

Antibacterial Activity of *Albizia coriaria* Ethanolic Extract Against Archived Methicillin-Resistant *Staphylococcus aureus* and Carbapenem-Resistant *Pseudomonas aeruginosa* Isolates from Pakwach District, Uganda

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ABSTRACT

Background:

Antimicrobial resistance is a major public health concern, particularly with methicillin-resistant *Staphylococcus aureus* (MRSA) and carbapenem-resistant *Pseudomonas aeruginosa* (CRPA), which are associated with limited treatment options and poor clinical outcomes. This study assessed the antibacterial activity of its ethanolic extract against archived MRSA and CRPA isolates.

Methods:

This was a laboratory-based experimental study. Stem bark of *A. coriaria* was collected from two sites in Pakwach District, Uganda, air-dried, powdered, and extracted using ethanol via cold maceration. Qualitative phytochemical screening was performed following standard protocols. Antibacterial activity against archived MRSA and CRPA isolates was determined using the agar well diffusion method, and the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined using broth microdilution with resazurin dye reduction.

Results:

The extract screened positive for alkaloids, flavonoids, tannins, saponins, and phenols. It produced a significantly larger mean zone of inhibition against MRSA (23.25 ± 0.48 mm) than against CRPA (11.50 ± 0.50 mm). Conversely, the extract required a lower concentration to inhibit and kill CRPA (MIC = 62.5 mg/mL; MBC = 125 mg/mL) than to inhibit and kill MRSA (MIC = 125 mg/mL; MBC = 250 mg/mL). Both organisms produced an MBC/MIC ratio of 2, confirming bactericidal rather than bacteriostatic activity.

Conclusion:

Albizia coriaria ethanolic extract possesses a rich phytochemical profile that confers bactericidal activity against both MRSA and CRPA through what appears to be a multi-target mechanism largely independent of conventional beta-lactam and carbapenem resistance pathways.

Recommendation:

Further studies are recommended to isolate and characterize the specific bioactive compounds responsible for the observed antibacterial activity, and to evaluate the extract's safety, toxicity, and efficacy through in vivo and clinical studies before it can be considered for pharmaceutical development or enrollment in the market.

Keywords: *Albizia coriaria*, methicillin-resistant *Staphylococcus aureus*, carbapenem-resistant *Pseudomonas aeruginosa*, antibacterial activity, phytochemicals, minimum inhibitory concentration.

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INTRODUCTION

Antimicrobial resistance (AMR) is one of the most pressing global public health challenges of the twenty-first century.

Methicillin-resistant *Staphylococcus aureus* (MRSA) and carbapenem-resistant *Pseudomonas aeruginosa* (CRPA) are among the World Health Organization's high-priority pathogens for the development of novel antibiotics, owing to their limited treatment options and association with poor clinical outcomes (Alibi et al., 2021). Globally, MRSA-attributable deaths rose from 57,200 in 1990 to approximately 130,000 in 2021 (GBD 2021 Antimicrobial Resistance Collaborators, 2024), while a 2025 meta-analysis of 163 studies comprising 58,344 isolates from 39 countries reported an overall pooled CRPA prevalence of 34.7%, rising to 38.5% in Africa during 2022–2024 (Ramatla et al., 2025). Within East Africa, MRSA prevalence among patients and healthcare workers has been reported at 31.5–42% in Uganda (Wangai et al., 2019).

Albizia coriaria Welw. ex Oliv. (Fabaceae) is a medicinal plant widely distributed across the savannah regions of Uganda, where decoctions of its stem bark are traditionally used to manage cough, fever, headache, inflammation, diarrhoea, and other ailments (Omara et al., 2023; Palanisamy et al., 2025). Prior phytochemical studies have identified terpenoids, alkaloids, flavonoids, tannins, and saponins in the plant, alongside isolated compounds such as coriariosides A and B, betulinic acid, lupeol, and catechin, which have demonstrated analgesic, anti-inflammatory, anti-tumour, anti-diabetic, and antimicrobial activity (Obakiro et al., 2022). Ethyl acetate and ethanol flower extracts of *A. coriaria* have previously shown moderate antibacterial activity against *Pseudomonas aeruginosa*, *Salmonella typhi*, *Escherichia coli*, and *Staphylococcus aureus*, with inhibition zones ranging from 7.00 to 22.00 mm (Obakiro et al., 2022).

Despite these promising traditional and preliminary scientific reports, standardized quantitative data on the minimum inhibitory and bactericidal concentrations of *A. coriaria* against archived, clinically defined MRSA and CRPA isolates remain limited. Plant-derived antimicrobials are attractive alternatives to synthetic antibiotics owing to their comparatively low cost, reduced toxicity, and lower propensity for resistance development (Guedes et al., 2024). This study therefore evaluated the phytochemical composition and in vitro antibacterial activity of *A. coriaria* ethanolic stem bark extract against archived MRSA and CRPA isolates, with the specific objectives of (i) screening for the presence of major phytochemical classes, (ii) determining zones of inhibition produced by the extract using the agar well diffusion assay, and (iii) determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extract using broth microdilution.

MATERIALS AND METHODS

Study Design and Area

A laboratory-based experimental design was used, involving deliberate manipulation of extract concentration (independent variable) to determine effects on MRSA and CRPA isolates (dependent variable). The study was conducted over two months (2 June–28 July 2026). Stem bark of *Albizia coriaria* was collected from Pakwach Town Council, Pakwach District, in the West Nile sub-region of Northern Uganda (2.46194°N, 31.49833°E; elevation ≈623 m). Archived MRSA and CRPA isolates were obtained from the Microbiology Laboratory, College of Veterinary Medicine, Animal Resources and Biosecurity (CoVAB), Makerere University; extract preparation and antibacterial testing were carried out at the Veterinary Microbiology Research Laboratory, CoVAB.

Study duration

The study was conducted between 02/June/2026 and 28/July/2026.

Plant Collection, Identification, and Preparation

Stem bark samples were purposively collected from two sites within Pakwach Town Council (a roadside open-woodland site and a riverbank site along the Lake Albert shore) on 12 and 14 April 2026 and identified by a qualified botanist. Samples were chopped into small pieces and air-dried under shade at room temperature (25.0 ± 2.0 °C) for one month until a constant mass was obtained, then ground into powder using an electric grinder and stored in labelled paper envelopes at room temperature until extraction.

Extraction

The dried powdered stem bark was extracted using ethanol via cold maceration for 72 hours at room temperature with intermittent shaking. The mixture was filtered using filter paper and muslin cloth, and the filtrate was concentrated using a water bath to obtain the dry crude extract, which was stored at 4 °C until use. Percentage yield was calculated as (weight of dry crude extract/weight of powder macerated) × 100.

Qualitative Phytochemical Screening

The extract was screened for alkaloids (Wagner's reagent), flavonoids (sodium hydroxide/dilute hydrochloric acid), tannins (ferric chloride), phenols (lead acetate), and saponins (froth test), following standard qualitative procedures.

Antibacterial Susceptibility Testing

Antibacterial activity was determined using the agar well diffusion method. Mueller-Hinton agar plates were seeded with bacterial inoculum standardized to 0.5 McFarland. Wells (6 mm diameter) were punched using a cork borer, and 100 μ L of extract (0.1 g/mL) was added per well. Ciprofloxacin served as the positive control and DMSO as the negative control. Plates were incubated at 37 $^{\circ}$ C for 24 hours, and inhibition zone diameters were measured in millimetres. Zones >20 mm were classified as strong activity, 10–20 mm as moderate, and <10 mm as weak (CLSI, 2021).

Determination of MIC and MBC

MIC was determined by broth microdilution in 96-well plates using two-fold serial dilutions of the extract, with resazurin dye added to detect bacterial growth (colour change from blue/purple to pink). The lowest concentration preventing a colour change was recorded as the MIC. MBC was determined by subculturing aliquots from wells showing no visible growth onto Mueller-Hinton agar; the lowest concentration yielding no bacterial colonies after 24 hours at 37 $^{\circ}$ C was recorded as the MBC. The MBC/MIC ratio was calculated for each organism to classify the extract as bactericidal (ratio $\leq$ 4) or bacteriostatic (ratio > 4).

Data Analysis and Ethical Considerations

Descriptive statistics (mean, standard deviation, standard error) were calculated in Microsoft Excel and presented in tables. The study used archived bacterial isolates and botanist-identified plant material; standard biosafety procedures were followed throughout. Ethical approval was granted under UVRI REC approval number GC/127/004.

RESULTS

Phytochemical Composition

The phytochemical screening of the *Albizia coriaria* ethanolic extract revealed the presence of five major classes of bioactive compounds: alkaloids, flavonoids, tannins, saponins, and phenols, each varying in relative abundance. Alkaloids and phenols were the most abundant compounds detected, both recording a high relative abundance (+++), suggesting that these compound classes are particularly concentrated in the extract. Flavonoids and tannins were present in moderate abundance (++), indicating a substantial but comparatively lower concentration relative to alkaloids and phenols. Saponins showed the lowest relative abundance (+) among all the phytochemicals screened, suggesting they are present only in trace or mild amounts, as summarized in Table 1.

Table 1: Phytochemicals Present in the *Albizia coriaria* Plant Extract.

Phytochemical	Relative Abundance
Alkaloids	+++
Flavonoids	++
Tannins	++
Saponins	+
Phenols	+++

Note. +++ = *highly abundant*; ++ = *moderately abundant*; + = *mildly abundant*.

Zone of Inhibition

The antibacterial activity of the ethanolic extract of *Albizia coriaria* was evaluated against archived clinical isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) and

carbapenem-resistant *Pseudomonas aeruginosa* (CRPA) using the agar well diffusion method. Zones of inhibition were measured in millimeters and compared against a positive antibiotic control and a DMSO negative control, as summarized in Table 2.

Table 2: Zone of Inhibition and Biological Interpretation of Albizia coriaria Extract Against MRSA and CRPA Isolates.

Organism	Isolate/Code	Treatment (0.1 g/mL)	Zone (mm)	Biological Interpretation
<i>P. aeruginosa</i> (CRPA)	491/25	Antibiotic (Positive Control)	13	Active
<i>P. aeruginosa</i> (CRPA)	491/25	Extract	27	Active
<i>P. aeruginosa</i> (CRPA)	491/25	DMSO (Negative Control)	0	Not active
<i>P. aeruginosa</i> (CRPA)	544/25	Antibiotic (Positive Control)	11	Active
<i>P. aeruginosa</i> (CRPA)	544/25	Extract	16	Active
<i>P. aeruginosa</i> (CRPA)	544/25	DMSO (Negative Control)	0	Not active
<i>P. aeruginosa</i> (CRPA)	544/25	Antibiotic (Positive Control)	11	Active
<i>P. aeruginosa</i> (CRPA)	544/25	Extract	14	Active
<i>P. aeruginosa</i> (CRPA)	544/25	DMSO (Negative Control)	0	Not active
<i>P. aeruginosa</i> (CRPA)	491/25	Antibiotic (Positive Control)	11	Active
<i>P. aeruginosa</i> (CRPA)	491/25	Extract	26	Active
<i>P. aeruginosa</i> (CRPA)	491/25	DMSO (Negative Control)	0	Not active
<i>Staphylococcus aureus</i> (MRSA)	2224	Antibiotic (Positive Control)	23	Active
<i>Staphylococcus aureus</i> (MRSA)	2224	Extract	18	Active
<i>Staphylococcus aureus</i> (MRSA)	2224	DMSO (Negative Control)	0	Not active
<i>Staphylococcus aureus</i> (MRSA)	2224	Antibiotic (Positive Control)	22	Active
<i>Staphylococcus aureus</i> (MRSA)	2224	Extract	19	Active
<i>Staphylococcus aureus</i> (MRSA)	2224	DMSO (Negative Control)	0	Not active
<i>Staphylococcus aureus</i> (MRSA)	400/25	Antibiotic (Positive Control)	24	Active
<i>Staphylococcus aureus</i> (MRSA)	400/25	Extract	20	Active
<i>Staphylococcus aureus</i> (MRSA)	400/25	DMSO (Negative Control)	0	Not active
<i>Staphylococcus aureus</i> (MRSA)	400/25	Antibiotic (Positive Control)	24	Active
<i>Staphylococcus aureus</i> (MRSA)	400/25	Extract	20	Active
<i>Staphylococcus aureus</i> (MRSA)	400/25	DMSO (Negative Control)	0	Not active

Note. CRPA = carbapenem-resistant *Pseudomonas aeruginosa*; MRSA = methicillin-resistant *Staphylococcus aureus*; DMSO = dimethyl sulfoxide.

Mean Zone of Inhibition

The extract produced a markedly larger mean zone of inhibition against MRSA (23.25 ± 0.48 mm) than against CRPA (11.50 ± 0.50 mm), indicating substantially greater sensitivity in the Gram-positive MRSA isolates compared with the Gram-negative CRPA isolates, as summarized in Table 3.

Table 3: Mean Zone of Inhibition (mm) of the Albizia coriaria Extract Against CRPA and MRSA Isolates.

Organism	Mean (mm)	Standard (SD)	Deviation	Standard Error (SE)	Mean $\pm$ SEM (mm)
CRPA	11.50	1.000		0.500	11.50 $\pm$ 0.50
MRSA	23.25	0.957		0.479	23.25 $\pm$ 0.48

Note. CRPA = carbapenem-resistant Pseudomonas aeruginosa; MRSA = methicillin-resistant Staphylococcus aureus; SEM = standard error of the mean.

Quality Control: Comparison with CLSI Breakpoints

The ciprofloxacin positive control zones for both MRSA (19.25 $\pm$ 0.48 mm) and CRPA (20.75 $\pm$ 0.50 mm) fell within

the CLSI Intermediate breakpoint range for their respective organisms, confirming both that the assay was functioning correctly and that the archived isolates behaved as clinically resistant, non-fully susceptible strains, as summarized in Table 4

Table 4: Comparison of Ciprofloxacin Positive Control Zones of Inhibition Against CLSI (2025/2026) Disk Diffusion Breakpoints.

Organism	Positive Control (mm)	CLSI Susceptible	CLSI Intermediate	CLSI Resistant	Interpretation
MRSA (<i>S. aureus</i>)	19.25 $\pm$ 0.48	≥ 21	16–22	≤ 15	Intermediate
CRPA (<i>P. aeruginosa</i>)	20.75 $\pm$ 0.50	≥ 25	19–24	≤ 18	Intermediate

Note. CLSI = Clinical and Laboratory Standards Institute.

Minimum Inhibitory and Minimum Bactericidal Concentrations

The extract required a lower concentration to inhibit and kill CRPA (MIC = 62.5 mg/mL; MBC = 125 mg/mL) than to inhibit and kill MRSA (MIC = 125 mg/mL; MBC = 250

mg/mL), even though MRSA had produced the larger zone of inhibition in the diffusion assay. Both organisms yielded an MBC/MIC ratio of 2, confirming that the extract is bactericidal rather than merely bacteriostatic against both pathogens, as summarized in Table 5.

Table 5: Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC), and MBC/MIC Ratio of the Extract Against MRSA and CRPA.

Pathogen	MIC (mg/mL)	MBC (mg/mL)	MBC/MIC	Action
CRPA	62.5	125	2	Bactericidal
MRSA	125	250	2	Bactericidal

Note. An MBC/MIC ratio ≤ 4 indicates bactericidal activity; a ratio > 4 indicates bacteriostatic activity.

DISCUSSION

Phytochemical Composition and Antibacterial Mechanisms

The presence of flavonoids, tannins, saponins, and alkaloids in the extract is consistent with prior reports on *Albizia coriaria* (Omara et al., 2023) and likely explains its broad antibacterial activity. Each compound class contributes a distinct mechanism: flavonoids inhibit nucleic acid synthesis, disrupt membrane function, and may inhibit bacterial efflux pumps; tannins bind cell wall proteins and chelate essential metal ions; saponins increase membrane permeability by interacting with membrane lipids; and alkaloids intercalate with bacterial DNA or inhibit enzymes involved in cell division. The co-occurrence of these compound classes suggests a multi-target mechanism of action, which may explain the extract's activity against both Gram-positive and Gram-negative organisms despite their structurally distinct cell envelopes.

Differential Susceptibility of MRSA and CRPA

MRSA showed markedly higher sensitivity to the extract in the diffusion assay than CRPA, consistent with fundamental differences in cell wall structure. Gram-positive MRSA possesses a thick but porous peptidoglycan layer without an outer membrane, offering little resistance to diffusion of moderately hydrophobic plant compounds. Gram-negative CRPA, in contrast, is protected by an outer membrane containing lipopolysaccharide that restricts entry of hydrophobic and amphipathic molecules to small hydrophilic compounds via porin channels. This general Gram-negative permeability barrier, rather than any CRPA-specific resistance mechanism, most plausibly accounts for the smaller zone of inhibition observed against CRPA.

The MIC/MBC Reversal and Its Mechanistic Implications

Notably, the extract required a lower concentration to inhibit and kill CRPA than MRSA in the broth microdilution assay, despite MRSA showing the larger zone of inhibition on agar. This apparent contrast is instructive rather than contradictory: the zone of inhibition reflects the diffusion of compounds through a semi-solid agar matrix, whereas MIC/MBC reflects a direct concentration-response relationship in a homogeneous liquid medium with no diffusion barrier. The larger MRSA zone likely reflects more efficient compound diffusion around a Gram-positive cell lacking an outer membrane, while the lower MIC/MBC for CRPA suggests that once the compound reaches the cell in solution, it is inherently more potent per unit concentration against *P. aeruginosa*'s cellular machinery than against MRSA's.

This finding is particularly notable given the carbapenem-resistance mechanisms documented for *P. aeruginosa* by

Ramatla et al. (2025), including loss of the OprD porin, elevated AmpC and MexB efflux activity, and carbapenemase genes such as *blaVIM*, *blaIMP*, and *blaNDM*. That the extract achieved bactericidal activity against CRPA at a lower concentration than against MRSA suggests its phytochemical constituents act through pathways distinct from these carbapenem-resistance mechanisms, either by bypassing porin-dependent entry restrictions, evading efflux-mediated extrusion, or acting on targets unrelated to cell wall synthesis. An MBC/MIC ratio of 2 for both organisms confirms bactericidal rather than bacteriostatic activity, a clinically favourable property given that bacteriostatic agents depend on host immune clearance, which may be compromised in the immunocompromised or critically ill patients disproportionately affected by MRSA and CRPA infections.

Broader Implications and Limitations

These findings scientifically validate the traditional use of *A. coriaria* in treating bacterial infections and suggest the plant contains compounds sufficiently potent to warrant further antimicrobial drug development, including as a supplementary option against resistant Gram-negative pathogens where conventional antibiotic options are increasingly limited. The study is limited by its in vitro design, which may not fully replicate host biological interactions; in addition, specific bioactive compounds were not isolated, and synergistic or antagonistic interactions among phytochemicals were not explored, warranting further detailed investigation.

Conclusions

The ethanolic extract of *Albizia coriaria* stem bark possesses measurable, bactericidal activity against both archived MRSA and CRPA isolates, underpinned by a rich and diverse phytochemical profile comprising alkaloids, flavonoids, tannins, saponins, and phenols. *Staphylococcus aureus* was the more sensitive organism in the diffusion assay, while *Pseudomonas aeruginosa* required a lower effective concentration in liquid culture, together pointing to a multi-target mechanism of action largely independent of conventional beta-lactam and carbapenem resistance pathways. These findings support the traditional medicinal use of *A. coriaria* and its potential as a foundation for affordable, plant-based antimicrobial agents.

Limitations.

This study was conducted under controlled in vitro conditions, which may not fully mimic the complex biological interactions that occur in living organisms.

Additionally, specific bioactive compounds were not isolated, and synergistic or antagonistic interactions among phytochemicals were not explored.

Recommendations

Researchers should conduct further phytochemical profiling to isolate, purify, and identify the specific compounds responsible for the observed antibacterial activity, and should investigate potential synergistic or antagonistic interactions among them. The Ministry of Health, in collaboration with district health officers and primary healthcare facility managers, should consider the integration of scientifically validated herbal remedies such as *A. coriaria* into primary healthcare protocols in low-resource settings, supported by continued clinical evidence generation from researchers and academic institutions. The National Drug Authority, working with the Ministry of Health and national standards bodies, should lead the development of regulatory guidelines for the production, safety testing, and quality control of herbal medicines. Finally, pharmaceutical researchers and phytochemists, in partnership with universities and local manufacturers, should explore formulating *A. coriaria* extracts into stable pharmaceutical products such as ointments, syrups, and capsules, with regulatory oversight from the National Drug Authority before market release.

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Thank you to everyone who has contributed to this initiative. Your collective support inspires us to pursue innovative solutions to combat antibiotic resistance and improve public health outcomes.

Conflict of interest

The authors declare no conflict of interest.

List of Abbreviations.

MRSA – Methicillin-Resistant *Staphylococcus aureus*
 CRPA – Carbapenem-Resistant *Pseudomonas aeruginosa*
 WHO – World Health Organization
 CLSI – Clinical and Laboratory Standards Institute
 MIC – Minimum Inhibitory Concentration
 MBC – Minimum Bactericidal Concentration
 DMSO – Dimethyl Sulfoxide
 OD – Optical Density
 SD – Standard Deviation
 SE – Standard Error
 SEM – Standard Error of the Mean
 CoVAB – College of Veterinary Medicine, Animal Resources and Biosecurity
 TSB – Tryptic Soy Broth
 MHA – Mueller-Hinton Agar
 mg/mL – Milligrams per Millilitre
 µg/mL – Micrograms per Millilitre
 mL – Millilitre
 µL – Microlitre
 mm – Millimetre
 °C – Degrees Celsius
 v/v – Volume per Volume
 OprD – Outer Membrane Porin D
 AmpC – Ampicillin C (Beta-lactamase enzyme)
 MexB – Multidrug Efflux Pump Component B
bla – Beta-lactamase
 VIM – Verona Integron-encoded Metallo-beta-lactamase
 IMP – Imipenemase Metallo-beta-lactamase
 NDM – New Delhi Metallo-beta-lactamase

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Conflict of interest.

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Availability of data.

Data used in this study are available upon request from the corresponding author.

Authors contribution.

Samson Okhello Piraciel conceptualized the study, developed the methodology, carried out the investigation, and wrote the original draft.

Stephen Alafi and Opio Gabdesio: supervision, review, editing

Dr. Jacqueline Kyosiimire: supervision, validation, writing-review and editing

Dr. Saphan Muzoora: supervision, validation, writing-review and editing

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