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ANTIBACTERIAL ACTIVITY OF BITTER LEAF (*Vernonia amygdalina*) EXTRACTS ON *Escherichia coli* AND *Staphylococcus aureus*

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ABSTRACT

In recent years, there has been a growing global health concern, necessitating the search for potent, resistance-free medicinal plants with antibacterial properties. This study evaluates the phytochemical and antibacterial properties of *Vernonia amygdalina* (bitter leaf) extracts as an alternative treatment for bacterial infections. Ethanolic and aqueous extracts of *V. amygdalina* leaves were prepared and subjected to phytochemical screening, revealing the presence of quinones, tannins, steroids, saponins, terpenoids, alkaloids, phenols, and flavonoids. The antibacterial activity was assessed against *Staphylococcus aureus* (gram-positive) and *Escherichia coli* (gram-negative) using the agar well diffusion method. Results showed that the highest inhibition zone (20 mm) was observed for the ethanolic extract at 200 mg/mL against *S. aureus*, while the aqueous extract exhibited a 10 mm inhibition zone against *E. coli* at the same concentration. The aqueous extract demonstrated moderate antibacterial activity, with inhibition zones of 20 mm and 12 mm against *S. aureus* and *E. coli*, respectively. In contrast, gentamicin, a standard antibiotic, produced inhibition zones of 36 mm and 33 mm. The study suggests that *V. amygdalina* exhibits stronger antibacterial activity against gram-positive *S. aureus* than gram-negative *E. coli*, likely due to tannins, saponins, and alkaloids. These findings support the medicinal potential of *V. amygdalina* and provide a scientific basis for its traditional use in bacterial infection treatment. Further research is recommended to isolate and characterize the bioactive compounds responsible for its antimicrobial effects.

Keywords: Antibacterial activity, Antimicrobial resistance, Medicinal plant, Phytochemicals, *Vernonia amygdalina*

INTRODUCTION

Antimicrobial resistance (AMR) has emerged as a pressing global health challenge, significantly threatening the efficacy of conventional antibiotics and complicating the management of infectious diseases. The widespread misuse and overuse of antibiotics in healthcare and agriculture have accelerated the evolution and dissemination of multidrug-resistant (MDR) pathogens, rendering many standard treatments ineffective (Ahmad *et al.*, 2024). As a result, infections caused by drug-resistant bacteria, fungi, and viruses have become increasingly difficult to treat, leading to prolonged illnesses, higher medical costs, and increased mortality rates. Given the urgency of the situation, there is an increasing need to identify novel antimicrobial agents that can serve as alternative therapeutic solutions. Natural sources, particularly medicinal plants, have long been recognized as reservoirs of bioactive compounds with potent antimicrobial properties. The authors submitted further that, many plant-derived compounds, including alkaloids, flavonoids, tannins, and essential oils, have demonstrated significant antibacterial, antifungal, and antiviral activities. These natural products have played a crucial role in both traditional and modern medicine, offering promising leads for new drug development. Harnessing the potential of medicinal plants for antimicrobial discovery could provide sustainable and effective strategies to combat AMR, reducing reliance on conventional antibiotics and mitigating the global threat posed by resistant pathogens. Among these, *Vernonia amygdalina*, commonly referred to as bitter leaf, has garnered considerable attention for its therapeutic applications and pharmacological significance.

Vernonia amygdalina is a widely distributed tropical plant belonging to the Asteraceae family, native to various African regions. It thrives in diverse ecological conditions, making it a readily available medicinal resource. For centuries, this plant has played a vital role in traditional medicine, where its leaves, roots, and bark have been employed for therapeutic purposes. Ethnobotanical studies have extensively documented its use in treating a broad spectrum of ailments, including malaria, gastrointestinal disorders, respiratory infections, skin diseases, diabetes, and fever (Etim *et al.*, 2017; Habtamu *et al.*, 2018). The plant's effectiveness is largely attributed to its rich phytochemical composition, which includes alkaloids, flavonoids, saponins, terpenoids, tannins, and phenolic compounds (Beentje, 2018). These bioactive constituents contribute to its antimicrobial, anti-inflammatory, antioxidant, antimalarial, and hypoglycemic properties, making it a promising candidate for modern pharmacological applications. The characteristic bitter taste of *V. amygdalina* leaves is linked to the presence of sesquiterpene lactones, compounds known for their potent medicinal properties. Beyond its medicinal applications, *V. amygdalina* is also consumed as a vegetable in many African countries, either cooked in soups or processed into tonics. Given its extensive therapeutic potential, further research is necessary to explore its pharmacological mechanisms and clinical applications in modern medicine. In many African communities, the leaves of *V. amygdalina* are either consumed as a vegetable or prepared as aqueous extracts for medicinal purposes. The bitterness of the leaves can be reduced through boiling or soaking in multiple washes of water, making them more palatable while retaining their medicinal properties (Ali *et al.*, 2019). Beyond its traditional uses, *V. amygdalina* has been integrated into contemporary

formulations for dietary supplements and botanical drugs, reinforcing its potential as a natural therapeutic agent.

The World Health Organization (WHO) estimates that approximately 80% of the global population relies on traditional medicine for primary healthcare needs (Muhammad *et al*., 2019). The increasing prevalence of MDR bacterial strains has intensified the need for alternative antimicrobial solutions derived from medicinal plants. Scientific investigations have revealed that *V. amygdalina* exhibits potent antimicrobial activity against a range of bacterial and fungal pathogens. Studies have demonstrated its efficacy against Gram-negative bacteria such as *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Shigella dysenteriae*, and *Proteus vulgaris*, as well as Gram-positive bacteria including *Bacillus cereus*, *Bacillus pumilus*, *Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Micrococcus kristinae*, and *Clostridium* species (Swee *et al.,* 2010). Additionally, phytochemical analyses have confirmed the antifungal potential of *V. amygdalina* against pathogenic fungi such as *Aspergillus flavus*, *Mucor hiemalis*, *Fusarium oxysporum*, *Penicillium notatum*, and *Aspergillus niger*. These effects have been observed to be comparable to standard antifungal drugs like Nystatin at concentrations of 0.1 mg/mL and above (Ijeh & Ejike, 2011). The antimicrobial efficacy of *V. amygdalina* is largely attributed to its bioactive metabolites, which interfere with microbial cell wall integrity, protein synthesis, and metabolic pathways, thereby inhibiting pathogen growth and proliferation (Jin *et al.,* 2017).

One of the major challenges confronting global public health is mitigating the impact of drug-resistant microorganisms. The indiscriminate and prolonged use of antibiotics has facilitated the emergence of resistant bacterial strains, particularly in developing countries where access to quality healthcare and antibiotic stewardship programs remains limited (Uzoigwe *et al.,* 2017). This underscores the necessity of exploring alternative antimicrobial agents that are not only effective but also affordable, sustainable, and readily available. Given the promising antimicrobial properties of *V. amygdalina*, further investigations are warranted to assess its efficacy against clinically relevant bacterial strains such as *Escherichia coli* and *Staphylococcus aureus*, which are common causative agents of various human infections. These bacterial pathogens have been increasingly associated with MDR profiles, necessitating innovative approaches to combat their resistance mechanisms. In light of the growing global burden of AMR and the need for novel bioactive compounds, this study aims to evaluate the antibacterial activity of *V. amygdalina* leaf extracts against clinical isolates of *E. coli* and *S. aureus*. By elucidating its antimicrobial efficacy, this research seeks to contribute valuable insights into the potential integration of *V. amygdalina* as a natural therapeutic agent in the fight against drug-resistant bacterial infections.

MATERIALS AND METHODS

Collection and Authentication of Plant Samples

Fresh leaves of *Vernonia amygdalina* were collected from Dutse Ultra-modern Market, Jigawa State, Nigeria. The plant samples were identified and authenticated by botanists and subsequently transported to the Microbiology Laboratory, Department of Microbiology and Biotechnology, Faculty of Life Sciences, Federal University Dutse. The leaves were air-dried under shade, ground into fine powder using a laboratory mortar and pestle, and stored in airtight containers until further analysis.

Collection and Confirmation of Clinical Isolates

Clinical isolates of *Escherichia coli* and *Staphylococcus aureus* were obtained from Dutse General Hospital, Jigawa State. The isolates were reconfirmed based on their colonial morphology on MacConkey Agar (MA) and Mannitol Salt Agar (MSA), Gram staining reaction, and biochemical tests, including coagulase test, citrate utilization test, methyl red, indole, and oxidase tests, as described by Chessbrough (2005). The isolates were stored on nutrient agar slants until further use as described by Kate & Lucky (2012).

Preparation and Extraction of *V. amygdalina* Leaves

The dried leaves were cleaned with distilled water, shade-dried, and pulverized into fine powder. Fifty (50) grams of the powdered leaves were soaked in 250 mL of ethanol, stirred, and covered for 48 hours. The mixture was filtered using Whatman No. 1 filter paper, and the filtrate was evaporated using a water bath to remove ethanol. The resultant residue was weighed and stored in a refrigerator at 4°C until use, following the procedure documented by Udochukwu *et al.* (2015). For aqueous extraction, 50 g of the powdered leaves were soaked in 500 mL of distilled water, stirred, covered for 48 hours, and filtered. The filtrate was evaporated using a water bath to remove the water content. The crude aqueous and ethanolic extracts were stored at 4°C for subsequent phytochemical and antimicrobial analysis.

Preparation of Extract Concentrations

A stock solution of 200 mg/mL was prepared by dissolving 1 g of both ethanolic and aqueous extracts in 5 mL of ethanol and distilled water, respectively. Serial dilutions were performed to obtain concentrations of 100 mg/mL, 50 mg/mL, and 25 mg/mL following the double dilution method documented by Ahmad *et al.* (2024).

Phytochemical Screening of *V. amygdalina* Extracts

The phytochemical constituents of the extracts were determined using standard procedures outlined by Evbuomwan *et al.* (2018). Alkaloids were identified by the formation of white turbidity or precipitate upon the addition of Mayer's reagent. Saponins were detected by the formation of a 1 cm persistent foam layer after agitation. Steroids were confirmed by the development of a reddish-brown color upon treatment with chloroform, concentrated H_2SO_4, and acetic anhydride. The presence of tannins was indicated by a dark blue coloration after the addition of 5% ferric chloride. Phenols were detected by the appearance of a blue-green color upon treatment with 10% ferric chloride. Quinones were identified by a red coloration following the addition of concentrated H_2SO_4. Terpenoids were indicated by a yellow color after the addition of chloroform and sulfuric acid. Cardiac glycosides were detected by the appearance of a blue color upon the addition of glacial acetic acid, ferric chloride, and sulfuric acid. Flavonoids were confirmed by a yellow solution turning colorless after the addition of concentrated sulfuric acid. The presence of phytosterols was indicated by a bluish-green coloration upon treatment with chloroform and concentrated H_2SO_4.

Antibacterial Activity Testing

Preparation of McFarland Standard and Standard Inoculum

The 0.5 McFarland standard was prepared by mixing 99.5 mL of 1% sulfuric acid with 0.5 mL of 1% barium chloride solution and stored in a bijou bottle (Chessbrough, 2005). Confirmed isolates of *E. coli* and *S. aureus* were suspended in

distilled water and adjusted to match the 0.5 McFarland standard (Ali & Yahaya, 2017).

Preparation of V. amygdalina Extract Concentrations
Stock solutions (200 mg/mL) of both ethanolic and aqueous extracts were prepared by dissolving 2 g of each extract in 10 mL of sterile dimethyl sulfoxide (DMSO). Serial dilutions were performed to obtain 100 mg/mL, 50 mg/mL, and 25 mg/mL concentrations (Manandhar *et al.,* 2019).

Susceptibility Testing by Agar Well Diffusion Method
Mueller-Hinton agar plates were inoculated with the standardized bacterial suspensions. A 4 mm cork borer was used to create wells in the agar, and 2 drops of *V. amygdalina* extracts at varying concentrations (200 mg/mL, 100 mg/mL, 50 mg/mL, and 25 mg/mL) were introduced into the wells. Plates were incubated at 37°C for 24 hours, and the zones of inhibition were measured. A gentamicin (10 µg) disc served as a positive control (Gobezie *et al.,* 2020).

Determination of Minimum Inhibitory Concentration (MIC)
A loopful of each test organism was inoculated into test tubes containing 5 mL of sterile nutrient broth with varying extract concentrations (200 mg/mL, 100 mg/mL, 50 mg/mL, and 25 mg/mL). The tubes were incubated at 37°C for 24 hours, and the MIC was determined as the lowest concentration that inhibited visible bacterial growth (Evbuomwan *et al.,* 2018).

Determination of Minimum Bactericidal Concentration (MBC)
Tubes showing no visible growth in the MIC test were sub cultured onto fresh Mueller-Hinton agar plates and incubated at 37°C for 48 hours. The MBC was recorded as the lowest concentration at which no bacterial growth was observed following the method described by Evbuomwan *et al.* (2018).

RESULTS AND DISCUSSION

Results

The phytochemical analysis of *Vernonia amygdalina* leaves revealed the presence of various bioactive constituents in both ethanolic and aqueous extracts. The ethanolic extract demonstrated a rich composition, including saponins, tannins, alkaloids, quinones, phenols, terpenoids, flavonoids, and steroids. In contrast, the aqueous extract exhibited a comparatively limited range of bioactive compounds, containing tannins, saponins, phenols, and phytosteroids, as summarized in Table 1.

Table 1: Phytochemical components of *V. amydalina* leaf extract

Bioactive Constituents	Ethanolic Extract	Aqueous Extract
Quinones	+	_
Flavonoids	+	_
Tannins	+	+
Alkaloids	+	_
Phytosterols	_	_
Steroids	+	_
Cardiac Glycosides	+	_
Saponins	+	+
Terpenoid	+	_
Phenol	+	+

Key: positive: +, negative: -

The ethanolic extracts of *V. amygdalina* demonstrated a larger zone of inhibition than the aqueous extract against the two isolates. Compared to the aqueous extract, the ethanolic extracts showed zones of inhibition as high as 20 mm in *S. aureus* isolate, while the aqueous extract showed a zone of inhibition as high as 10 mm to *E. coli* (Table 2).

Table 2: Antibacterial activity of Ethanolic extracts of *Vernonia amygdalina* leaves against *E. coli and S. aureus*

Organism	Control	200 mg/mL (mm)	100 mg/mL (mm)	50 mg/mL (mm)	25 mg/mL (mm)
E. coli	38	14	10	7	2
S. aureus	36	20	15	9	5

The antibacterial activity of *Vernonia amygdalina* leaf extracts was assessed against *Escherichia coli* and *Staphylococcus aureus*. The ethanolic extract exhibited a larger zone of inhibition compared to the aqueous extract. Notably, the ethanolic extract produced a zone of inhibition of up to 20 mm against *S. aureus*, whereas the aqueous extract showed a maximum inhibition zone of 10 mm against *E. coli* (Tables 2 and 3). The inhibitory effect of the ethanolic extract was concentration-dependent, with the highest inhibition observed at 200 mg/mL, where *E. coli* and *S. aureus* exhibited inhibition zones of 14 mm and 20 mm, respectively. Lower concentrations (100 mg/mL, 50 mg/mL, and 25 mg/mL) showed progressively smaller inhibition zones. The control antibiotic, gentamycin, demonstrated the highest antibacterial activity, with inhibition zones of 38 mm for *E. coli* and 36 mm for *S. aureus*. These results indicate differential antibacterial potency based on extract type and concentration (Table 3).

Table 3: Antibacterial Activities of Aqueous Extracts of *V. amydalina against E coli.* and *S. aureus*

Organism	Control	200 mg/mL (mm)	100 mg/mL (mm)	50 mg/mL (mm)	25 mg/mL (mm)
E. coli	25	8	5	2	-
S. aureus	34	10	6	3	2

The lowest concentration of the leaf that inhibited the growth of the test bacteria after a 24-hour incubation period was determined to be the minimum inhibitory concentration (MIC), and the lowest concentration of the extract that the organism didn't recover and grow was determined to be the minimum bactericidal concentration (MBC). The minimum inhibitory concentration (MIC) of ethanolic extract was found to range from 25mg/ml in *E. coli* to 100mg/ml in *S. aureus.* While that of aqueous extract was 50mg/ml in *S. aureus*. Minimum bactericidal concentration of the ethanol extract was 100mg/ml in *E. coli* and 200mg/ml in *S. aureus.* MBC of 200mg/ml was observed for *S. aureus* in the aqueous fraction of the plant while no MBC was detected for *E. coli* as summarized in Table 4. The potency of an antibacterial agent is an inverse measurement of its MIC and MBC. Plant extract or drugs that have low MIC and MBC against bacteria are said to be very potent. The reverse is also true for antimicrobial agents (Table 4).

Table 4: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the *V. amygdalina* Leaves Extract against *E. coli* and *S. aureus*

Organism	Ethanolic E. MIC (mg/mL)	E. Extract MBC (mg/mL)	Aqueous E. MIC (mg/mL)	Aqueous E. MBC (mg/mL)
E. coli	25	100	-	-
S. aureus	100	200	50	200

Discussion

Vernonia amygdalina serves both as a vegetable and a food seasoning, contributing significantly to nutritional value. Beyond its nutritive benefits, it has demonstrated considerable antimicrobial potential by inhibiting the growth of various microorganisms (Femi *et al.*, 2021). Extensive research has investigated its proximate composition, phytochemical constituents, and antimicrobial properties. This study further corroborates findings on the presence of bioactive compounds in *V. amygdalina*, highlighting the differential extraction of these compounds in ethanolic and aqueous solvents. The importance of *V. amygdalina* extends beyond nutrition and antimicrobial activities, but also been explored for its antioxidant, anti-inflammatory, and anticancer properties. The growing interest in medicinal plants for alternative therapeutic applications underlines the need for further exploration of *V. amygdalina's* pharmacological activities. Additionally, its role in food preservation has gained attention, with studies suggesting that its bioactive compounds can serve as natural preservatives against food spoilage microorganisms. These properties make *V. amygdalina* a promising candidate for use in both traditional medicine and modern pharmaceutical applications.

Phytochemical screening revealed the presence of flavonoids, cardiac glycosides, terpenoids, saponins, quinones, alkaloids, and steroids in the ethanolic extract of *V. amygdalina* in Table 1. However, the aqueous extract lacked flavonoids, steroids, quinones, alkaloids, and cardiac glycosides, while phytosterol was absent in both aqueous and ethanolic extracts. These results align with previous studies by Ali *et al.* (2019) and Ahmad *et al.* (2024), who identified similar bioactive components, including saponins, alkaloids, tannins, phenols, terpenoids, steroids, and cardiac glycosides in *V. amygdalina* leaf extracts. The variations in phytochemical profiles between solvent extracts indicate that ethanol is a more efficient solvent for extracting a broader range of bioactive compounds compared to water. Differences in the solubility of phytochemicals in various solvents can impact their efficacy and biological activities. Several studies have suggested that flavonoids and alkaloids possess significant antimicrobial properties, which may contribute to the observed differences in antibacterial activity between ethanolic and aqueous extracts. The presence of bioactive compounds in higher concentrations in ethanolic extracts suggests that ethanol facilitates the dissolution of complex secondary metabolites, making it a preferred solvent for plant-based antimicrobial research (Table 2). Additionally, environmental factors, plant maturity, and geographical variations can influence the phytochemical composition of *V. amygdalina*, which may explain discrepancies among different studies.

The antibacterial efficacy of *V. amygdalina* was found to be solvent-dependent, with ethanolic extracts exhibiting greater antibacterial activity than aqueous extracts. This is attributed to ethanol's superior ability to extract phytochemicals with antibacterial properties. The zones of inhibition produced by the ethanolic extract ranged from 2.0 mm at 25 mg/ml to 14 mm at 200 mg/ml against *Escherichia coli* and from 5.0 mm at 25 mg/ml to 20 mm at 200 mg/ml against *Staphylococcus aureus* (Table 2). *S. aureus* was the most sensitive bacterial species, while *E. coli* displayed the least sensitivity to the ethanolic extract. This observation corroborates previous findings by Udochukwu *et al.* (2015) and Ahmad *et al.* (2024), which demonstrated the phytochemical and antibacterial activity of *V. amygdalina*. The role of solvent polarity in influencing antibacterial efficacy is well-documented, with ethanol being more effective in dissolving a wide range of bioactive compounds compared to water. Additionally, studies suggest that phenolic compounds and alkaloids, which are more effectively extracted with ethanol, contribute to the observed antimicrobial activities. The varying susceptibility of bacterial species to plant extracts can be attributed to differences in bacterial cell wall structures, enzyme activity, and resistance mechanisms. The effectiveness of *V. amygdalina* extracts suggests potential applications in the development of natural antibacterial agents, particularly for treating bacterial infections resistant to conventional antibiotics. However, further research is necessary to determine the synergistic interactions between its bioactive components and their mechanisms of action against pathogenic bacteria.

Conversely, the aqueous extract showed limited antibacterial activity, particularly against *E. coli*, which exhibited complete resistance at lower concentrations. However, at higher concentrations, inhibition zones were observed, ranging from 2.0 mm at 25 mg/mL to 10 mm at 200 mg/mL against *S. aureus*, and from 5.0 mm at 100 mg/mL to 8.0 mm at 200 mg/mL against *E. coli* (Table 3). These results are in agreement with those reported by Evbuomwan *et al.* (2018) and David and Musyoki (2024), reinforcing the reduced efficacy of aqueous extracts in comparison to ethanolic extracts. The poor performance of aqueous extracts in antibacterial assays may be linked to the low solubility of certain phytochemicals in water. Water primarily extracts polar compounds, which may not include the most potent antimicrobial agents found in *V. amygdalina*. The results highlight the importance of solvent selection when evaluating plant-based antimicrobial agents. The resistance of *E. coli* to lower concentrations of the extract suggests that additional

research is needed to optimize extraction techniques and enhance the efficacy of aqueous extracts. Moreover, bacterial resistance mechanisms, such as efflux pumps and enzyme-mediated inactivation of bioactive compounds, may play a role in the observed variations in susceptibility. Investigating these mechanisms can provide insights into how plant extracts can be improved for enhanced antimicrobial applications. Additionally, exploring the combination of *V. amygdalina* extracts with conventional antibiotics may provide an alternative approach to overcoming bacterial resistance and improving treatment outcomes.

The differential antibacterial effects of *V. amygdalina* extracts on *E. coli* and *S. aureus* can be attributed to differences in bacterial cell wall composition. Gram-positive bacteria, such as *S. aureus*, possess a thick peptidoglycan layer that allows greater susceptibility to antibacterial agents. In contrast, Gram-negative bacteria, such as *E. coli*, have an outer lipopolysaccharide layer that acts as a barrier, reducing permeability to bioactive compounds, The outcome of this findings is in agreement with Zubairu *et al.* (2019) submission. Additionally, the resistance of *E. coli* to lower concentrations of the plant extract may stem from drug/phytochemical inactivating enzymes, further complicating its susceptibility to antimicrobial agents. The composition of bacterial cell membranes influences the effectiveness of plant-based antimicrobials, highlighting the need for targeted approaches in developing natural antibiotics, as also reported by Lee *et al.* (2019). For instance, Gram-negative bacteria are particularly challenging due to their outer membrane and efflux pump systems, which actively expel antimicrobial agents and reduce their efficacy (Liu *et al.*, 2024). These mechanisms highlight the need for further research into bioactive compounds with improved permeability against Gram-negative bacteria. One promising approach involves the use of nanotechnology-based delivery systems, which have been shown to enhance the solubility, stability, and cellular uptake of plant extracts, such as those derived from *Vernonia amygdalina* (Adewumi *et al.*, 2021). Additionally, comparative studies with other medicinal plants, such as *Azadirachta indica* (neem) and *Ocimum gratissimum* (scent leaf), could provide valuable insights into the relative antibacterial potency of *V. amygdalina* (Nwonuwa *et al.*, 2019).

Comparing the plant extracts with a standard antibiotic, gentamycin exhibited the highest antibacterial activity, producing zones of inhibition of 34 mm against *S. aureus* and 25 mm against *E. coli*. These results confirm the superior efficacy of gentamycin over plant-based extracts, although *V. amygdalina* remains a viable natural alternative, particularly for Gram-positive bacterial infections (David and Musyoki, 2024). Distilled water, used as a negative control, showed no inhibitory effects on either bacterial strain, reinforcing the specificity of the observed antibacterial activity to the bioactive constituents of *V. amygdalina*. Similar findings have been reported by Udeh *et al.* (2021), who demonstrated that distilled water had no antibacterial effects, further supporting its role as an appropriate negative control. While gentamycin remains the gold standard for bacterial inhibition, concerns about antibiotic resistance necessitate the exploration of alternative treatments, including plant-derived antimicrobials. A study by Ashokkumar *et al.* (2021) have emphasized the antibacterial properties of medicinal plants, including *V. amygdalina*, against drug-resistant bacterial strains. This study is in agreement with the present findings, suggesting that plant-based antimicrobials could be viable alternatives to conventional antibiotics. The increasing prevalence of multidrug-resistant bacterial strains has prompted research into natural products with antibacterial potential. Research by Evbuomwan *et al.* (2018) revealed that bioactive compounds from *V. amygdalina* exhibited comparable antibacterial activity to standard antibiotics against *Escherichia coli* and *Staphylococcus aureus*. This aligns with the present study, which highlights the potential of plant-derived antimicrobials in mitigating MDR infections. Future studies should explore the synergistic effects of *V. amygdalina* extracts with existing antibiotics to determine potential combination therapies that enhance antimicrobial efficacy while reducing the risk of resistance development. Additionally, in vivo studies assessing the safety, efficacy, and pharmacokinetics of *V. amygdalina* extracts will be essential for translating these findings into clinical applications. Further exploration of its bioactive compounds through advanced analytical techniques such as mass spectrometry and nuclear magnetic resonance spectroscopy could provide deeper insights into their mechanisms of action, paving the way for new antibacterial drug development.

CONCLUSION

This study confirms that *Vernonia amygdalina* contains bioactive phytochemicals responsible for its antibacterial properties, with ethanolic extracts exhibiting stronger activity than aqueous extracts. Its effectiveness against *Staphylococcus aureus* and *Escherichia coli* highlights its potential as a natural antimicrobial agent, with differences in bacterial susceptibility attributed to variations in cell wall composition. Further research is needed to isolate and characterize the specific bioactive compounds using advanced analytical techniques and to understand their mechanisms of action for optimized therapeutic applications. Given the growing concern over antibiotic resistance, *Vernonia amygdalina* offers a promising alternative to synthetic drugs. Future studies should focus on purification, formulation, and clinical trials to assess its safety and efficacy. Integrating *Vernonia amygdalina*-derived compounds into pharmaceutical research could provide a sustainable and effective approach to combating bacterial infections while addressing the challenge of antimicrobial resistance.

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