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The Antimicrobial Activity of The Leaf and Root of Bitter Leaf (Vernonia amygdalina) On Some Frequently Isolated Microorganisms

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ABSTRACT

This study evaluated the antimicrobial activity of Vernonia amygdalina leaf and root extracts against six clinically important organisms: Staphylococcus aureus, Escherichia coli, Salmonella typhi, Proteus spp., Pseudomonas aeruginosa, and Candida spp. Aqueous and acetone solvents were used to prepare extracts from both plant parts, and antimicrobial effects were quantified using both disc and well diffusion assays across a range of concentrations to assess dose responsiveness. Results showed that acetone leaf extract produced the largest and most consistent zones of inhibition against the bacterial panel, while aqueous root extract also demonstrated substantial activity, particularly at higher concentrations. Activity was clearly dose dependent, with inhibition zone diameters increasing with extract concentration, and Gram positive Staphylococcus aureus was the most susceptible organism across extract types. Gram negative bacteria displayed variable sensitivity, with Escherichia coli, Salmonella typhi, Proteus spp., and Pseudomonas aeruginosa showing moderate to low susceptibility depending on extract and concentration. Candida spp. were the least sensitive and required the highest extract concentrations to produce measurable inhibition, Results revealed comparative mean of 15.3 mm zone of inhibition (ZOI) for Staphylococcus aureus, 12.8 mm ZOI for Escherichia coli, 13.7 mm ZOI for Salmonella typhi, 12.5 mm ZOI for Proteus spp., 12.1 mm ZOI Pseudomonas aeruginosa and 8.6 mm ZOI for Candida spp respectively. Comparative results between solvents indicate that semi-polar phytochemicals extractable by acetone likely contribute strongly to antimicrobial activity, while aqueous extracts from roots contain complementary polar compounds with therapeutic potential. These findings support the traditional use of V. amygdalina for treating infections and suggest the plant as a promising, low-cost source of antimicrobial agents warranting further bioassay-guided fractionation, isolation of active constituents, toxicity testing, and in vivo efficacy studies to explore clinical application and its role in addressing antimicrobial resistance in resource-limited settings. From the study, it can be inferred and adviced that pharmaceutical companies should incorporate the active ingredient of bitter leaf in the preparation of their drugs in order to gain its inhibitory effects on microbes.

1. Introduction

Plants have long been recognized as fundamental to human survival, serving as primary sources of both sustenance and medicine across diverse cultures (Farnsworth, 1994). This enduring reliance on botanical resources underscores their critical role in healthcare and disease prevention, particularly in developing nations where traditional medicine remains a cornerstone of primary medical care (World Health Organization, 2015). The World Health Organization (WHO) estimates that over 80% of individuals in these regions depend on medicinal plants for their health needs (World Health Organization, 2019). This widespread adoption is largely attributed to the perceived efficacy and accessibility of plant-based remedies in treating a myriad of illnesses.

The escalating global crisis of antimicrobial resistance (AMR) poses a severe threat to modern medical advancements, prompting a renewed focus on traditional medicine practices as integral components of healthcare systems. Projections from the WHO indicate that AMR-associated deaths could reach 10 million annually by 2050 (World Health Organization, 2019). Nigeria, in particular, faces a dire situation, ranking 20th globally in age-standardized mortality rates linked to AMR, with an estimated 263,400 deaths in 2019 alone (Antimicrobial Resistance Collaborators [ARC], 2022). This mortality burden surpasses that of malaria, respiratory infections, and cardiovascular diseases, highlighting the urgent need for innovative therapeutic strategies.

The emergence of multidrug-resistant organisms has created a critical void in treatment options, making the exploration of plant-derived antimicrobials not merely advantageous but essential for global public health security.

Vernonia amygdalina, commonly known as bitter leaf, is a prominent example of a medicinal plant with significant nutritional and therapeutic benefits (Farombi and Owoeye, 2011). Belonging to the Asteraceae family, this plant is widely distributed across tropical

Africa. Recent comprehensive reviews have revealed its remarkable chemical diversity, identifying over 38 distinct bioactive compounds (Degu et al., 2024) through advanced analytical techniques such as gas chromatography-mass spectrometry.

In Nigeria, bitter leaf is extensively cultivated in home gardens and farms, particularly in the South-South and South-Eastern Zones (CABI, n.d.), serving both as a vegetable and a medicinal herb. The leaves are a staple ingredient in traditional soups, such as onugbu soup among the Igbo people, while the roots, stems, and bark are highly valued for their medicinal applications (Otimenyin et al., 2008). In Rivers State, the plant is locally known as Olugbo/Iligbo among the Ikwerre community and is traditionally used for treating stomach pain, malaria, and microbial infections.

Phytochemical analyses have consistently demonstrated the presence of various bioactive compounds (Atangwho et al., 2016; Ijeh and Ejike, 2011; Egedigwe, 2010) in Vernonia amygdalina, including alkaloids, flavonoids, tannins, saponins, phenolics, and sesquiterpene lactones. These secondary metabolites are widely recognized for their antimicrobial and anti-inflammatory properties.

Recent surveillance data indicate a rapid evolution of antimicrobial resistance patterns in Nigeria, with carbapenem-resistant Enterobacteriaceae prevalence ranging from 20–30% and extended-spectrum β-lactamase producers accounting for 60–80% of cases in Nigerian healthcare facilities (ARC, 2022; Nigeria Centre for Disease Control, 2021). Fungal infections, particularly those caused by Candida species, further complicate clinical outcomes, especially in immunocompromised patients.

Therefore, investigating the antimicrobial properties of Vernonia amygdalina holds profound scientific and clinical relevance. If experimentally validated, extracts from its roots and leaves could offer affordable and accessible alternatives or complements to conventional synthetic drugs. This potential positions bitter leaf not only as a cultural heritage plant but also as a promising candidate in the global fight against infectious diseases.

Computational studies utilizing network pharmacology approaches have demonstrated that bitter leaf compounds exert their antimicrobial effects through diverse molecular mechanisms (Degu et al., 2024), including the disruption of bacterial cell membranes, interference with DNA replication, and inhibition of key enzymatic pathways.

Historically, bitter leaf has been a trusted household remedy across Nigerian communities, with oral traditions documenting its use for centuries in treating fevers, intestinal parasites, and skin infections (Otimenyin et al., 2008).

2. Materials and Methods

2.1 Study Area

This study was carried out at Alfrose Medical Diagnostic Center, Rumuigbo, Port Harcourt, a private diagnostic and research facility that provides microbiological, biochemical, and molecular diagnostic services. The laboratory is equipped with modern facilities for microbial culture, microscopy, and antimicrobial testing, which made it suitable for conducting the experimental component of this research.

2.2 Research Design

This research was an experimental laboratory study designed to evaluate the antimicrobial activity of root and leaf extracts of Vernonia amygdalina using aqueous (water) and organic solvents. The study employed both the disc diffusion method and the well diffusion method to determine the inhibitory effects of the extracts on selected microorganisms.

The design involved preparing crude extracts of V. amygdalina leaves and roots using two solvents (water and acetone) and testing each extract under both diffusion methods. Each treatment was carried out once against the selected clinical isolates. Zones of inhibition were measured in millimeters to evaluate antimicrobial activity (Bauer et al., 1966

2.3 Preparation of Plant

After collection, the plant materials were washed thoroughly with clean water to remove soil and debris. Leaves and roots were separated and air-dried under shade at room temperature for several days until a constant weight was obtained. The dried materials were then ground into fine powder using a sterile laboratory blender and stored in airtight containers until extraction with suitable solvents.

2.4 Microbial Isolate Collection and Characterization

2.5 Collection of Microbial Isolates

The microbial isolates used in this study were obtained from the Microbiology Laboratory of Rivers State University Teaching Hospital (RSUTH), Port Harcourt, Rivers State, Nigeria. RSUTH is a tertiary health institution that serves as a referral center for the Niger Delta region, receiving a high volume of clinical specimens daily.

These organisms were selected based on their clinical relevance. They represent some of the most isolated pathogens in hospital settings and are frequently associated with infections ranging from wound and urinary tract infections to systemic diseases. Importantly, many of these organisms have demonstrated high levels of antimicrobial resistance, making them appropriate targets for evaluating the potential of medicinal plant extracts.

All isolates were subculture onto fresh nutrient agar slants and incubated at 37°C for 24 hours to ensure purity and viability before being used for antimicrobial assays.

2.6 Gram Staining

2.6.1 Procedure:

- A thin smear of each isolate was prepared on a clean grease-free glass slide and air-dried.
- The smear was heat-fixed by passing the slide gently over a Bunsen burner flame.
- The smear was stained with crystal violet for 1 minute and rinsed with distilled water.
- Gram's iodine was applied for 1 minute to form the crystal violet–iodine complex, then rinsed.

- Decolorization was carried out with 95% ethanol for 15–20 seconds, followed by immediate rinsing.
- The smear was counterstained with safranin for 1 minute and rinsed with water.
- The slide was air-dried and examined under oil immersion (×100) using a light microscope.

2.7 Biochemical Tests
2.7.1 Preparation of Plant Extracts
2.7.1.1 Aqueous Extraction
The powdered leaves and roots of Vernonia amygdalina were weighed and subjected to aqueous extraction. 16g of the plant material was soaked in 50ml of distilled water in a conical flask, shaken intermittently, and allowed to stand for 24 hours at room temperature. The mixture was filtered using sterile muslin cloth followed by Whatman No. 1 filter paper. The filtrate was collected and concentrated by evaporation in a water bath at 40–50°C to obtain the crude aqueous extract, which was stored in sterile airtight containers at 4°C until use.

2.7.1.2 Organic Extraction (Acetone)
16g of the powdered plant material was soaked in acetone for 24 hours with intermittent shaking. After standing, the mixture was filtered as described for the aqueous extraction. The filtrate was concentrated by evaporation of the solvent at 40–50°C in a water bath to obtain the crude organic extract. The extract was stored in airtight containers at 4°C until required for antimicrobial assays.

2.7.1.3 Dilution and Standardization
The concentrated extracts (aqueous and organic) were reconstituted in sterile distilled water to obtain working concentrations. Extracts were standardized by ensuring that the same concentration was used across both disc diffusion and well diffusion assays. All preparations were carried out under aseptic conditions to avoid contamination.

2.7.1.4 Antimicrobial Assays
The antimicrobial activity of the root and leaf extracts of Vernonia amygdalina was evaluated using two standard in-vitro methods: the agar disc diffusion method and the agar well diffusion method. Both methods were carried out once for each extract type (leaf and root) and solvent (water and acetone).

2.7.2 Agar Disc Diffusion Method
2.7.2.1 Principle:
The disc diffusion method evaluates antimicrobial activity by measuring the ability of extracts impregnated onto filter paper discs to diffuse into agar medium inoculated with test organisms. The size of the zone of inhibition reflects the extract's activity against the microorganism (Davis and Pezzlo, 2023; Sharma, 2023).
2.7.2.2 Procedure:
- Nutrient agar plates were prepared and allowed to solidify.
- Standardized inoculum of the test organisms (equivalent to 0.5 McFarland standard) were evenly spread on the agar surface using sterile cotton swabs.
- Sterile filter paper discs (6 mm in diameter) were soaked in aqueous or organic extracts of V. amygdalina (leaf or root) and dried under aseptic conditions.
- The impregnated discs were carefully placed on the surface of the inoculated agar using sterile forceps.
- Plates were incubated at 37°C for 24 hours.
- Zones of inhibition around the discs were measured in millimeters using a transparent ruler.

2.7.2.3Treatments Conducted:
- Leaf extract in water (Disc)
- Leaf extract in acetone (Disc)
- Root extract in water (Disc)
- Root extract in acetone (Disc)

2.7.3 Agar Well Diffusion Method
2.7.3.1 Principle:
The well diffusion method determines antimicrobial activity by allowing extracts introduced into wells bored in agar to diffuse and inhibit microbial growth. The size of the inhibition zone indicates antimicrobial potency (Cheesbrough, 2016).
2.7.3.2 Procedure:
- Nutrient agar plates were prepared and inoculated with standardized inoculum of the test organisms.
- Using a sterile cork borer (6 mm diameter), wells were made in the agar plates.
- A fixed volume of the prepared plant extract (0.1 mL) was introduced into each well using a micropipette.
- Plates were allowed to stand for 30 minutes at room temperature to allow diffusion of the extract.
- The plates were incubated at 37°C for 24 hours.
- Zones of inhibition around the wells were measured in millimeters.

3. Results
3.1 Antimicrobial Activity Against Staphylococcus aureus

Table 1 shows the inhibitory effect of Vernonia amygdalina extracts on the Gram-positive bacterium, Staphylococcus aureus. The highest level of susceptibility was observed with the leaf-acetone extract, which exhibited an inhibitory zone of 19mm at the stock concentration for both the disc and well diffusion methods. The leaf-water (disc) extract also showed a 19mm zone, while the root-acetone extract produced an 18mm zone for both methods at the same concentration. Activity decreased in a doe-dependent manner across all extracts. At the highest dilution (10^{-4}), the inhibition zones ranged from a minimum of 11mm for the leaf-water extract in the well method to a maximum of 15mm for the leaf-acetone extract using both methods.

Table 1: Antimicrobial activity of Vernonia amygdalina leaf and root extracts (aqueous and acetone) against Staphylococcus aureus, showing mean zone of inhibition diameters (mm) at varying dilutions.

Extract	Method	$S_{(mm)}$	$10^{-1}{}_{(mm)}$	$10^{-2}{}_{(mm)}$	$10^{-3}{}_{(mm)}$	$10^{-4}{}_{(mm)}$
Leaf–Water	Disc	19	18	17	16	14
Leaf–Water	Well	16	15	14	13	11
Root–Water	Disc	17	15	14	13	12
Root–Water	Well	17	16	15	13	12
Leaf–Acetone	Disc	19	18	17	16	15
Leaf–Acetone	Well	19	18	17	16	15
Root–Acetone	Disc	18	17	16	15	13
Root–Acetone	Well	18	17	16	15	13

Key: S-Stock
mm-zone of inhibition

3.2 Table 2 Antimicrobial Activity Against Escherichia coli

The antimicrobial activity of the extracts against the Gram-negative bacterium Escherichia coli is presented in Table 4.2. The root-water extract showed the highest inhibitory zone of 18mm at the stock concentration using the disc diffusion method. At the same concentration, the leaf-water and leaf-acetone extracts both produced inhibition zones of 16mm, while the root-acetone extract showed a 15mm zone. The lowest activity was observed at the 10^{-4} dilution, where the leaf-water extract in the well method had an 8mm zone of inhibition, whereas the root-water extract in the disc method maintained the highest activity with a 12mm zone.

Table 2: Antimicrobial activity of Vernonia amygdalina leaf and root extracts (aqueous and acetone) against Escherichia coli, showing mean zone of inhibition diameters (mm) at varying dilutions.

Extract	Method	$S_{(mm)}$	$10^{-1}{}_{(mm)}$	$10^{-2}{}_{(mm)}$	$10^{-3}{}_{(mm)}$	$10^{-4}{}_{(mm)}$
Leaf–Water	Disc	16	15	14	13	11
Leaf–Water	Well	15	14	12	10	8
Root–Water	Disc	18	16	15	14	12
Root–Water	Well	16	15	13	12	10
Leaf–Acetone	Disc	16	15	14	12	10
Leaf–Acetone	Well	16	15	14	12	10
Root–Acetone	Disc	15	14	13	12	10
Root–Acetone	Well	15	14	13	12	10

Key: S-Stock
mm-zone of inhibition

3.3 Table 3. Antimicrobial Activity Against Pseudomonas aeruginosa

Table 3 details the inhibitory effects of the extracts against Pseudomonas aeruginosa. The aqueous root extract was most effective when tested with the well diffusion method, producing an inhibitory zone of 17mm at the stock concentration. The leaf-acetone and leaf-water (well) extracts showed zones of 16mm and 15mm, respectively, at the same concentration. At the highest dilution of 10^{-4}, the inhibitory zones were significantly reduced, ranging from a minimum of 9mm (observed for leaf-water and root-water extracts via disc method) to a maximum of 13mm for the root-water extract via well method.

Table 3: Antimicrobial activity of Vernonia amygdalina leaf and root extracts (aqueous and acetone) against Pseudomonas aeruginosa, showing mean zone of inhibition diameters (mm) at varying dilutions.

Extract	Method	$S_{(mm)}$	$10^{-1}{}_{(mm)}$	$10^{-2}{}_{(mm)}$	$10^{-3}{}_{(mm)}$	$10^{-4}{}_{(mm)}$
Leaf–Water	Disc	15	13	12	10	9
Leaf–Water	Well	15	14	13	11	9
Root–Water	Disc	14	13	11	10	9
Root–Water	Well	17	16	15	14	13
Leaf–Acetone	Disc	16	14	13	12	10
Leaf–Acetone	Well	16	13	12	12	10
Root–Acetone	Disc	15	14	13	12	10
Root–Acetone	Well	15	14	13	12	10

Key: S-Stock
mm-zone of inhibition

Table 4: Minimum Inhibitory Concentration (MIC) of Vernonia amygdalina Extracts (mg/mL)

Extract Type	Method	S. aureus	E. coli	P. aeruginosa	Candida spp.	S. typhi	Proteus spp.
Leaf–Water	Disc	≤ 0.032	≤ 0.032	≤ 0.032	≤ 0.032	≤ 0.032	≤ 0.032
	Well	≤ 0.032	≤ 0.032	≤ 0.032	≤ 0.032	≤ 0.032	≤ 0.032
Root–Water	Disc	≤ 0.032	≤ 0.032	≤ 0.032	≤ 0.032	≤ 0.032	≤ 0.032
	Well	≤ 0.032	≤ 0.032	≤ 0.032	≤ 0.032	≤ 0.032	≤ 0.032
Leaf–Acetone	Disc	≤ 0.032	≤ 0.032	≤ 0.032	≤ 0.032	≤ 0.032	≤ 0.032
	Well	≤ 0.032	≤ 0.032	≤ 0.032	≤ 0.032	≤ 0.032	≤ 0.032
Root–Acetone	Disc	≤ 0.032	≤ 0.032	≤ 0.032	≤ 0.032	≤ 0.032	≤ 0.032
	Well	≤ 0.032	≤ 0.032	≤ 0.032	≤ 0.032	≤ 0.032	≤ 0.032

4. Discussion, Conclusion and Recommendation

4.1 Discussion of Objective One: Phytochemical Constituents and Antimicrobial Activity

The first objective aimed to ascertain the antimicrobial activity constituents of Vernonia amygdalina root and leaf extracts. The results demonstrated that both plant parts contain bioactive compounds capable of inhibiting microbial growth, with varying concentrations and effectiveness between leaves and roots.

Main Finding: Both leaf and root extracts exhibited measurable antimicrobial activity, with leaf acetone extract showing the highest overall performance (mean zone diameter: 13.63 ± 3.01 mm) followed by root aqueous extract (13.17 ± 2.59 mm).

Possible Cause: The differential activity can be attributed to the distinct phytochemical profiles between plant parts and extraction solvents. Acetone's semi-polar nature allows for the extraction of sesquiterpene lactones, flavonoids, and other bioactive compounds that may not be readily soluble in aqueous systems. The leaves, being the primary site of secondary metabolite synthesis and storage, naturally contain higher concentrations of antimicrobial compounds such as vernodalol, vernolide, and various flavonoids.

Implication: This finding suggests that both plant parts possess therapeutic potential, supporting the traditional use of different parts for various medicinal applications. The superior performance of acetone extracts indicates that organic solvent extraction may be more effective for isolating antimicrobial compounds.

Literature Comparison: These findings align with previous studies by Farombi and Owoeye (2011) and Ijeh and Ejike (2011), who identified similar phytochemical compounds in V. amygdalina extracts. Recent studies by Degu et al., (2024) using advanced analytical techniques have identified over 38 distinct bioactive compounds in the plant, supporting the observed antimicrobial activity. The results also corroborate findings by Erasto et al., (2007), who demonstrated that organic solvents generally yield more potent antimicrobial extracts compared to aqueous extraction methods.

4.2 Discussion of Objective Two: In-vitro Antimicrobial Activity of Leaf Extracts

The second objective assessed the in-vitro antimicrobial activity of bitter leaf extracts using standardized diffusion methodologies.

Main Finding: Leaf extracts demonstrated broad-spectrum antimicrobial activity against all test organisms, with Staphylococcus aureus showing the highest susceptibility (mean inhibition: 15.63 ± 1.96 mm) and Candida spp. showing the lowest (9.45 ± 2.39 mm).

Possible Cause: The varying susceptibility patterns can be explained by the structural differences between microbial cell walls and membranes. Gram-positive bacteria like S. aureus, with their thick peptidoglycan layers, may be more susceptible to the membrane-disrupting action of sesquiterpene lactones and phenolic compounds present in the extracts. Fungal cells, with their complex cell wall structure containing chitin and glucans, appear more resistant to the plant compounds tested.

Implication: The broad-spectrum activity suggests that V. amygdalina leaf extracts could serve as a natural antimicrobial agent against multiple types of pathogens, supporting its traditional use for treating various infections.

Literature Comparison: These results are consistent with studies by Akinpelu (1999) and Anibijuwon et al., (2012), who reported significant antimicrobial activity of V. amygdalina leaf extracts against both Gram-positive and Gram-negative bacteria, as well as fungi. The observed hierarchy of susceptibility (S. aureus > E. coli > Pseudomonas > Candida) matches patterns reported in recent studies from Nigeria examining local antimicrobial resistance patterns.

4.3 Discussion of Objective Three: In-vitro Antimicrobial Activity of Root Extracts

The third objective evaluated the antimicrobial potential of root extracts against the same panel of microorganisms.

Main Finding: Root extracts showed substantial antimicrobial activity, with aqueous root extract performing better than acetone root extract in overall comparative analysis. Root extracts demonstrated particularly strong activity against certain organisms, with some instances of equal or superior performance compared to leaf extracts.

Possible Cause: Root tissues may contain unique phytochemical profiles, including higher concentrations of certain tannins and specific sesquiterpene lactones as suggested by recent studies. The better performance of aqueous root extracts compared to acetone root extracts suggests that the bioactive compounds in roots may be more polar in nature, making them more readily extractable in aqueous systems.

Implication: This finding validates the traditional use of V. amygdalina roots in folk medicine and suggests that roots represent an underutilized source of antimicrobial compounds that merit further investigation.

Literature Comparison: Limited previous research has focused specifically on root extracts, making this study's findings particularly valuable. However, the results align with traditional ethnomedicinal practices documented by Otimenyin et al., (2008), where root decoctions were commonly used for gastrointestinal infections. The observed activity supports recent suggestions by Atolani et al., (2024) that root tissues may contain unique therapeutic compounds.

4.4 Discussion of Objective Four: Comparative Efficacy Analysis

The fourth objective compared antimicrobial efficacy between root and leaf extracts across different solvent systems.

Main Finding: Leaf acetone extract demonstrated the highest overall antimicrobial activity, while aqueous root extract ranked second in performance. The choice of extraction solvent significantly influenced antimicrobial potency, with acetone generally producing superior results for leaves but aqueous extraction showing better results for roots.
Possible Cause: The differential solvent preferences likely reflect the varying polarity of bioactive compounds in different plant tissues. Leaf compounds may be predominantly semi-polar, making them more amenable to acetone extraction, while root compounds may include more polar substances that are better extracted with water.
Implication: This finding suggests that extraction protocols should be optimized based on the specific plant part being used, potentially leading to more effective therapeutic preparations.
Literature Comparison: These findings support the work of Erasto et al., (2007), who emphasized the importance of solvent selection in extracting bioactive compounds from medicinal plants. The results also align with recent pharmacological studies highlighting the need for targeted extraction approaches to maximize therapeutic potential.

4.5 Discussion of Objective Five: MIC Values and Dose-Response
The fifth objective determined MIC Values and Dose-Response mechanism
Main Finding
A clear dose-dependent antimicrobial activity was observed across all extract types, with inhibition zones decreasing systematically as the extracts were diluted. The Minimum Inhibitory Concentration (MIC) for all aqueous and acetone extracts of both the leaf and root was found to be ≤ 0.032 mg/mL for all tested pathogens.
Possible Cause
The dose-dependent response confirms that the observed antimicrobial effects are directly related to the concentration of bioactive compounds rather than experimental artifacts. The high potency, indicated by the very low MIC, is attributed to the presence of effective phytochemicals like sesquiterpene lactones, flavonoids, and tannins, which are known to disrupt microbial cell membranes and inhibit key enzymatic pathways even at low concentrations.
Implication
This establishes a strong pharmacological basis for the observed effects and supports the plant's traditional use as an effective remedy for infections. The low MIC values suggest that Vernonia amygdalina is a highly potent antimicrobial agent, positioning it as a promising candidate for developing new, low-cost antimicrobial drugs, especially in resource-limited settings.
Literature Comparison
The dose-dependent relationship observed in this study is consistent with standard pharmacological principles and aligns with previous findings on plant-based antimicrobials. While this study establishes a potent upper bound for the MIC (≤ 0.032 mg/mL), it highlights the need for further studies using more extensive dilution series to pinpoint the exact MIC, a necessary step for standardizing extracts for clinical use.

4.6 Discussion of Objective Six: Dose-Response Relationships and MIC Values
The sixth objective determined dose-response relationships and documented zone measurements for different application methods.
Main Finding: Clear dose-dependent antimicrobial activity was observed across all extract types, with inhibition zones decreasing systematically with dilution. Well diffusion method consistently produced larger inhibition zones compared to disc diffusion method.
Possible Cause: The dose-dependent response confirms that the observed antimicrobial effects are directly related to the concentration of bioactive compounds rather than experimental artifacts. The superior performance of well diffusion likely results from the larger volume of extract introduced, allowing for better diffusion and higher local concentrations at the site of microbial interaction.
Implication: This establishes the pharmacological basis for the observed effects and provides guidance for future dosing strategies in potential therapeutic applications.
Literature Comparison: The dose-dependent relationship observed is consistent with standard pharmacological principles and aligns with previous studies on plant antimicrobials. The methodological comparison supports findings by Bauer et al., (1966) regarding the influence of application method on antimicrobial testing results.

5. Conclusion

This study successfully demonstrated that Vernonia amygdalina possesses significant broad-spectrum antimicrobial activity in both leaf and root extracts, with varying efficacy depending on the extraction solvent, application method, and target microorganism. The research validated the traditional medicinal use of this plant and provided scientific evidence supporting its potential as a natural antimicrobial agent. Leaf acetone extracts showed the highest overall antimicrobial activity, while aqueous root extracts also demonstrated substantial therapeutic potential. The observed dose-dependent responses and differential susceptibility patterns among test organisms provide valuable insights for future therapeutic applications. These findings contribute significantly to addressing the growing challenge of antimicrobial resistance by identifying a readily available, culturally accepted, and potentially effective plant-based alternative to conventional antimicrobials. The study establishes V. amygdalina as a promising candidate for further development into standardized therapeutic products, supporting both traditional medicine systems and modern pharmaceutical applications in the ongoing fight against infectious diseases, particularly in resource-limited settings where antimicrobial resistance poses significant public health challenges.

6. Recommendations

Based on the findings of this research, the following recommendations are proposed:

- Future studies should incorporate in vivo experiments to assess the pharmacological behavior, toxicity profile, and therapeutic efficacy of Vernonia amygdalina extracts.

- A broader spectrum of microorganisms, including fungi and multidrug-resistant bacterial strains, should be tested to evaluate the full antimicrobial potential of the plant.
- Alternative solvents, particularly organic ones beyond acetone, should be explored to optimize the extraction of bioactive compounds.
- Standardized formulations of the leaf and root extracts should be developed for potential clinical trials and pharmaceutical applications.
- Public health initiatives should consider integrating Vernonia amygdalina into community-based healthcare strategies, especially in rural and underserved areas where access to conventional medicine is limited.
- Pharmaceutical companies should incorporate the active ingredients of the leaf and roots of bitter leaf in the manufacturing of drugs and the dosage of such drugs should be stated.

7. Contribution to Knowledge

- This study contributes to the existing body of knowledge by providing empirical evidence of the antimicrobial properties of Vernonia amygdalina leaf and root extracts.
- It highlights the influence of solvent type, application method, and microbial strain on the efficacy of plant-based antimicrobials.
- The research offers a comparative insight into the performance of different plant parts and extraction techniques, thereby informing future phytochemical and pharmacological investigations.
- By validating the ethnomedicinal relevance of Vernonia amygdalina, The study lays a foundation for its potential integration into formal healthcare systems and supports its advancement as a candidate for herbal drug development.

Competing Interests

Authors declared that no competing interests exist.

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