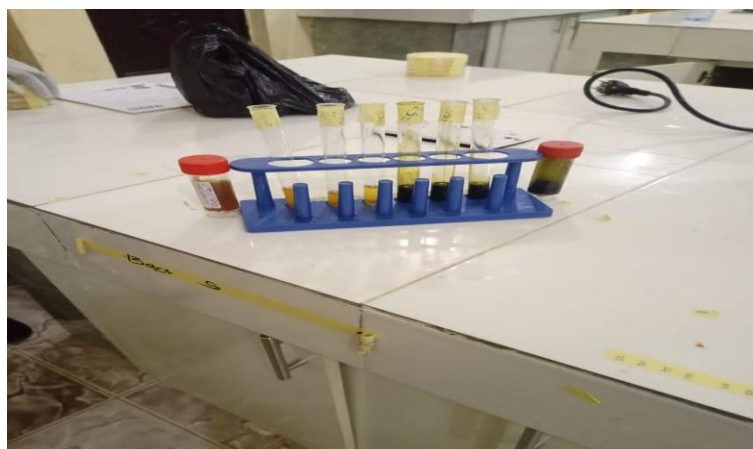


Figure 3: *Senna siamea* extracts

Antibacterial Activity of *Senna siamea* Against *S. aureus* via Disc Diffusion Method

The outcomes of the antibacterial activity assessment using the disc diffusion method with aqueous and ethanolic extracts are detailed in Table 3. The findings indicate that the highest inhibition zone was 11mm at a concentration of 100mg/mL for the ethanolic extract, while the lowest inhibition was observed with the aqueous extract, registering 0mm at concentrations of 25mg/mL and 50mg/ml. Control antibiotics (Amoxicillin 50mg/mL) displayed inhibition zones of 21mm and 20mm for the aqueous and ethanolic extracts, respectively

Table 3: Antistaphylococcal Activity of Aqueous and Ethanolic Extracts of *Senna siamea* against *S. aureus* via Disc Diffusion Method.

Extract	25(mg/mL)	50(mg/mL)	75(mg/mL)	100(mg/m)	Control
Aqueous	0	0	4	7	21
Ethanolic	6	8	9	11	20

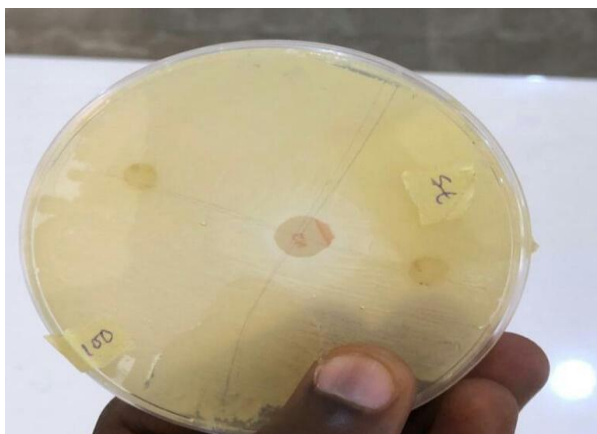


Figure 4: Zone of Inhibition Observed from the Ethanolic Extract at 75mg/mL and 100mg/mL concentrations.



Figure 5: Zone of Inhibition Observed from the aqueous extract at 25mg/mL concentration



Figure 6: Zone of Inhibition observed from the aqueous extract at 50mg/mL concentration

Antibacterial Activity of *Senna seamea* Against *S. aureus* via Agar Well Diffusion Method

The results of the antibacterial activity assessment using the agar well diffusion method with aqueous and ethanolic extracts of *Senna siamea* are outlined in Table 2. It was observed that the highest inhibition zone was 17mm at a concentration of 100mg/ml for the ethanolic extract, whereas the lowest inhibition zone was 6mm at a concentration of 25mg/ml for the aqueous extract. Control antibiotics (erythromycin 50mg/ml) exhibited inhibition zones of 22mm and 21mm for the aqueous and ethanolic extracts, respectively.

Table 4: Antibacterial Activity of the Extracts of *Senna siamea* against *S. Aureus* via Agar Well Diffusion Method.

Extracts	25mg/mL	50mg/mL	75mg/mL	100mg/mL	Control
Aqueous	6	8	11	12	22
Ethanolic	12	13	15	17	21

MIC and MBC of the Extracts

The MIC & MBC of the extracts of *Senna siamea* is depicted in Table 4. The results indicate a lower MIC (6.25 mg/ml) for the ethanolic extract, while the MBC results for both extracts are identical (50mg/ml).

Table 4: Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentration (MBC) of the *Senna siamea* Extracts against *S. aureus*.

Aqueous extract		Ethanolic extract	
MIC(mg/mL)	MBC(mg/mL).	MIC(mg/mL)	MBC(mg/mL).
12.5	50	6.25	50

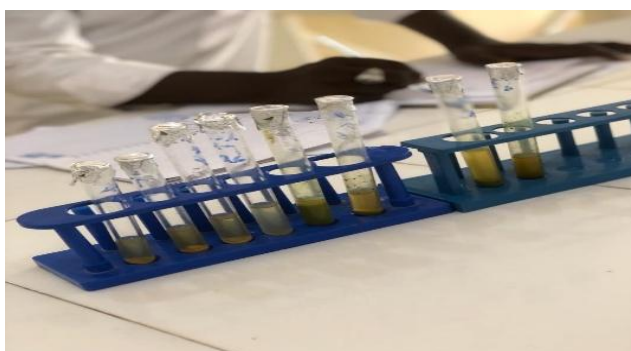


Figure 8: MIC and MBC of the extracts against the *S. aureus*

DISCUSSION

The phytochemical screening of *Senna siamea* leaves confirmed the presence of several bioactive compounds, including tannins, alkaloids, saponins, glycosides, flavonoids, phenols, and terpenoids. These secondary metabolites are known to contribute significantly to the antimicrobial properties of medicinal plants. Their presence in the extracts suggests a strong potential for therapeutic applications, particularly in bacterial inhibition.

The ethanolic extract demonstrated greater antibacterial activity than the aqueous extract against *Staphylococcus aureus*. The inhibition zones for the ethanolic extract ranged from 6 mm at 25 mg/mL to 11 mm at 100 mg/mL, while the aqueous extract showed little to no activity at lower concentrations and only 7 mm at the highest concentration tested. This difference in activity may be attributed to the higher solubility of active phytochemicals in ethanol, which facilitates more effective extraction of antimicrobial constituents.

The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values further support the efficacy of the ethanolic extract, with MIC recorded at 6.25 mg/mL and MBC at 12.5 mg/mL. These values indicate that the extract is not only bacteriostatic but also bactericidal at higher concentrations.

Comparing these findings with previous studies, our phytochemical results are consistent with Nas et al. (2018), who also reported the presence of flavonoids, tannins, polyphenols, anthraquinones, saponins, and glycosides in *S. siamea* leaves. However, they contrast with Bukar et al. (2009), who did not detect saponins, alkaloids, and flavonoids in the ethanolic extract. The stronger antibacterial effect of the ethanolic extract observed in our study aligns with earlier work by Nas et al. (2018), who similarly reported superior efficacy of ethanol-based extractions against *S. aureus*. The MIC and MBC values reported here also corroborate the findings of Bukar et al. (2009), Ahmad et al. (2007), and Nas et al. (2018), who recorded similar inhibitory and bactericidal concentrations against *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

These findings support the potential of *S. siamea*, particularly its ethanolic extract, as a source of natural antimicrobial agents and underscore the relevance of solvent selection in phytochemical extraction and antibacterial testing.

CONCLUSION

This study demonstrates the antibacterial potential of *Senna siamea* leaf extracts, particularly against *Staphylococcus aureus*. The observed inhibitory effects are likely due to the presence of bioactive phytochemicals such as alkaloids, saponins, tannins, and flavonoids. Notably, the ethanolic extract exhibited stronger antibacterial activity than the aqueous extract, emphasizing the importance of solvent choice in phytochemical extraction. These findings support the traditional use of *S. siamea* in herbal medicine and suggest its potential as a natural source of antimicrobial agents for combating bacterial infections.

RECOMMENDATIONS

It is recommended that:

- Future studies focus on isolating and characterizing the specific phytochemicals responsible for the antibacterial activity observed.
- Toxicological assessments should be carried out to determine the safety profile of *Senna siamea* extracts.
- Further investigations involving a wider range of pathogenic organisms are encouraged to assess the broad-spectrum efficacy of the extracts.
- Lastly, in vivo studies should be conducted to validate the antibacterial potential under physiological conditions and support possible therapeutic applications.

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