



Antibacterial Activity and Qualitative Phytochemical Constituents of the Ethyl acetate Extract of *Cleome viscosa* against selected Skin Disease-Causing Bacteria

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ABSTRACT

Skin infections caused by bacteria and other pathogens remain a major global health concern, worsened by the rising resistance of microorganisms to conventional antibiotics. This study evaluated the antibacterial activity and qualitative phytochemical constituents of the ethyl acetate whole plants extract of *Cleome viscosa* against selected skin disease-causing bacteria. Antibacterial activity was assessed at extract concentrations of 200, 300, and 400 mg/mL using the disk diffusion method. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were also determined, while qualitative phytochemical screening was performed to identify major classes of secondary metabolites. The extract exhibited concentration-dependent antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. At 400 mg/mL, inhibition zones of 11.00 ± 1.00 mm and 9.50 ± 0.50 mm were recorded against *S. aureus* and *P. aeruginosa*, respectively. At 200 mg/mL, the corresponding inhibition zones decreased to 7.50 ± 0.50 mm and 6.50 ± 0.50 mm. The MIC of the extract against *S. aureus* was reported as 200 mg/mL, whereas the MIC and MBC endpoints for *P. aeruginosa* were not determined under the conditions tested. Phytochemical screening revealed the presence of phenols, flavonoids, tannins, saponins, alkaloids, and steroids. These findings demonstrate preliminary *in vitro* antibacterial activity of the ethyl acetate extract of *C. viscosa*. Further studies are required to characterize the active constituents and evaluate safety before the extract can be considered for topical antimicrobial development.

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INTRODUCTION

Skin diseases constitute a major global public health concern, contributing substantially to morbidity, disability, and reduced quality of life. The Global Burden of Disease Study 2021 estimated approximately 4.69 billion incident cases of skin and subcutaneous diseases, accounting for 41.9 million disability-adjusted life years (Oseni *et al.*, 2024; Rashki *et al.*, 2025; Li *et al.*, 2026). Bacterial pathogens, including

Staphylococcus aureus, *Pseudomonas aeruginosa*, and *Escherichia coli*, are important causes of skin and soft-tissue infections, which may result in delayed wound healing and other complications (Aslam *et al.*, 2025).

Various plant species have been used in treating several human diseases (Hassan *et al.*, 2021; Aina *et al.*, 2024; Ekeyi *et al.*, 2025; Upahi *et al.*, 2026; Muhammad *et al.*, 2026). However, *Cleome viscosa* L., a member of the family

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Cleomaceae, is a medicinal herb commonly known as wild mustard or sticky cleome. It is widely distributed across tropical and subtropical regions, including parts of Africa, India, and Southeast Asia, where it has been traditionally used for medicinal and nutritional purposes. In traditional medicine, the plant is employed in the management of wounds, ulcers, and various skin disorders, suggesting its potential relevance to the treatment of cutaneous infections (Sathiyavani *et al.*, 2026).

Phytochemical investigations have identified several classes of secondary metabolites in *C. viscosa*, including alkaloids, flavonoids, phenolic compounds, tannins, saponins, and terpenoids, many of which have been associated with antimicrobial and other biological activities (Shalini, 2026). Emerging evidence also supports the antibacterial potential of specific constituents of the plant. Lakshmanan *et al.* (2024) reported the isolation of imperatorin, a coumarin derivative, from the aerial parts of *C. viscosa* and demonstrated its antibacterial activity against *S. aureus*, *Bacillus subtilis*, *Klebsiella pneumoniae*, and *E. coli*. Similarly, Jana *et al.* (2011) identified lactam nonanoic acid in root exudates of the plant, reporting complete inhibition of *P. aeruginosa* and *S. aureus* at concentrations of 500 ppm and above.

Despite these promising findings, additional investigations are necessary to validate the antibacterial efficacy of *Cleome viscosa* against clinically relevant bacterial pathogens associated with skin infections. Therefore, this study aimed to evaluate the antibacterial activity of *C. viscosa* extracts against selected skin infection-associated bacteria. The findings are expected to provide scientific evidence supporting the plant's traditional medicinal applications and establish a basis for further research into its bioactive constituents as potential sources of alternative therapeutic agents for managing bacterial skin infections.

MATERIALS AND METHODS.

Study Area

This study was conducted in microbiology laboratory of Confluence University of Science and Technology, Osara, Kogi State.

Identification of Plant

Whole *Cleome viscosa* whole plant was collected from Osara Campus, Confluence University of Science and Technology (CUSTECH), Kogi State, Nigeria. The plant was identified and authenticated by a botanist, Prof. Akinnibosun Henry Adewale in the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Edo State. The plant was identified as *Cleome viscosa* (Plate I) from the family Cleomaceae with voucher number UBH-C146 for reference purposes.



Plate I: Photograph of *C. viscosa* in the field

Processing of plant Materials

The *C. viscosa* whole plants were collected, washed with clean water and dried at room temperature in the Microbiology Laboratory at CUSTECH. The mortar and pestle were first used to reduce the sample size, and the blender was used to pulverise it into a powdery form and was stored in polythene bags until ready for extraction.

Extraction of powdered Plant Material

A 50 g quantity of the powdered plant was soaked in 100 mL of ethyl acetate. The mixture was allowed to stand for 7 days at room temperature ($28 \pm 2^\circ\text{C}$) with occasional agitation. The extract was sieved through a muslin cloth and filtered through a Whatman (No. 1) filter paper

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(Zode and Chakole, 2020). The extract was concentrated by placing it on a water bath at 50°C to obtain a greasy mass. The greasy mass obtained as the final material from ethyl acetate extraction was transferred into a cleaned sample bottle, labelled and stored in a refrigerator (2 - 5°C) until used.

Reconstitution of the extract

The extract was prepared at three different concentrations (400 mg/mL, 300 mg/mL, and 200 mg/mL) using dimethyl sulfoxide (DMSO) as the solvent. Briefly, 0.4 g, 0.3 g, and 0.2 g of the extract were weighed separately and each dissolved in 1 mL of DMSO to obtain concentrations of 400 mg/mL, 300 mg/mL, and 200 mg/mL, respectively. Each preparation was mixed thoroughly to ensure adequate dissolution and uniform distribution of the extract in the solvent

Test Organisms

The test organisms used in this study included *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and which are commonly associated with skin infections. These bacteria were obtained from the Department of Microbiology culture collection unit at Confluence University of Science and Technology, Kogi State, Nigeria. The organisms were maintained on nutrient agar slants at 4°C until further use for antimicrobial screening.

Standardization of Test Organisms

Pure culture of the test organisms was transferred into 5ml of nutrient broth and incubated for 24 hours. 0.1ml of the overnight culture was transferred into 9.9ml of sterile distilled water in a test tube using a sterile needle and syringe and then mixed by shaking which was later compared with Mcfarland standard.

Antibacterial Susceptibility Testing

Twenty milliliters of Mueller Hinton Agar (MHA) were aseptically poured into a sterile Petri dish and allowed to solidify. Subsequently, sterile swab sticks were immersed in a liquid suspension containing the standardised test organisms. Each

swab was then gently and uniformly used to inoculate the surface of the solidified MHA medium. Prepared antimicrobial discs, impregnated with various concentrations of the plant extracts, were carefully positioned onto the inoculated agar surface. Similarly, a positive control disc (Chloramphenicol) and a negative control disc (Dimethyl Sulfoxide - DMSO) were also placed on the dish. The Petri dishes were then incubated at 37°C for 24 hours, after which the diameters of the zones of inhibition were measured and recorded according to Attah *et al.* (2021).

Minimum Inhibitory Concentration (MIC) Determination

The Minimum Inhibitory Concentration (MIC) of the extracts against the test organisms was determined using the broth dilution method. Aliquots of 1ml of stock extract at the concentration of 200mg/ml was added to 1ml of fresh nutrient broth and serially diluted to obtained extract concentrations of 100mg/ml, 50mg/ml, 25mg/ml, and 12.5mg/ml in four different test tubes. Each dilution is then inoculated with the test organisms and then incubated at 37°C for 24 hours. The MIC is the lowest concentration of the extract that prevent visible growth of the microorganism after incubation

Minimum Bactericidal Concentration (MBC) Determination

To determine the Minimum Bactericidal Concentration (MBC), the solutions from the MIC test tubes that exhibited no visible microbial growth after incubation were utilized. From each of these clear test tubes, 1 ml of the solution was transferred into a fresh nutrient broth and incubated at 37°C for 24 hours. Following incubation, the MBC value was identified as the lowest concentration of the extract that resulted in no bacterial growth, indicating a bactericidal effect.

Qualitative Phytochemical Screening of *C. viscosa*'s Extract

Phytochemical screening of the different extracts was carried out to determine the

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presence of the phytoconstituents such as saponins, phenols, flavonoids, tannins, alkaloids, cardiac glycoside, reducing sugars, steroids according to the standard method described by Singleton *et al.* (1999) Oloyed (2005) and Jato *et al.* (2018).

Data Analysis

The statistical evaluation of antibacterial activity results was performed using IBM SPSS statistics software, version 23. Data obtained were presented as mean $\pm$ standard Error of Mean (SEM) from two independent duplicates. Within the same column, values carrying identical superscripts showed no significant difference at a probability level greater than 0.05 ($p > 0.05$).

RESULTS

Antibacterial susceptibility testing

The ethyl acetate extract of *Cleome viscosa* demonstrated concentration-dependent antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The highest inhibition zones were recorded at 400 mg/mL, measuring 11.00 ± 1.00 mm and 9.50 ± 0.50 mm, respectively. These decreased to 9.00 ± 1.00 mm and 7.50 ± 0.50 mm at 300 mg/mL, and 7.50 ± 0.50 mm and 6.50 ± 0.50 mm at 200 mg/mL while the control produced inhibition zones of (15.00 ± 0.00) mm against *S. aureus* and (10.00 ± 0.00) mm against *P. aeruginosa* as shown in Table 3.1

Table 3.1: Zones of inhibition of ethyl-acetate extract of *Cleome viscosa*

Concentration/Organisms	<i>Staphylococcus aureus</i> (mm)	<i>Pseudomonas aeruginosa</i> (mm)
400 mg/mL	11.00 ± 1.00^b	9.50 ± 0.50^b
300 mg/mL	9.00 ± 1.00^{ab}	7.50 ± 0.50^a
200 mg/mL	7.50 ± 0.50^a	6.50 ± 0.50^a
Control	15.00 ± 0.00^c	10.00 ± 0.00^b

Key: Control (20 mg/mL of Chloramphenicol), Results Represent Mean $\pm$ Standard Error Mean of duplicate determinations. Values with the same subscript on the same column and not significantly different at ($P < 0.05$).

Minimum Inhibitory Concentration (MIC) and Minimum Inhibitory Concentration

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the ethyl acetate extract of *Cleome*

viscosa against the selected bacterial isolates, *Staphylococcus aureus* and *Pseudomonas aeruginosa*, are presented in Table 3.2. The extract exhibited an MIC of 200 mg/mL against *S. aureus*, while no MIC or MBC was recorded for *P. aeruginosa* under the conditions tested.

Table 3.2: Minimum Inhibitory Concentration (MIC) and Minimum Inhibitory Concentration Determination

Bacteria	MIC	MBC
<i>Staphylococcus aureus</i>	200mg/ml	Nil
<i>Pseudomonas aeruginosa</i> .	Nil	Nil

Keys: Nil – No MIC and MBC

Qualitative Phytochemical Screening of *Cleome viscosa*

The qualitative phytochemical screening of *Cleome viscosa* revealed the

presence of phenols, flavonoids, tannins, saponins, alkaloids, and steroids, while reducing sugars and cardiac glycosides were absent as shown in Table 3.4 .

Table 3.4: Qualitative Phytochemical Screening of *Cleome Viscosa*

S/N	Phytochemicals	Result
1	Phenol	+ve
2	Flavonoid	+ve

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S/N	Phytochemicals	Result
3	Tannins	+ve
4	Saponin	+ve
5	Alkaloids	+ve
6	Reducing sugar	-ve
7	Steroids	+ve
8	Cardiac glycoside	-ve

Key: Present (+), Negative (-)

DISCUSSION OF FINDINGS

Bacterial skin and soft tissue infections constitute a major global public health burden, and the continued rise of antimicrobial resistance (AMR) further complicates clinical management (Monk *et al.*, 2024). This present study demonstrated preliminary in vitro antibacterial activity of the ethyl acetate whole plant extract of *Cleome viscosa* against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The activity increased with extract concentration, with maximum inhibition zones of 11.00 ± 1.00 mm and 9.50 ± 0.50 mm, respectively, at 400 mg/mL. The results align with previous reports of antibacterial constituents in *C. viscosa* by Jana and Biswas (2011) reported antibacterial activity of lactam nonanoic acid isolated from the plant, while Lakshmanan *et al.* (2024) identified imperatorin and investigated its antibacterial activity against selected Gram-positive and Gram-negative bacteria. However, the activity observed in the present study cannot be attributed to these specific compounds without chemical characterization of the extract.

The extract demonstrated greater inhibition against *S. aureus* than *P. aeruginosa* at all tested concentrations. This difference may reflect variations in bacterial envelope permeability, efflux activity, and other defence mechanisms, particularly the protective outer membrane of Gram-negative bacteria (Maher and Hassan, 2023). Nevertheless, these mechanisms were not investigated directly, and differences in the solubility and agar diffusion of extract constituents may also have influenced the results (Gonzalez-Pastor *et al.*, 2023). Although chloramphenicol produced larger inhibition zones against both organisms, the findings indicate that the extract possesses preliminary antibacterial

activity. The inhibition-zone diameters may not be directly compared because crude extracts and conventional antibiotics differ in composition, concentration, and diffusion properties.

The MIC value of 200 mg/mL recorded for *S. aureus* indicates that a relatively high concentration of the *Cleome viscosa* extract was necessary to inhibit bacterial growth. However, neither an MIC nor an MBC endpoint was established for *P. aeruginosa*, while the MBC for *S. aureus* remained undetermined. The relatively small inhibition zones observed at the tested concentrations (6.50 ± 0.50 to 7.50 ± 0.50 mm) suggest limited antibacterial activity against the tested organisms. According to the inhibition-zone classification described by Muharni *et al.* (2017), zones measuring 5–10 mm indicate moderate activity, whereas those below 5 mm, between 10 and 20 mm, and above 20 mm are interpreted as weak, strong, and very strong activity, respectively. Thus, the observed results fall within the moderate activity range under this classification.

Qualitative phytochemical screening revealed phenols, flavonoids, phenols tannins, saponins, alkaloids, and steroids, while reducing sugars and cardiac glycosides were not detected. These metabolite classes may contain compounds with potential antibacterial properties (Attah *et al.*, 2020). These phytochemicals may affect bacterial membranes, proteins, enzymes, or other cellular processes (Attah *et al.*, 2020). However, qualitative screening establishes neither the identities nor the concentrations of individual compounds and cannot demonstrate which constituents contributed to the observed activity.

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CONCLUSION

In conclusion, the ethyl acetate extract of the whole plant of *Cleome viscosa* demonstrated concentration-dependent antibacterial activity against *S. aureus* and *P. aeruginosa*, with greater inhibitory effects against *S. aureus*. Phytochemical screening identified phenols, flavonoids, tannins, saponins, alkaloids, and steroids, which may contribute to its observed antibacterial properties. These findings provide preliminary scientific evidence supporting the traditional use of *C. viscosa* in managing skin conditions. However, further studies involving chemical characterization, toxicity evaluation, and in vivo investigations are necessary to establish its safety and efficacy and determine its suitability for developing standardized topical formulations.

Conflict of Interest

The authors declared absence of conflict of interest

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