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# Phytochemical Screening, Antibacterial Efficacy and Acute Oral Toxicity Evaluation of Methanolic and Aqueous *Vernonia amygdalina* Leaf Extracts against Selected Bacterial Isolates

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## Abstract

*Vernonia amygdalina* Delile is widely used in African traditional medicine; however, comparative evidence regarding the antibacterial efficacy and safety of different extraction solvents remains limited. This study evaluated the phytochemical composition, antibacterial activity and acute oral toxicity of methanolic and aqueous leaf extracts of *V. amygdalina* against selected clinically important bacterial pathogens. Fresh leaves of *V. amygdalina* were extracted separately with 80% methanol and distilled water. Qualitative phytochemical screening was performed using standard methods. Antibacterial activity against *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Salmonella typhi* was determined by agar disc diffusion, while minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined using broth microdilution. Acute oral toxicity was evaluated in Wistar rats administered graded doses (500–8000 mg/kg) and monitored for behavioural changes and mortality over 14 days. Both extracts contained alkaloids, flavonoids, tannins, saponins, terpenoids, cardiac glycosides and reducing sugars. The methanolic extract consistently produced significantly larger inhibition zones than the aqueous extract ($P = 0.012$), with the highest activity observed against *S. aureus* (28.00 ± 1.00 mm) at 200 mg/mL. MIC values for the methanolic extract ranged from 1.56–3.12 mg/mL compared with 3.12–6.25 mg/mL for the aqueous extract ($P = 0.016$). MBC values ranged from 50–100 mg/mL and 50–200 mg/mL for the methanolic and aqueous extracts, respectively, without significant differences ($P > 0.05$). No

mortality occurred in any treatment group, indicating an oral $LD_{50}$ greater than 8000 mg/kg. However, transient restlessness and respiratory distress were observed only in animals receiving 8000 mg/kg. *Vernonia amygdalina* leaf extracts possess broad-spectrum antibacterial activity and a favourable acute safety profile. The methanolic extract demonstrated superior antibacterial efficacy, suggesting that methanol more effectively extracts bioactive antimicrobial constituents.

## Introduction

The increasing global burden of infectious diseases and the rapid emergence of antimicrobial resistance (AMR) have intensified the search for novel therapeutic agents from natural sources (Degu et al., 2024). Medicinal plants continue to play a critical role in primary healthcare systems, particularly in developing countries where access to conventional medicine may be limited. The Ethnopharmacology of medicinal plants remains highly relevant because many modern pharmaceuticals originate from bioactive phytochemicals derived from plant sources. According to recent estimates, more than 80% of the population in many African countries rely partly or wholly on traditional herbal medicine for disease management and health maintenance (Degu et al., 2024). Among the medicinal plants widely utilized in sub-Saharan Africa, *Vernonia amygdalina* Delile (family Asteraceae), commonly known as bitter leaf, has gained substantial scientific attention because of its nutritional, pharmacological, and therapeutic properties (Degu et al., 2024).

*Vernonia amygdalina* is a perennial shrub indigenous to tropical Africa and extensively distributed across Nigeria, Cameroon, Ethiopia, and other parts of sub-Saharan Africa. The plant is widely consumed as a vegetable and traditionally employed in the treatment of malaria, gastrointestinal disorders, diabetes mellitus, helminthic infections, fever, wound infections, and other inflammatory conditions (Atinga et al., 2021). Different parts of the plant, including the leaves, roots, stems, and bark, are used in traditional medicine either singly or in combination with other medicinal herbs. Its widespread ethnomedicinal application is attributed largely to the presence of numerous bioactive secondary metabolites with diverse biological activities (Mishra et al., 2023; Degu et al., 2024).

Phytochemical investigations have revealed that *V. amygdalina* contains abundant biologically active compounds such as flavonoids, alkaloids, tannins, saponins, terpenoids, phenolic compounds, cardiac glycosides, steroidal glycosides, sesquiterpene lactones, and anthraquinones. Several specific compounds including vernodalin, vernolepin, vernomygdin, luteolin, and vernoamyosides have also been isolated and characterized from the plant. These phytochemicals contribute significantly to the antioxidant, anti-inflammatory, anticancer, antidiabetic, antiparasitic, antifungal, and antibacterial properties associated with the plant (Atinga et al., 2021).

The antibacterial potential of medicinal plants has become increasingly important in view of the alarming rise in multidrug-resistant bacterial pathogens. Antimicrobial resistance is currently recognized as one of the most serious public health threats worldwide, contributing substantially to morbidity, mortality, and healthcare costs. Plant-derived antimicrobial compounds are being explored as alternative or complementary therapeutic agents because many exhibit broad-spectrum antibacterial activity and multiple mechanisms of action, thereby reducing the likelihood of resistance development (Angelini, 2024). In this regard, *V. amygdalina* has demonstrated inhibitory activity against several clinically important Gram-positive and Gram-negative bacteria, including *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, and *Klebsiella pneumoniae* (Onifade et al., 2024).

Despite the growing evidence supporting the therapeutic potential of *V. amygdalina*, the safety profile of the plant remains an important concern. Although traditional usage suggests relative safety, scientific validation through toxicological evaluation is essential before its incorporation into standardized pharmaceutical formulations. Acute oral toxicity studies provide critical information regarding the safety margin, lethal dose ($LD_{50}$), and possible adverse effects associated with plant extracts. Toxicological assessments are particularly important because bioactive phytochemicals, while therapeutically beneficial, may exert toxic effects at higher concentrations or with prolonged exposure (Olubunmi et al., 2024).

In addition, variations in extraction solvents, geographical origin, environmental conditions, and processing methods may influence the phytochemical composition and biological activity of medicinal plants. Therefore, comprehensive investigations integrating phytochemical analysis, antibacterial evaluation, and toxicity assessment are necessary to establish the efficacy and safety of *V. amygdalina* for potential therapeutic applications. Such studies are essential for the development of evidence-based herbal medicines and may contribute significantly to the discovery of novel antimicrobial agents capable of combating resistant bacterial pathogens. Therefore, this study was designed to evaluate the phytochemical constituents and antibacterial activity of *Vernonia amygdalina* extracts against selected bacterial isolates while also assessing the acute oral toxicity profile in experimental animal models. The findings of this study could provide scientific justification for the traditional use of the plant and contribute to the development of safe, effective, and affordable plant-derived antimicrobial agents.

## Materials and Methods

### Plant collection and authentication

Fresh, mature leaves of *Vernonia amygdalina* Delile were collected from Sardauna Road Azare, Bauchi State, Nigeria, during the June 2025 growing season. The plant material was authenticated by Dr. Usman A. M. in the Department of Biological Sciences, Federal University of Health Sciences Azare, where a voucher specimen (Voucher No. FUHSA/V002/2025) was deposited in the departmental herbarium for future reference. The leaves were washed thoroughly under running tap water followed by distilled water to remove adhering soil and debris before processing.

### Preparation of plant extract

The cleaned leaves were shade-dried at ambient laboratory temperature (25–30°C) for approximately two weeks until a constant weight was achieved. The dried samples were pulverized into fine powder using a sterile laboratory grinder and stored in airtight amber containers until extraction.

Five hundred grams of the powdered plant material were extracted with distilled water and 80% methanol using cold maceration for 72 h with intermittent agitation. The extracts were filtered successively through muslin cloth and Whatman No. 1 filter paper before being concentrated under reduced pressure using a rotary evaporator at temperatures below 40°C. The concentrated extracts were further dried in a hot-air oven (40°C) to obtain a constant weight (Edeoga et al., 2005).

The dried crude extract was stored in sterile amber bottles at 4°C until required for further analyses.

### Preliminary phytochemical screening

Qualitative phytochemical screening of the crude extract was carried out to detect the presence of alkaloids, flavonoids, tannins, saponins, terpenoids, steroids, cardiac glycosides, phenolic compounds and anthraquinones using standardized phytochemical procedures described by Sofowora (2008) and Trease & Evans (2009). Appropriate positive and negative controls were included throughout the analyses. Each assay was performed in triplicate to ensure reproducibility.

### Bacterial isolates

Clinical bacterial isolates comprising *Staphylococcus aureus*, *Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa* and *Salmonella typhi* were obtained from the Microbiology Laboratory of Extreme Hospital Azare, Bauchi State, Nigeria. Ethical approval for the use of clinical isolates was obtained from the Research Ethics Committee (Approval No. EXT/233.2A).

All animal experiments were approved under the same committee's approval number (EXT/233.2A-II), and carried out following ARRIVE guidelines

The isolates were subcultured on nutrient agar and confirmed using colonial morphology, Gram staining and conventional biochemical tests (including catalase, coagulase, urease, indole, oxidase, citrate and nitrate tests) following contemporary clinical microbiology procedures and standardized biochemical identification guidelines (Dunn, 2023). Pure cultures were maintained on nutrient agar slants at 4°C until use.

## Preparation of bacterial inoculum

Fresh bacterial cultures (18–24 h) were suspended in sterile normal saline and adjusted to the turbidity of a 0.5 McFarland standard, corresponding to approximately $1.5 \times 10^8$ CFU/mL. The inoculum was further diluted according to CLSI recommendations to obtain the required working inoculum for antimicrobial susceptibility testing (CLSI, 2024).

## Antibacterial susceptibility assay

The antibacterial activity of the plant extract was initially evaluated using the agar disc diffusion technique following CLSI recommendations with slight modifications. Sterile Mueller–Hinton agar plates were inoculated uniformly using sterile cotton swabs dipped into standardized bacterial suspensions.

Sterile 6-mm Whatman filter paper discs impregnated with predetermined concentrations (12.50, 25.00, 50.00, 100.00 and 200.00 mg/mL) of each of the methanolic and aqueous plant extracts were aseptically placed on the inoculated agar surface. Discs containing the extraction solvent served as negative control, while Erythromycin (10 µg/disc) served as a positive control.

The plates were incubated aerobically at 35 ± 2°C for 18–24 h. Zones of inhibition were measured in millimetres using a digital Vernier caliper (CLSI, 2024). All experiments were performed in triplicate, and the results were expressed as mean ± standard deviation.

## Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration was determined using the broth microdilution method in sterile 96-well microtitre plates according to the Clinical and Laboratory Standards Institute.

Two-fold serial dilutions of the crude extracts (200, 100, 50, 25, 12.5, 6.25, 3.125, 1.562 and 0.781 mg/mL) were prepared in cation-adjusted Mueller–Hinton broth to obtain the required concentration range. Each well received equal volumes of extracts and standardized bacterial suspension, resulting in a final inoculum of approximately $5 \times 10^5$ CFU/mL.

Sterility control wells containing broth only, growth control wells containing broth plus inoculum without extract, solvent control wells, and antibiotic control wells were included.

Following incubation at 35 ± 2°C for 18–20 h, bacterial growth was assessed visually. The MIC was defined as the lowest concentration of the extract showing complete inhibition of visible bacterial growth (CLSI, 2024).

## Determination of minimum bactericidal concentration (MBC)

The minimum bactericidal concentration was determined according to CLSI guidelines. Aliquots (10–20 µL) from wells showing no visible growth during MIC determination were subcultured onto sterile Mueller–Hinton agar plates and incubated aerobically at 35 ± 2°C for 24 h.

The MBC was defined as the lowest extract concentration producing no visible bacterial

colony following incubation, indicating complete bacterial killing (CLSI, 2024). All determinations were carried out in triplicate.

### Acute oral toxicity study

Acute oral toxicity of the crude extract was evaluated following the principles of the Organisation for Economic Co-operation and Development guideline (OECD 423) for acute oral toxicity (OECD, 2001). Healthy adult Wistar albino rats of both sexes weighing 130–200 g were obtained from the animal house of Federal University of Health Sciences Azare.

Animals were acclimatized for seven days under standard laboratory conditions (temperature 22 ± 2°C; relative humidity 50–60%; 12 h light/dark cycle) with unrestricted access to standard feed and clean drinking water.

Prior the administration of the extracts, the animals were kept fasting overnight. The extract was administered orally by gavage at graded doses up to 8000 mg/kg body weight. The first group of the animals (n = 3) received 8000 mg/kg, the second group (n = 3) received 6000 mg/kg, the third group (n = 3) received 4000 mg/kg, the fourth group (n = 3) received 2000 mg/kg, the fifth group (n = 3) received 1000 mg/kg and the sixth group (n = 3) received 500 mg/kg. A group ($7^{th}$) (n = 3) served as control group received distilled water. The animals were observed continuously during the first 4 h after administration and periodically thereafter for 24 h, followed by daily observations for 14 consecutive days. Clinical signs including changes in behaviour, locomotion, salivation, piloerection, tremors, convulsions, respiratory distress, diarrhoea, food intake, water consumption, body weight and mortality were recorded (Magaji et al., 2025).

The median lethal dose ($LD_{50}$) was estimated based on mortality and clinical observations. All animal procedures complied with institutional ethical guidelines for laboratory animal care and use.

### Statistical analysis

All experiments were performed independently in triplicate. Data were expressed as mean ± standard deviation (SD). Statistical analyses were conducted using SPSS version 23.0 (IBM Corp., Armonk, NY, USA). Comparisons among treatment groups were performed using one-way analysis of variance (ANOVA). Differences were considered statistically significant at $P < 0.05$.

## Results

### Phytochemical composition of *Vernonia amygdalina* extracts

The qualitative phytochemical screening revealed that both methanolic and aqueous extracts of *Vernonia amygdalina* contained similar classes of secondary metabolites. Alkaloids, saponins, tannins, cardiac glycosides, flavonoids, terpenoids, and reducing sugars were detected in both extracts (Table 1), indicating that the extraction solvents recovered comparable phytochemical constituents.

**Table 1:** Phytochemical composition of *V. amygdalina* methanolic and aqueous extracts

| Compositions | Methanolic extract | Aqueous extract |
|---|---|---|
| Alkaloids | + | + |
| Saponins | + | + |
| Tannins | + | + |
| Cardiac glycosides | + | + |
| Flavonoids | + | + |
| Terpenoids | + | + |
| Reducing sugar | + | + |

**Note: "**+" = Present, "-" = Absent

### Antibacterial activity of *Vernonia amygdalina* extracts

Both methanolic and aqueous extracts exhibited concentration-dependent antibacterial activity against all five tested bacterial isolates (Table 2). Generally, inhibition zones increased significantly with increasing extract concentration from 12.5 to 200 mg/mL (Table 2).

The methanolic extract consistently produced larger inhibition zones than the aqueous extract across all tested concentrations. At the highest concentration (200 mg/mL), the methanolic extract demonstrated the greatest activity against

*Staphylococcus aureus* (28.00 ± 1.00 mm), followed by *Klebsiella pneumoniae* (27.17 ± 1.26 mm), *Pseudomonas aeruginosa* (25.00 ± 0.80 mm), *Escherichia coli* (24.17 ± 0.39 mm), and *Salmonella typhi* (23.00 ± 0.00 mm) (Table 2).

Similarly, the aqueous extract showed maximum inhibition at 200 mg/mL, with inhibition zones of 21.00 ± 0.70 mm, 21.67 ± 0.58 mm, 20.00 ± 0.00 mm, 18.00 ± 0.00 mm, and 18.00 ± 0.00 mm against *S. aureus*, *K. pneumoniae*, *P. aeruginosa*, *E. coli*, and *S. typhi*, respectively (Table 2).

Analysis of variance demonstrated a statistically significant difference in antibacterial activity between the methanolic and aqueous extracts ($F = 6.828$, $p = 0.012$), indicating superior antibacterial efficacy of the methanolic extract (Table 2).

### Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The MIC values of the methanolic extract ranged from 1.56 to 3.12 mg/mL, whereas the aqueous extract required relatively higher concentrations ranging from 3.12 to 6.25 mg/mL to inhibit bacterial growth (Table 3).

The lowest MIC (1.56 mg/mL) for the methanolic extract was observed against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, while the highest MIC (3.12 mg/mL) was recorded against *Salmonella typhi*. In contrast, the aqueous extract exhibited MIC values of 3.12 mg/mL against *S. aureus*, *K. pneumoniae*, and *P. aeruginosa*, increasing to 6.25 mg/mL against *S. typhi*.

Statistical analysis showed a significant difference in MIC values between the two extracts ($F = 9.139$, $p = 0.016$), with the methanolic extract demonstrating significantly greater antibacterial potency (Table 3).

The MBC values for the methanolic extract ranged between 50 and 100 mg/mL, while those of the aqueous extract ranged from 50 to 200 mg/mL. The methanolic extract achieved bactericidal activity at 50 mg/mL against *S. aureus* and *P. aeruginosa*, whereas concentrations of 100 mg/mL were required against *E. coli*, *K. pneumoniae*, and *S. typhi*. For the aqueous extract, bactericidal concentrations varied between 50 and 200 mg/mL. However, no statistically significant difference was observed in MBC values between the two extracts ($F = 2.381$, $p = 0.161$) (Table 3).

### Acute Toxicity Assessment

The oral acute toxicity assessment results for both methanolic and aqueous extracts of *V. amygdelina* showed no mortality across all the dosses administered. Although, adverse physiological changes were observed in experimental animals administered with the highest dose tested (8000 mg/kg) of both extracts (Table 4). Parameters such as respiration rate and behavior of the animals changed at 8000 mg/kg, indicating that, the extracts are safe at relatively low doses.

## Discussion

### Phytochemical Composition of *V. amygdalina*

The present study demonstrated that both methanolic and aqueous extracts of *Vernonia amygdalina* contained alkaloids, flavonoids, tannins, saponins, terpenoids, cardiac glycosides and reducing sugars. These findings corroborate previous reports describing *V. amygdalina* as a rich source of diverse phytochemicals responsible for its extensive medicinal applications (Magaji et al., 2023; Yunusa et al., 2024). The phytochemical profile observed in this study is also consistent with that reported by Tordzagla et al. (2024), who identified similar bioactive compounds in Ghanaian *V. amygdalina* leaf extracts and attributed their antibacterial activity to the synergistic interaction of these secondary metabolites.

The antibacterial activity observed in the *Vernonia amygdalina* extract may be attributed, at least in part, to the phytochemical constituents detected, including flavonoids, tannins, alkaloids, saponins, terpenoids and cardiac glycosides. Previous studies have proposed several mechanisms by which these classes of compounds exert antimicrobial effects. Flavonoids have been reported to disrupt

bacterial cell membranes, inhibit nucleic acid synthesis and interfere with energy metabolism. Tannins are thought to precipitate proteins, disrupt bacterial cell wall integrity and inhibit microbial enzymes. Alkaloids have been associated with inhibition of DNA replication, protein synthesis and other essential cellular processes. Saponins are proposed to increase membrane permeability, leading to leakage of intracellular contents, whereas terpenoids may interfere with membrane integrity and cellular respiration. Cardiac glycosides have also been reported to exhibit antimicrobial activity through mechanisms that may involve disruption of membrane function and interference with key metabolic pathways.

**Table 2:** Antibacterial activity of *V. amygdalina* methanolic and aqueous extracts

| Organisms | | *S. aureus* | *E. coli* | *K. pneumonia* | *P. aeruginosa* | *S. typhi* |
|---|---|---|---|---|---|---|
| Methanolic | 12.5 mg/mL | 8.67 ± 0.58 | 8.00 ± 0.00 | 9.33 ± 0.39 | 8.17 ± 0.39 | 7.00 ± 0.00 |
| Extract | 25 mg/mL | 13.00 ± 0.00 | 11.67 ± 0.58 | 13.00 ± 1.00 | 12.00 ± 0.00 | 10.00 ± 1.00 |
| | 50 mg/mL | 18.33 ± 0.58 | 15.00 ± 1.00 | 17.00 ± 0.00 | 17.67 ± 0.58 | 14.00 ± 0.60 |
| | 100 mg/mL | 22.67 ± 0.58 | 20.17 ± 1.26 | 22.67 ± 0.58 | 21.00 ± 0.60 | 18.03 ± 0.71 |
| | 200 mg/mL | 28.00 ± 1.00 | 24.17 ± 0.39 | 27.17 ± 1.26 | 25.00 ± 0.80 | 23.00 ± 0.00 |
| Aqueous | 12.5 mg/mL | 6.00 ± 1.00 | 5.67 ± 0.58 | 6.00 ± 0.00 | 5.00 ± 0.00 | 5.00 ± 1.00 |
| Extract | 25 mg/mL | 9.67 ± 0.58 | 7.00 ± 0.00 | 9.00 ± 0.00 | 8.00 ± 1.00 | 7.00 ± 0.00 |
| | 50 mg/mL | 13.00 ± 0.60 | 10.33 ± 0.39 | 13.67 ± 0.58 | 12.33 ± 0.39 | 10.17 ± 0.39 |
| | 100 mg/mL | 17.00± 00 | 14.67 ± 0.58 | 17.00 ± 1.00 | 16.00 ± 0.00 | 14.00 ± 0.00 |
| | 200 mg/mL | 21.00 ± 0.70 | 18.00± 00 | 21.67 ± 0.58 | 20.00 ± 0.00 | 18.00 ± 0.00 |
| Control | + | 27.00 | 26.00 | 28.00 | 26.00 | 24.00 |
| | - | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| F | 6.828 | | | | | |
| P | 0.012* | | | | | |

Key: * is significant at $p < 0.05$

**Table 3:** Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of *V. amygdalina* methanolic and aqueous extracts

| Organisms | Range tested (mg/mL) | Minimum Inhibitory Concentration (mg/mL) | | | | Minimum Bactericidal Concentration (mg/mL) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | ME | AE | F | P | ME | AE | F | P |
| *Staphylococcus aureus* | 1.56 – 200 | 1.56 | 3.12 | 9.139 | 0.016* | 50 | 50 | 2.381 | 0.161 |
| *Escherichia coli* | 1.56 – 200 | 3.12 | 6.25 | | | 100 | 200 | | |
| *Klebsiella pneumonia* | 1.56 – 200 | 3.12 | 3.12 | | | 100 | 100 | | |
| *Pseudomonas aeruginosa* | 1.56 – 200 | 1.56 | 3.12 | | | 50 | 100 | | |
| *Salmonella typhi* | 1.56 – 200 | 3.12 | 6.25 | | | 100 | 200 | | |

Key: * is significant at $p < 0.05$, ME: Methanol extract, AE: aqueous extract

**Table 4:** Oral Acute Toxicity of *V. amygdalina* methanolic and aqueous extracts

| | Methanolic Extracts | | | Aqueous Extracts | | |
|---|---|---|---|---|---|---|
| Dose ($mgkg^{-1}$) | Mortality | Behavioral changes | Clinical Signs | Mortality | Behavioral changes | Clinical Signs |
| 8000 | 0/3 | Restlessness | Respiratory distress | 0/3 | Restlessness | Respiratory distress |
| 6000 | 0/3 | No change | - | 0/3 | No change | - |
| 4000 | 0/3 | No change | - | 0/3 | No change | - |
| 2000 | 0/3 | No change | - | 0/3 | No change | - |
| 1000 | 0/3 | No change | - | 0/3 | No change | - |
| 500 | 0/3 | No change | - | 0/3 | No change | - |
| Distilled water | 0/3 | No change | - | 0/3 | No change | - |

Note: Clinical signs include; Respiration Rate, Urination, Body Temperature, Eye Color, Body Weight, and Food Intake; + : Changed; - : Not Changed

However, these mechanisms were not investigated experimentally in the present study and are presented only as plausible explanations based on the known biological activities of the phytochemical classes identified in the extract. Further mechanistic studies are required to confirm the specific pathways responsible for the antibacterial activity of *V. amygdalina* observed in this investigation.

## Antibacterial Activity of Methanolic and Aqueous Extracts of *V. amygdalina*

Both methanolic and aqueous extracts exhibited concentration-dependent antibacterial activity against all tested bacterial isolates. The progressive increase in inhibition zones with increasing extract concentration suggests that antibacterial efficacy depends on the quantity of bioactive constituents available to interact with bacterial cells. Similar concentration-dependent inhibition has been consistently reported for medicinal plant extracts and reflects cumulative interactions between multiple antimicrobial compounds (Tordzagla et al., 2024). Unlike conventional antibiotics that often possess a single molecular target, phytochemicals act through several complementary mechanisms, thereby reducing the likelihood of resistance development (Cheesman et al., 2017).

The methanolic extract demonstrated significantly greater antibacterial activity than the aqueous extract against all tested organisms. This finding agrees with previous investigations reporting superior antimicrobial efficacy of methanolic extracts of *V. amygdalina* (Adoyi et al., 2025; Tordzagla et al., 2024). Methanol possesses intermediate polarity, enabling efficient extraction of both moderately polar and relatively non-polar phytochemicals including flavonoids, phenolic acids, terpenoids and sesquiterpene lactones. In contrast, water primarily extracts highly polar constituents, resulting in comparatively lower recovery of lipophilic antimicrobial compounds (Do et al., 2014). Consequently, methanolic extracts usually contain higher concentrations of biologically active metabolites capable of exerting stronger antibacterial effects.

Among the tested organisms, *Staphylococcus aureus* exhibited the highest susceptibility whereas *Salmonella typhi* showed comparatively lower susceptibility. This observation agrees with several previous studies reporting greater sensitivity of Gram-positive bacteria to plant-derived antimicrobials than Gram-negative bacteria (Magaji et al., 2023; Tordzagla et al., 2024). The increased susceptibility of *S. aureus* may be explained by structural differences in bacterial cell envelopes. Gram-positive

organisms possess a thick peptidoglycan layer but lack the outer lipopolysaccharide membrane characteristic of Gram-negative bacteria. The absence of this permeability barrier facilitates diffusion of phytochemicals into the bacterial cytoplasm where they interfere with essential metabolic processes (Silhavy et al., 2010).

Conversely, Gram-negative bacteria such as *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Salmonella typhi* possess an additional outer membrane rich in lipopolysaccharides that restricts penetration of many antimicrobial compounds. These organisms also express multidrug efflux pumps, porin modifications and enzymatic resistance mechanisms that reduce intracellular accumulation of antibacterial agents (Breijyeh et al., 2020). Nevertheless, the appreciable inhibition observed against these organisms indicates that *V. amygdalina* contains bioactive compounds capable of overcoming some intrinsic resistance mechanisms. This observation is particularly important considering the increasing prevalence of multidrug-resistant Gram-negative pathogens worldwide (Murray et al., 2022).

The MIC values obtained in this study further demonstrated the superior potency of the methanolic extract. The relatively low MIC values (1.562–3.125 mg/mL) indicate that small concentrations of the extract were sufficient to inhibit bacterial growth. Similar MIC ranges have been reported by Tordzagla et al. (2024), who observed MIC values of 1.562–3.125 mg/mL against *S. aureus*, *K. pneumoniae*, *E. coli* and *P. aeruginosa*. The close agreement between the two studies suggests consistency in the antibacterial potential of *V. amygdalina* despite differences in geographical origin.

The present findings have important implications for combating antimicrobial resistance (AMR). The World Health Organization has identified AMR as one of the most serious global public health threats because of increasing resistance among clinically important pathogens including *S. aureus*, *K. pneumoniae*, *P. aeruginosa* and *E. coli* (WHO, 2024). Plant-derived antimicrobials such as *V. amygdalina* represent promising alternative or complementary therapeutic agents because they possess multiple bioactive compounds acting through diverse molecular pathways. Such multitarget mechanisms reduce selective pressure on individual bacterial proteins and may delay the emergence of resistance compared with conventional antibiotics (Cheesman et al., 2017; WHO, 2024). Furthermore, recent studies have demonstrated synergistic interactions between *V. amygdalina* extracts and conventional antibiotics, suggesting that combination therapy may restore antibiotic susceptibility in resistant bacterial strains (Tordzagla et al., 2024).

The MBC:MIC ratios obtained in this study were generally greater than 4, indicating that the *Vernonia amygdalina* extract exhibited predominantly bacteriostatic rather than bactericidal activity against the tested bacterial isolates. According to established interpretative criteria, an MBC/MIC ratio of ≤4 is indicative of bactericidal activity, whereas values >4 suggest a bacteriostatic effect. This finding implies that the extract is more effective at inhibiting bacterial growth than directly killing bacterial cells. Such activity may still have therapeutic value, particularly when host immune defenses are intact, but it also suggests that caution should be exercised when inferring bactericidal efficacy from the antibacterial results. Further purification of the bioactive constituents or investigation of synergistic combinations with conventional antibiotics may enhance the bactericidal potential of the extract.

## Acute Oral Toxicity

The acute oral toxicity assessment demonstrated that neither the methanolic nor aqueous extract of *Vernonia amygdalina* produced mortality in experimental animals at any of the administered doses, including the highest dose of 8000 mg/kg body weight. These findings indicate that the median lethal dose ($LD_{50}$) is greater than 8000 mg/kg, suggesting a very high margin of safety according to the Organisation for Economic Co-operation and Development (OECD) toxicity classification, where substances with oral $LD_{50}$

values exceeding 5000 mg/kg are regarded as practically non-toxic (OECD, 2001). This observation is consistent with the findings of Yunusa et al. (2024), who similarly reported no mortality in rats following oral administration of methanolic *V. amygdalina* leaf extract at doses up to 5000 mg/kg, concluding that the plant possesses a favourable acute safety profile.

Similarly, previous toxicological investigations on *Vernonia* species have consistently reported high $LD_{50}$ values with no significant mortality following acute oral administration, supporting the traditional use of the plant as a medicinal herb when consumed within therapeutic limits. Earlier work on *Vernonia bipontini* also demonstrated the absence of mortality and major histopathological lesions after repeated administration, further suggesting that members of the genus possess relatively low acute toxicity (Alebachew et al., 2013).

Despite the absence of mortality, animals that received the highest dose (8000 mg/kg) exhibited transient behavioural alterations characterized by restlessness, laboured respiration and audible respiratory sounds. Although these clinical signs resolved without death, they indicate that extremely high doses may elicit reversible toxic or pharmacological responses. Such behavioural manifestations may reflect transient effects on the central nervous system or respiratory system resulting from high systemic concentrations of biologically active phytochemicals. Sesquiterpene lactones, alkaloids and saponins, which are abundant in *V. amygdalina*, have been reported to exert dose-dependent physiological effects capable of altering neuromuscular activity, autonomic function and membrane permeability. At excessive concentrations, these compounds may temporarily stimulate or depress respiratory centres, induce smooth muscle responses or alter airway secretions, thereby contributing to the observed respiratory distress. This is supported by the findings of Tordzagla et al. (2024), who mild behavioral changes at 5000 mg/kg.

The observed restlessness may also represent an adaptive physiological response to the bitter sesquiterpene lactones and other secondary metabolites present in the extract. These compounds have diverse pharmacological activities, and at supratherapeutic doses may stimulate sympathetic activity or induce transient stress responses before metabolic detoxification occurs. Likewise, the respiratory difficulty and audible breathing could be associated with temporary irritation of the respiratory tract, mild bronchoconstriction or increased respiratory effort secondary to systemic pharmacological effects rather than irreversible tissue injury. Since no mortality occurred and the animals survived throughout the observation period, these responses appear to represent reversible signs of acute toxicity rather than lethal toxic effects.

The acute oral toxicity findings suggest that the *Vernonia amygdalina* extract has relatively low acute toxicity under the conditions of this study. No mortality was observed at any of the administered doses, although transient behavioural changes, including restlessness and laboured respiration, were noted at the highest dose, indicating that mild dose-related effects may occur at very high exposure levels. These findings are consistent with a favourable acute safety profile and suggest a potentially wide therapeutic window. However, this conclusion should be interpreted with caution, as the present assessment was based solely on mortality and clinical observations. Biochemical, haematological and histopathological evaluations were not performed; therefore, possible subclinical or organ-specific toxic effects cannot be excluded. Further subacute and chronic toxicity studies incorporating comprehensive organ function and histopathological assessments are required to establish the safety profile of the extract more conclusively.

## Limitations of the Study

The present study has several limitations that should be considered when interpreting the findings. The antibacterial activity was evaluated using clinical bacterial isolates without the inclusion of standard reference strains, which

would have enhanced comparability with published studies and strengthened quality assurance. In addition, no conventional antibiotic was included as a positive control during the MIC and MBC assays, limiting direct comparison of the antibacterial efficacy of the extract with established antimicrobial agents. The extraction yield of the *Vernonia amygdalina* extract was not determined, preventing assessment of extraction efficiency and limiting reproducibility. Furthermore, the safety evaluation was based solely on an acute oral toxicity study involving mortality and clinical observations, without biochemical, haematological or histopathological assessments; therefore, potential subclinical or organ-specific toxic effects could not be evaluated. Despite these limitations, the study provides valuable preliminary evidence of the phytochemical composition, antibacterial activity and acute safety profile of *V. amygdalina*. Future studies should incorporate standard reference strains, appropriate antibiotic controls, extraction yield determination, comprehensive toxicity evaluations and mechanistic investigations to further validate and extend the present findings.

## Conclusion

The present study demonstrated that methanolic extracts of *Vernonia amygdalina* leaves contain several bioactive phytochemical constituents, including flavonoids, tannins, alkaloids, saponins, terpenoids and cardiac glycosides, and exhibited measurable in vitro antibacterial activity against the bacterial isolates evaluated. The MBC/MIC ratios obtained indicate that the extract exhibited predominantly bacteriostatic rather than bactericidal activity under the experimental conditions employed. The acute oral toxicity assessment revealed no mortality at the tested doses, suggesting relatively low acute toxicity under the conditions of this study; however, this finding should be interpreted cautiously because the safety evaluation was limited to clinical observations without biochemical, haematological or histopathological investigations. These findings indicate that the tested *V. amygdalina* extract possesses antibacterial properties against the bacterial isolates included in this study and merits further investigation. However, the results should not be interpreted as evidence of established therapeutic or clinical efficacy. Future studies should include standard reference bacterial strains, appropriate antibiotic positive controls, determination of extraction yield, detailed mechanistic investigations, and comprehensive subacute and chronic toxicity studies incorporating biochemical, haematological and histopathological assessments to better define the efficacy and safety profile of the extract before considering clinical application.

## Author Contributions

Auwal Magaji designed the work, collected samples and did laboratory analysis, Nathaniel Luka Kwarau prepared the manuscript, Sani Garba and Abdullahi Musa proofread the entire work. While Mohammed Mohammed Usman did grammar check.

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## Conflict of Interests

The authors declare that, there is no conflict of interest

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