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Original Research Article

Phytochemical Characterization of the Aerial Parts of *Aneilema lanceolatum* (Benth.) (Commelinaceae) and Its Antibacterial Activity Against *Staphylococcus aureus*

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ABSTRACT

This work examined the chemical makeup and antibacterial potential of chromatographic fractions derived from ethyl acetate and methanol extracts of *Aneilema lanceolatum* aerial parts. Plant material was sequentially macerated in cold hexane, ethyl acetate, and methanol. The resulting extracts were characterized through qualitative phytochemical tests, thin-layer chromatography (TLC), column chromatography, and ultraviolet-visible (UV–Vis) spectroscopy, and their antibacterial potential against *Staphylococcus aureus* was determined using the agar well diffusion technique. Screening tests confirmed pharmacologically relevant constituents, including flavonoids, tannins, alkaloids, saponins, steroids, and triterpenes. After chromatographic separation, UV–Vis scanning revealed several distinct fractions, with absorption profiles indicating high phenolic content in several. All fractions inhibited *S. aureus* growth in a dose-dependent fashion, with inhibition zones spanning 9–28 mm. Notably, at the highest test concentration, some fractions outperformed the standard antibiotic gentamicin, which produced a 26 mm zone of inhibition. This antibacterial performance likely stems from the combined effect of phenolics and other bioactive constituents in the plant material.

Keywords: Aneilema lanceolatum; Aerial parts, Phytochemicals, Antimicrobial activity, *Staphylococcus aureus*

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Introduction

Medicinal plants have long played a central role in preventing, managing, and treating disease, and they remain indispensable to healthcare systems worldwide. Despite remarkable progress in synthetic pharmaceuticals, plant-derived agents remain among the most valuable sources of therapeutics because of their vast chemical diversity and broad spectrum of biological effects. A substantial proportion of drugs in current clinical use were either isolated directly from plants or designed based on plant-derived lead compounds[1].

A key factor sustaining interest in plant-based therapeutics is the escalating problem of antimicrobial resistance. Resistant infections represent a serious and growing public health challenge, driving up healthcare costs, extending illness duration, raising mortality, and narrowing available treatment options[2,3].

Staphylococcus aureus ranks among the most clinically significant bacterial pathogens, implicated in a wide range of infections, including skin and soft tissue infections, pneumonia, septicemia, osteomyelitis, and endocarditis[4,5].

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Because they synthesize antimicrobial secondary metabolites, medicinal plants offer a promising avenue for addressing this challenge. Unlike conventional antibiotics, which typically act on a single target, many plant-derived compounds exert antimicrobial effects through several simultaneous mechanisms[6,7,8].

Aneilema lanceolatum (Benth.), a herbaceous member of the Commelinaceae family, holds notable ethnomedicinal value. Known as Day Flower in English and referred to locally as Kayar-Galma in northern Nigeria, the plant is used as livestock fodder. It is better known in traditional medicine circles for treating skin conditions, inflammation, infertility, pain, wounds, erectile dysfunction, and a variety of other ailments[9,10].

Earlier investigations have documented antimicrobial and antitussive properties in *A. lanceolatum*, and phytochemical work has identified constituents such as tannins, flavonoids, and triterpenes, all recognized for their pharmacological relevance[11,12].

Even so, comparatively little is known about how the phytochemical composition of chromatographically separated fractions of *A. lanceolatum* relates to antibacterial performance. Existing research has largely focused on crude extracts, leaving a gap in understanding which specific constituents drive the plant's biological activity. Fractionating crude extracts allows compounds of similar polarity to be concentrated together, making it easier to pinpoint the metabolites most likely responsible for antibacterial effects.

This study aimed to characterize the phytochemical profile of *A. lanceolatum* aerial parts through qualitative screening, TLC, column chromatography, and UV–Vis spectroscopy, and to assess the antibacterial activity of the resulting fractions against *S. aureus*.

Materials and Methods

Plant Collection and Identification

Aerial parts of *A. lanceolatum* (Benth.), including leaves, stems, flowers, and seeds, were collected in October 2024 from Kubanni, Sabon Gari Local Government Area, Kaduna State, Nigeria. Identification and authentication were performed at the Herbarium

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Unit, Department of Botany, Ahmadu Bello University, Zaria, where a voucher specimen (reference number ABU0533) was subsequently deposited for future verification.

Preparation of Plant Material and Extraction

The harvested material was first cleaned of debris and left to dry in the shade at ambient temperature; shade drying was chosen to minimize degradation of heat-labile phytochemicals that direct sunlight or elevated temperatures might otherwise cause. Once fully dry, the material was mechanically ground into a coarse powder to maximize surface area for solvent contact.

Cold maceration was carried out using solvents of increasing polarity: hexane first (to recover non-polar constituents), followed by ethyl acetate, and finally methanol (for compounds of intermediate to high polarity). Each solvent was left in contact with the plant material for 72 hours with intermittent agitation to enhance extraction efficiency. After each 72-hour interval, mixtures were filtered to separate the liquid extract from the residue, and the filtrates were concentrated over a 60 ℃ water bath until the solvent fully evaporated. The resulting concentrated extracts were weighed, labeled, and stored in airtight containers pending further analysis.

Preliminary Phytochemical Screening

Qualitative screening of the ethyl acetate and methanol extracts followed established protocols described by Evans (2009) and Sofowora (2008),[13,14] methods widely recognized for reliably detecting common classes of plant secondary metabolites. Each extract was tested with appropriate chemical reagents, and a positive result was recorded when a characteristic color change or precipitate formed. These preliminary results provided an early indication of extract composition before chromatographic fractionation.

Thin-Layer Chromatography (TLC)

TLC was used to generate preliminary chromatographic fingerprints of the ethyl acetate and methanol extracts. Pre-coated silica gel 60 F_{254} plates served as the stationary phase, and one-dimensional ascending development was carried out in solvent systems chosen to give good separation of the extract components.

Developed plates were air-dried, sprayed uniformly with 10% sulphuric acid, and heated at 104 ℃ to enhance spot visibility. Distinct coloured spots appeared following heating, and retention factor (Rf) values were calculated as the ratio of the distance traveled by each compound to that traveled by the solvent front. These TLC profiles were later used as a reference for comparing column chromatography fractions and grouping those with related chemical signatures.

Column Chromatography

Crude extracts were further separated by silica gel column chromatography. Roughly 4.0 g of each extract was loaded onto a silica gel-packed column, and elution was carried out under a gradient of increasing polarity using hexane, ethyl acetate, and butanol in varying proportions. This gradient allowed compounds of differing polarity to elute at different rates, achieving effective separation.

Collected fractions were individually screened by TLC, and those displaying comparable spot patterns and Rf values were pooled. These pooled fractions were then carried forward for spectroscopic characterization and antibacterial testing.

Ultraviolet–Visible (UV–Vis) Spectroscopic Analysis

Pooled fractions were scanned by UV–Vis spectroscopy across a 200–750 nm range, with the corresponding solvent used as a blank for calibration. Recorded absorption maxima were compared against published reference spectra to tentatively assign the phytochemical classes present,[15] with particular attention paid to absorption regions typically associated with phenolic compounds, given their frequent association with antimicrobial and antioxidant properties. While UV–Vis spectroscopy cannot definitively identify individual compounds, it provided useful preliminary insight to guide further phytochemical work.

Antibacterial Assay

Antibacterial activity of the pooled fractions against *S. aureus* was assessed using the agar well diffusion method following Clinical and Laboratory Standards Institute guidelines.[16] A clinical *S. aureus* isolate was obtained from the Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, Gombe State University.

Bacterial suspensions were standardized to match a 0.5 McFarland turbidity standard before inoculation. Sterile Mueller–Hinton agar plates were evenly inoculated with the standardized culture, and 6 mm wells were aseptically cut into the agar. Fractions were introduced into the wells at concentrations of 100, 75, 50, and 25 mg/mL, with gentamicin (20 mg/mL) serving as the positive control for comparison against a standard antibiotic.

Plates were incubated at 37 ℃ for 24 hours, after which inhibition zone diameters around each well were measured with a calibrated Vernier caliper and recorded in millimeters.

Statistical Analysis

Antibacterial assay results were expressed as mean ± standard deviation (SD). Data were analyzed in IBM SPSS Statistics version 23, using descriptive statistics to summarise findings and compare antibacterial performance across fractions and concentrations. This approach provided a sound basis for interpreting activity trends and benchmarking the plant fractions against the reference antibiotic.

Results and Discussion

Preliminary Phytochemical Screening

Preliminary phytochemical screening offers an early window into the bioactive compounds present in a medicinal plant. It helps assess its potential therapeutic effects and guides the choice of methods for further chemical and biological evaluation.[17]

Qualitative analysis of the ethyl acetate and methanol extracts of *A. lanceolatum* detected saponins, flavonoids, tannins, alkaloids, steroids, and triterpenes in both extracts (Table I). Their presence confirms that the plant's aerial parts carry a rich and varied phytochemical profile. Numerous studies have linked these metabolite classes to antibacterial, antioxidant, anti-inflammatory, and wound-healing effects, underscoring their broader relevance to the medicinal properties of many plant species[18,19,20].

The concurrent presence of several phytochemical classes within the same extract points to a combined, rather than single-compound, basis for the biological activity of *A. lanceolatum*. Flavonoids and tannins, for instance, can disrupt microbial enzymes and compromise membrane integrity, while saponins can alter membrane permeability; alkaloids, steroids, and triterpenes have similarly been linked to antimicrobial and anti-inflammatory effects through distinct biochemical routes[21]. The interplay among these constituents may therefore explain the overall antibacterial activity observed in this study.

This phytochemical profile aligns with earlier reports on *A. lanceolatum* and related species within the same genus, which likewise identified flavonoids, tannins, steroids, and triterpenes as dominant constituents.[9,11,12] Such agreement with prior work reinforces confidence in the present findings and lends further support to the plant's traditional medicinal applications. Taken together, the phytochemical composition identified here offers a plausible chemical basis for the antibacterial activity observed.

Thin-Layer Chromatography (TLC)

TLC was used to establish a preliminary chemical fingerprint of the extracts ahead of further purification. Valued in pharmacognostic research for its speed and low cost, the technique is routinely used to separate complex mixtures and assess phytochemical diversity in medicinal plants.[22]

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Chromatographic profiling of the ethyl acetate and methanol extracts produced several distinct spots differing in color and retention factor (Rf) (Table II). Rf values ranged from 0.33 to 0.96 for the ethyl acetate extract and from 0.35 to 0.94 for the methanol extract. This wide spread of Rf values reflects compounds with markedly different polarities and chemical behavior.

Table I: Phytochemical screening of methanol and ethylacetate extracts of *A. lanceolatum*

S/N	Constituents	Test	Ethylacetate extract	Methanol extract
1	Saponin	Frothing test	+	+
2	Steroid/Triterpenes	Liebermann-burchard	+	+
		Salkowski	+	+
3	Tannins	Ferric chloride	+	+
4	Flavonoids	NaOH	+	+
		Shinoda	+	+
5	Alkaloids	Mayer's	+	+
		Dragendorff's	+	+

Key: + = Positive and - = Negative

Table II: Thin layer chromatographic profile of methanol and ethylacetate extracts of *A. lanceolatum* sprayed with 10% Sulphuric acid

Extracts	Solvent system	Rf Values	Colour of spot
Ethylacetate	Hexane : Ethyl Acetate : Methanol (6:2:2)	0.96	Reddish-bronw
		0.89	Pink
		0.77	pink
		0.75	Green
		0.57	Purple
	Hexane : Ethyl Acetate: Methanol (7:2:1)	0.95	Reddish-brown
		0.86	Pink
		0.58	Brown
		0.47	Green
		0.33	Purple
	Ethyl Acetate : Hexane (8:2)	0.82	Pink
		0.73	Green
		0.56	Purple
		0.5	Purple
Methanol	Hexane:Ethylacetate:Methanol (6:2:2)	0.92	Purple
		0.75	Green
		0.41	Brown
		0.35	Brown
	Hexane:Ethylacetate:Methanol (7:2:1)	0.94	Yellow
		0.92	Green
	Ethylacetate: Hexane (8:2)	0.83	Reddish-brown
		0.75	Green
		0.69	Purple
		0.46	Brown
		0.35	Brown

The multiple colored spots on the developed TLC plates confirms that the constituents of each extracts were successfully resolved. The differences observed in spot color and migration distance point to considerable chemical diversity and imply the presence of several distinct phytochemical classes. The chosen solvent systems achieved satisfactory resolution and proved suitable for the subsequent fractionation step[23].

Beyond illustrating the chemical complexity of the extracts, the TLC data also served a practical role during column chromatography, guiding the pooling of fractions that shared similar spot patterns and Rf values. Grouping chemically related fractions in this way

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streamlined the purification process and avoided unnecessary repetition in later stages of the work.[23,24]
Similar TLC profiles have been documented previously for *A. lanceolatum* and other medicinal plants sharing comparable phytochemical compositions.[12,22] The correspondence between the present results and earlier reports supports the appropriateness of the chromatographic approach used to resolve the constituents of the plant's aerial parts.

Column Chromatography and UV–Visible Spectroscopic Analysis
The UV–Vis spectroscopy is a technique frequently applied in early-stage phytochemical work because many naturally occurring compounds possess chromophores that absorb light within characteristic wavelength bands.[25,26] While UV–Vis spectroscopy does not allow definitive identification of individual compounds, it offers useful clues about the likely phytochemical classes present.
Ethyl acetate fractions absorbed maximally between 310 and 550 nm, while methanol fractions peaked between 400 and 535 nm (Table III). These ranges are consistent with literature values reported for phenolic compounds, including flavonoids, tannins, anthocyanins, carotenoids, and certain terpenoid derivatives.[27,28,29] The spectroscopic data thus corroborate the earlier screening results and further support the conclusion that the extracts contain substantial amounts of phenolic constituents.
Phenolic compounds are recognized for a wide range of biological effects. Beyond their antioxidant properties, they have shown notable antimicrobial activity through mechanisms such as bacterial membrane disruption, enzyme inhibition, interference with protein synthesis, and suppression of oxidative processes within microbial cells.[21] Their abundance in the chromatographic fractions may therefore help explain, at least in part, the antibacterial activity recorded in this study.

Table III: Thin Layer Chromatogram and UV-Visible Absorbance of the column chromatographic fractions from methanol and ethylacetate extracts of *A. lanceolatum*

Extracts	Solvent system	Rf values	Colour of spot	UV-Vis Absorbance (nm)
Ethylacetate	Hexane:Ethylacetate(9:1)	0.72	Reddish-Brown	370
		0.61	Pink	
	Hexane:Ethylacetate(7:3)	0.61	Purplish-brown	455
		0.53	Brown	
	100% Ethylacetate	0.81	Green	310
		0.71	Purplish-brown	
	Butanol:Ethylacetate(9:1)	0.90	Brown	550
		0.85	Purplish-brown	
		0.75	Purplish-brown	
		0.66	Brown	
		0.58	Green	
	Butanol:Ethylacetate(8:2)	0.86	Brown	450
		0.61	Brown	
		0.53	Brown	
	100% Butanol	0.7	Purplish-pink	470
		0.4	Brown	
Methanol	Butanol:Ethylacetate(8:2)	0.8	Pink	535
		0.33	Brown	
	Butanol:Ethylacetate(1:1)	0.7	Pink	520
		0.6	Brown	
	100% Butanol	0.86	Green	400
		0.72	Green	
		0.65	Brown	
		0.57	Green	
		0.49	Pink	

Antibacterial Activity
Antibacterial activity of the chromatographic fractions was tested against *Staphylococcus aureus*, a pathogen widely implicated in skin infections. The rising prevalence of antibiotic-resistant strains, particularly methicillin-resistant *S. aureus* (MRSA), continues to motivate research into medicinal plants as alternative sources of antimicrobial agents.[30,31,32]
A clear concentration-dependent trend emerged, with inhibition zones widening as fraction concentration increased (Table IV); a pattern consistent with how many plant-derived antimicrobial compounds are known to behave as their active-constituent load rises.

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Table IV: Zone of Inhibition of the column chromatographic fractions from methanol and ethylacetate extracts of *A. lanceolatum* against *S. aureus*

Extracts	Solvent system	Zone of Inhibition (mm) Concentration				
		100mg/ml	75mg/ml	50mg/ml	25mg/ml	20mg/ml
Ethylacetate	Hexane:Ethylacetate(9:1)	28 ± 0.2	22 ± 0.1	18 ± 0.6	13 ± 0.1	
	Hexane:Ethylacetate(7:3)	16 ± 0.1	16 ± 0.2	0.0	8 ± 0.1	
	100% Ethylacetate	12 ± 0.1	10 ± 0.1	11 ± 0.1	0.0	
	Butanol:Ethylacetate(9:1)	15 ± 0.1	13 ± 0.1	14 ± 0.1	11 ± 0.1	
	Butanol:Ethylacetate(8:2)	20 ± 0.3	12 ± 0.1	11 ± 0.1	9 ± 0.1	
	100% Butanol	21 ± 0.3	20 ± 0.1	15 ± 0.1	14 ± 0.1	
Methanol	Butanol:Ethylacetate(8:2)	20 ± 0.1	12 ± 0.1	13 ± 0.1	14 ± 0.1	
	Butanol:Ethylacetate(1:1)	16 ± 0.1	15 ± 0.1	11 ± 0.1	9 ± 0.1	
	100% Butanol	28 ± 0.1	22 ± 0.1	14 ± 0.1	12 ± 0.1	
Gentamycin						26 ± 0.1

The strongest antibacterial performance came from the fractions with absorption maxima near 370 nm (ethyl acetate extract) and 400 nm (methanol extract). At the highest concentration tested (100 mg/mL), these fractions produced inhibition zones of approximately 28 ± 0.20 mm and 28 ± 0.10 mm, respectively, marginally exceeding the 26 ± 0.10 mm zone recorded for gentamicin and indicating meaningful antibacterial potential under the conditions tested here.

This activity is likely driven by the combined presence of flavonoids, tannins, alkaloids, saponins, steroids, triterpenes, and other phenolic constituents identified in the phytochemical analysis. Rather than acting through a single mechanism, these metabolites may suppress bacterial growth through several complementary routes: membrane disruption, enzyme inhibition, interference with nucleic acid synthesis, reduced biofilm formation, and disruption of essential metabolic pathways.[21] Their combined effect could account for why certain fractions matched, or even slightly exceeded, the performance of the reference antibiotic.

These results align with earlier reports showing that phenolic-rich plant extracts can strongly inhibit *S. aureus*, including resistant clinical isolates.[32,33,34] Collectively, the findings add to the evidence that *A. lanceolatum* harbors bioactive constituents with promising antimicrobial properties, reinforcing its standing as a medicinal plant of pharmaceutical interest.

While this study clearly establishes the antibacterial potential of the chromatographic fractions, it does not pinpoint the specific compound(s) responsible. Future work should pursue bioassay-guided isolation of the active constituents alongside structural characterization using advanced analytical techniques. Further evaluation of minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), toxicity, pharmacokinetics, and in vivo efficacy will also be needed before these compounds can be considered candidates for therapeutic development.

Conclusion

This study adds to the scientific evidence supporting the medicinal value of the aerial parts of *Aneilema lanceolatum*. Qualitative phytochemical screening confirmed several biologically active secondary metabolites, including flavonoids, tannins, alkaloids, saponins, steroids, and triterpenes, underscoring the plant's rich phytochemical makeup and providing a chemical rationale for many of its traditional uses.

TLC and column chromatography showed that the extracts contained multiple constituents with varying physicochemical properties, and subsequent UV–Vis spectroscopy indicated that several pooled fractions were rich in phenolic compounds—a class widely known for antimicrobial and antioxidant activity. The antibacterial assay confirmed that the fractions inhibited *S. aureus* growth in a concentration-dependent manner, with the most active fractions producing inhibition zones comparable to, and in some cases marginally exceeding, those of the standard antibiotic gentamicin under the tested conditions.

Conflict Of Interest

The authors declare no conflict of interest

Authors' Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by us.

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