



Research Article

Antibacterial Activity and Phytochemical Composition of Ethanolic and Aqueous Leaf Extracts of *Vernonia galamensis* Against Selected Pathogenic Bacteria

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ABSTRACT

The rising prevalence of antimicrobial resistance has intensified the search for alternative therapeutic agents from medicinal plants. This study evaluated the antibacterial activity and qualitative phytochemical composition of ethanolic and aqueous leaf extracts of *Vernonia galamensis* against selected pathogenic bacteria. Fresh leaves of *V. galamensis* were collected, authenticated, air-dried, and extracted using ethanol and distilled water. Qualitative phytochemical screening was performed to detect alkaloids, tannins, flavonoids, saponins, phenols, phytate, and oxalate using standard chemical tests. Antibacterial activity of the extracts at concentrations of 25 mg/mL, 50 mg/mL, and 100 mg/mL was assessed against *Salmonella typhi* and *Staphylococcus aureus* using the disc diffusion method. Ciprofloxacin (250 mg) and dimethyl sulfoxide served as positive and negative controls, respectively. Phytochemical analysis revealed solvent-dependent variation in constituent distribution. Alkaloids were strongly present in the ethanolic extract but absent in the aqueous extract, while tannins, flavonoids, saponins, and phenols were more pronounced in the aqueous extract. Both extracts contained moderate levels of phytate, whereas oxalate was more abundant in the ethanolic extract. The antibacterial assay demonstrated concentration-dependent inhibition against both test organisms. The ethanolic extract showed higher antibacterial activity, producing inhibition zones of up to 15 mm against *S. typhi* and 14 mm against *S. aureus* at 100 mg/mL. Although ciprofloxacin exhibited greater activity, the findings highlight the antibacterial potential of *V. galamensis* and support its ethnomedicinal use as a source of bioactive compounds for complementary antimicrobial development.

Keywords: Antibacterial activity; *Vernonia galamensis*; Phytochemicals composition; *Salmonella typhi*; *Staphylococcus aureus*

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INTRODUCTION

The rapid emergence and global spread of antimicrobial resistance (AMR) have become a major public health challenge, threatening the effectiveness of existing antibiotics and increasing morbidity, mortality, and healthcare costs worldwide (Yogesh *et al.*, 2011). Pathogenic bacteria that were once easily treatable with conventional antibiotics are increasingly developing resistance due to factors such as indiscriminate drug use, incomplete treatment

regimens, and limited development of new antimicrobial agents (Ali *et al.*, 2019). As a result, there is growing scientific interest in identifying alternative sources of antimicrobial compounds, particularly from medicinal plants that have been traditionally used for the treatment of infectious diseases (Unegbu *et al.*, 2020).

Medicinal plants have historically played a crucial role in primary healthcare systems, especially in developing countries where access to modern

pharmaceuticals may be limited. Plants synthesize a wide array of secondary metabolites, including alkaloids, tannins, flavonoids, saponins, phenols, and other bioactive compounds, many of which possess documented antimicrobial, anti-inflammatory, antioxidant, and antiparasitic properties (Yogesh *et al.*, 2011). These phytochemicals often act through diverse mechanisms such as disruption of microbial cell membranes, inhibition of essential enzymes, interference with nucleic acid synthesis, and modulation of oxidative stress (Ali *et al.*, 2019). Consequently, plant-based antimicrobials are increasingly being explored as potential alternatives or complements to conventional antibiotics.

Vernonia galamensis is a perennial shrub belonging to the family Asteraceae and is widely distributed across tropical regions of Africa (Tafesse, 2018). The plant is traditionally valued for various ethnomedicinal applications. In several African communities such as Nigeria, its twigs are used as chewing sticks for oral hygiene, while the leaves and other plant parts are employed in the management of ailments such as infections, inflammation, metabolic disorders, and gastrointestinal disturbances (Egedigwe, 2010; Tafesse, 2018). Despite its widespread traditional use, scientific information on the antibacterial properties of *V. galamensis* remains relatively limited compared to other species within the *Vernonia* genus.

Bacterial pathogens such as *Salmonella typhi* and *Staphylococcus aureus* continue to pose significant health burdens, particularly in tropical and subtropical regions (Ali *et al.*, 2019; Ekeleme *et al.*, 2017). *Salmonella typhi* is the causative agent of typhoid fever, a systemic infection associated with poor sanitation and contaminated food or water, while *S. aureus* is a versatile pathogen responsible for a wide range of infections, including skin and soft tissue infections, wound infections, and life-threatening systemic conditions (Umamaheswari and Rama, 2014). The increasing resistance of these organisms to commonly used antibiotics has complicated treatment strategies and underscores the need for alternative antimicrobial agents.

Previous studies have demonstrated that solvent type plays a critical role in the extraction efficiency of phytochemicals from plant materials (Nwozo *et al.*, 2023). Organic solvents such as ethanol are often more effective in extracting alkaloids and moderately polar compounds, whereas aqueous solvents tend to

favour the extraction of highly polar constituents such as tannins, flavonoids, and Saponins (Tafesse, 2018). These differences in phytochemical composition may significantly influence the biological activities of plant extracts, including their antibacterial potential.

Given the ethnomedicinal relevance of *Vernonia galamensis* and the urgent need for novel antimicrobial agents, it is important to scientifically evaluate its antibacterial activity and phytochemical composition. Therefore, the present study aimed to investigate the antibacterial activity of aqueous and ethanolic leaf extracts of *V. galamensis* against *Salmonella typhi* and *Staphylococcus aureus*, and to qualitatively determine the phytochemical constituents present in the extracts. The findings of this study are expected to provide scientific justification for the traditional use of *V. galamensis* and contribute to the growing body of knowledge on plant-based antimicrobial agents.

MATERIALS AND METHODS

Sample Collection and Authentication

Fresh leaves of *Vernonia galamensis* were purchased from Katsina Central Market, Katsina State, Nigeria. The plant was authenticated at the Herbarium of Umaru Musa Yar'adua University, Katsina, and assigned Voucher Number UMYU/HERB/1819. The leaves were washed thoroughly, air-dried at room temperature for seven days, and ground into a fine powder.

Extraction of Plant Materials

The method described by Bandiola (2018) was adapted. Fifty grams (50 g) of powdered leaves were soaked in 300 ml of ethanol for 72 hours with intermittent shaking. The mixture was filtered and the filtrate concentrated using a rotary evaporator. For Aqueous Extraction; Fifty grams (50 g) of powdered leaves were soaked in 300 ml of distilled water for 72 hours, filtered, and evaporated at 50 °C to dryness.

Preparation of Extract Concentrations

Crude extracts were reconstituted in distilled water or dimethyl sulfoxide (DMSO) to obtain concentrations of 25mg/mL, 50mg/mL, and 100 mg/ml.

Phytochemical Screening

Qualitative phytochemical analyses for the detection of tannins, saponins, flavonoids, phenols, phytate,

oxalate and alkaloids were carried out using standard procedures;

Test for Alkaloids

An aliquot of each extract (20 µL) was applied onto a thin-layer chromatography (TLC) plate (Silica Gel 60G, 5 × 10 cm) and developed using a toluene–ethyl acetate–diethylamine solvent system (70:20:10). Alkaloids were detected by spraying the developed plates with Dragendorff's reagent, where the appearance of orange to brown spots indicated a positive reaction (Wagner and Bladt, 2001; Ekeleme *et al.*, 2017).

Test for Tannins

One millilitre (1 mL) of 5% ferric chloride solution was added to the extract. The formation of a bluish-black or greenish-black precipitate indicated the presence of tannins (Firdouse and Alam, 2011; Wagner and Bladt, 2001).

Test for Flavonoids

A few fragments (3–4 pieces) of magnesium ribbon were added to 1 mL of the extract, followed by the drop wise addition of concentrated hydrochloric acid. The development of a pink or red coloration confirmed the presence of flavonoids (Rathore *et al.*, 2013; Ekeleme *et al.*, 2017).

Test for Saponins

Two millilitres (2 mL) of distilled water were added to the extract suspended in ethanol and the mixture was shaken vigorously. The formation of a stable and persistent froth indicated the presence of saponins (Rathore *et al.*, 2013; Ekeleme *et al.*, 2017).

Phenolic Glycosides

The presence of phenolic glycosides was assessed using Fehling's test to detect salicin. Briefly, 0.1 g of the sample was dissolved in 5 mL of distilled water, and 1 mL each of Fehling's solutions A and B was added. The mixture was boiled, and no brick-red precipitate of cuprous oxide (Cu₂O) was observed. The absence of reduction indicates the presence of phenolic glycosides, as salicin does not reduce Fehling's solutions (Ebana *et al.*, 2016).

Test for Phytic Acid

Phytic acid extraction was carried out following the method described by Ebana *et al.* (2016). Ferric chloride was added to the extract to precipitate phytic acid in the form of ferric phytate.

Test for Oxalate

Oxalate in the extracts was determined according to the method of Ebana *et al.* (2016), where oxalic acid

was precipitated using calcium chloride to form insoluble calcium oxalate salts.

Bacterial Isolates

Clinical isolates of *Salmonella typhi* and *Staphylococcus aureus* were obtained from the Microbiology Laboratory, Department of Microbiology, Umaru Musa Yar'adua University Katsina.

Antibacterial Activity Assay

The antimicrobial activity was evaluated using the disc diffusion method on Mueller–Hinton agar according to EUCAST guidelines. Discs impregnated with different concentrations of the extracts were placed on inoculated plates, with ciprofloxacin (250 mg) and dimethyl sulfoxide (DMSO) serving as positive and negative controls, respectively. Plates were incubated at 37 °C for 18–24 h, and inhibition zones were measured in millimetres (Nigussie *et al.*, 2020).

RESULTS

Table 1 presents the extraction yield of *V. galamensis* leaf powder using ethanol and aqueous solvents. Using an equal starting mass of 50 g of powdered leaves, the ethanolic extraction yielded 6.81 g (2.83%), whereas the aqueous extraction yielded 5.63 g (2.33%).

Table 2 presents the qualitative phytochemical profile of the ethanolic and aqueous leaf extracts of *Vernonia galamensis*. The results indicate marked variations in the presence and relative intensity of phytochemical constituents depending on the extraction solvent used. Alkaloids were strongly detected (+++) in the ethanolic extract but were completely absent (–) in the aqueous extract, suggesting that these compounds are more efficiently extracted in ethanol. Tannins were present in both extracts; however, their intensity was higher in the aqueous extract (+++) compared to the ethanolic extract (++) reflecting their greater solubility in aqueous.

Flavonoids were detected at low intensity (+) in the aqueous extract but were absent (–) in the ethanolic extract. Saponins were present in both extracts, with a moderate intensity (++) in the aqueous extract and low intensity (+) in the ethanolic extract. Phenol glycosides compounds were moderately present (++) in both ethanolic and aqueous extracts. In addition, phytate and oxalate were detected in both extracts. Phytate was moderately present (++) in both

ethanolic and aqueous extracts, while oxalate showed moderate intensity (++) in the ethanolic extract and low intensity (+) in the aqueous extract. The aqueous and ethanolic leaf extracts of *Vernonia galamensis* demonstrated measurable antibacterial activity against both *Salmonella typhi* and *Staphylococcus aureus* (Table 3). In the aqueous extract, *S. typhi* showed zones of inhibition of 8.0 ± 0.12 mm, 10.0 ± 0.16 mm, and 13.0 ± 0.20 mm at concentrations of 25mL, 50mL, and 100 mg/mL, respectively. In contrast, *S. aureus* exhibited no inhibition at 25 mg/mL but showed moderate inhibition at 50 mg/mL (9.0 ± 0.25 mm) and the highest inhibition at 100 mg/mL (11.0 ± 0.31 mm).

The ethanolic extract exhibited comparatively higher antibacterial activity against both test organisms. For *S. typhi*, zones of inhibition increased from 8.0 ± 0.12 mm at 25 mg/mL to 15.0 ± 0.20 mm at 100 mg/mL. Similarly, *S. aureus* recorded zones of inhibition of 8.0 ± 0.13 mm, 10.0 ± 0.25 mm, and 14.0 ± 0.31 mm at 25, 50, and 100 mg/mL, respectively. Ciprofloxacin (250 mg), used as the positive control, produced significantly larger zones of inhibition ranging from 18–21 mm against the test organisms, while DMSO showed no inhibitory effect, confirming the validity of the assay (Table 3).

Table 1: Percentage yield from ethanolic and aqueous extracts of *Vernonia galamensis*

	Extraction Solvents Used	
	Ethanol	Aqueous
Weight of Leaf powdered used (g)	50	50
Extract Yield (g)	6.81	5.63
Percentage Yield (%)	2.83	2.33

KEY: g = Gram, % = Percentage

Table 2: Qualitative Phytochemical Screening of the ethanolic and aqueous extracts of *Vernonia galamensis*

Phytochemical Constituent Tested	Ethanol	Aqueous
Alkaloids	+++	-
Tannins	++	+++
Flavonoids	-	+
Saponins	+	++
Phenolic glycosides	++	++
Phytate	++	++
Oxalate	++	+

Key: Absence, +; low intensity, ++; moderate intensity, +++; strong intensity

Table 3: Antibacterial Activity of Aqueous and Ethanolic Leaf Extracts of *Vernonia galamensis* Against Selected Bacterial Pathogens

Test Organism	Extract Type	25	50	100	Ciprofloxacin (250 mg)	DMSO
<i>Salmonella typhi</i>	Aqueous	8.0 ± 0.12	10.0 ± 0.16	13.0 ± 0.20	20	0.00
<i>Salmonella typhi</i>	Ethanolic	8.0 ± 0.12	11.0 ± 0.18	15.0 ± 0.23	21	0.00
<i>Staphylococcus aureus</i>	Aqueous	0.0 ± 0.00	9.0 ± 0.25	11.0 ± 0.31	18	0.00
<i>Staphylococcus aureus</i>	Ethanolic	8.0 ± 0.13	10.0 ± 0.30	14.0 ± 0.34	18	0.00

Concentration mg/mL

Zone of Inhibition values are expressed as mean \pm SD, also in mm; DMSO = Dimethyl Sulfoxide

DISCUSSION

The higher percentage yield obtained with ethanol indicates that ethanol is a more efficient solvent for

extracting soluble constituents from *V. galamensis* leaves. This may be attributed to ethanol's ability to dissolve both polar and moderately non-polar

phytochemicals, thereby extracting a broader spectrum of secondary metabolites compared to water alone (Kumarasamy and Selvi, 2020). The relatively lower yield observed in the aqueous extract suggests limited solubility of some bioactive compounds in water. These findings support the subsequent antibacterial results where ethanolic extracts demonstrated superior biological activity (Tafesse, 2018; Trang *et al.*, 2024).

The results in Table 2 point to marked variation in phytochemical composition depending on the extraction solvent used. The sturdy alkaloids presence in ethanolic extract alone, suggests that these compounds are more readily extracted in organic solvents. Presence of tannins in both solvent extracts reflects their higher water solubility. Likewise, detection of flavonoids in only aqueous further emphasizes that solvent-dependent extraction efficiency. The presence of these phytochemicals is significant, as tannins, saponins, flavonoids, and phenols are well documented for their antimicrobial, antioxidant, and anti-inflammatory properties (Nwozo *et al.*, 2023). Taken as a whole, the phytochemical profile suggests that ethanol preferentially extracts alkaloid-rich fractions, while water favours the extraction of tannins, saponins, flavonoids, and phenolic compounds. This differential distribution may explain variations in antibacterial activity observed between the two extracts. These phytochemicals present in the leaves of *V. galamensis* may be directly linked to its wide range of reported pharmacological activities, including anti-carcinogenic, hypoglycemic, antioxidant, anti-inflammatory, antibacterial, antimalarial, and anti-helminthic effects, among several other therapeutic properties attributed to the plant (Dahanukar *et al.*, 2000).

The antibacterial activity of aqueous and ethanolic leaf extracts of *Vernonia galamensis* against *Salmonella typhi* and *Staphylococcus aureus* demonstrated a clear concentration-dependent inhibitory effect. However, the zones of inhibition produced by the plant extracts at all tested concentrations (25–100 mg/mL) were consistently lower than those recorded for ciprofloxacin (250 mg), which served as the positive control.

Ciprofloxacin exhibited superior antibacterial activity, producing inhibition zones ranging from 18 to 21 mm against the test organisms. This enhanced activity is

expected, as ciprofloxacin is a purified, broad-spectrum fluoroquinolones antibiotic with a well-defined mechanism of action, primarily involving inhibition of bacterial DNA gyrase and topoisomerase IV (Zaghary *et al.*, 2021). In contrast, the plant extracts represent crude mixtures of bioactive compounds whose concentrations, purity, and bioavailability are inherently lower and less standardized than those of a synthetic antibiotic.

Despite this disparity, the extracts of *V. galamensis* showed appreciable antibacterial activity, particularly at higher concentrations. The ethanolic extract exhibited greater inhibitory effects than the aqueous extract, achieving zones of inhibition of up to 15 mm against *S. typhi* and 14 mm against *S. aureus* at 100 mg/mL. This suggests that ethanol was more effective in extracting antibacterial constituents, possibly due to its ability to solubilize both polar and moderately non-polar compounds such as alkaloids, tannins, and Saponins as agreed by (Kumarasamy and Selvi, 2020). The lower antibacterial activity of the extracts relative to ciprofloxacin does not diminish their pharmacological relevance. Rather, it reflects the fundamental difference between crude plant extracts and purified pharmaceutical agents. Plant extracts often exert antibacterial effects through multiple synergistic mechanisms, including disruption of cell membranes, enzyme inhibition, and interference with protein synthesis (Al-amoot *et al.*, 2025). These effects may be weaker individually but can contribute collectively to antimicrobial activity, especially when used at higher concentrations or in combination with conventional antibiotics.

Furthermore, the absence of inhibitory effects in the DMSO negative control confirms that the observed antibacterial activity was attributable solely to the plant extracts. The concentration-dependent increase in inhibition zones indicates that higher extract concentrations enhance the availability of active compounds at the site of bacterial growth, reinforcing the biological plausibility of the results, this tends to corroborates with (Vaou *et al.*, 2022).

Although ciprofloxacin at 250 mg demonstrated significantly higher antibacterial activity, the measurable inhibition produced by *V. galamensis* extracts highlights their potential as alternative or complementary antimicrobial agents. These findings support the ethnomedicinal use of *V. galamensis* in managing bacterial infections and provide a scientific

basis for further studies aimed at isolating, purifying, and characterizing the specific compounds responsible for the observed antibacterial effects (Unegbu *et al.*, 2020).

CONCLUSION

The findings of this study confirm that *V. galamensis* leaf extracts possess notable antibacterial activity against *Salmonella typhi* and *Staphylococcus aureus*. The ethanol extract was more effective than the aqueous extract, likely due to improved extraction of bioactive compounds. The study provides scientific justification for the traditional use of *V. galamensis* in the treatment of bacterial infections and highlights its potential as a source of novel antimicrobial agents.

A limitation of this study is the use of crude plant extracts and qualitative phytochemical screening, which limits direct quantitative comparison with a purified antibiotic such as ciprofloxacin. Furthermore, the *in vitro* disc diffusion assay does not fully account for *in vivo* pharmacokinetic and pharmacodynamic factors. Further studies involving compound isolation, quantitative analysis, and *in vivo* evaluation are recommended.

Further studies should focus on the isolation and characterization of the specific bioactive compounds responsible for antibacterial activity and evaluation of antifungal and antiparasitic properties of *V. galamensis*. Also, *In vivo* toxicity and efficacy studies to establish safety and therapeutic potential is highly recommended

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