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Phytochemistry Screening and Anti-Bacterial Activity of *Vernonia amygdalina* (Del) (Bitter Leaf) Methanolic Stem Extract on Selected Clinical Bacteria**S.T., Soom¹ and J.T., Hemen²**¹Department of Biological Sciences, Faculty of Sciences, Benue state University, Makurdi²Department of Biological Sciences, Faculty of Sciences, Nigeria Police Academy, Kano, Nigeria

Abstract: *Vernonia amygdalina* (Del), commonly known as Bitter Leaf is a medicinal plant widely recognized for its therapeutic potential in traditional medicine. This study aimed to investigate the phytochemical composition and evaluate the anti-bacterial activity of methanolic stem extracts of *Vernonia amygdalina* against selected clinical bacteria. The phytochemical analysis of the extract revealed the presence of various bioactive compounds, including alkaloids, flavonoids, tannins, saponins, phenolics, and terpenoids. These phytochemicals are known for their diverse biological activities, including antimicrobial properties. The anti-bacterial activity of the *Vernonia amygdalina* methanolic stem extract was assessed against a panel of clinically relevant bacteria, including *Salmonella typhi*, *Escherichia coli*, *Candida albicans* and *Klebsiella pneumoniae*. The determination of minimum inhibitory concentration (MIC) and Dimethylsulfoxide (DMSO) were employed to evaluate the extract's antimicrobial potential by comparing its activity with that of an antibiotic (Gentamycin). The results demonstrated that the extract exhibited significant ($P < 0.05$) anti-bacterial activity against all tested clinical bacteria except *Salmonella typhi* (0.00 ± 0.00 mm). Additionally, the MIC values 25mg/ml for *Salmonella typhi*, *Escherichia coli* and *Klebsiella sp.*, while *C. albicans* was 50mg/ml. The MBC on the other hand was 100mg/ml for *S. typhi* and *E. coli* and 50mg/ml for *Klebsiella sp.* were determined, indicating the minimum concentration at which bacterial growth was completely inhibited. This study suggests that *Vernonia amygdalina* methanolic stem extract possesses promising anti-bacterial properties against clinically relevant bacteria. The presence of various phytochemicals suggests that they may be responsible for the observed antimicrobial effects.

Keywords: Anti-bacterial, Bitter leaf, Gentamycin, Methanolic, Phytochemicals,Email: solomonsoom@gmail.com**Received:** May 25, 2023**Revised:** August 28, 2023**Accepted:** September 25, 2023**About the Journal:** SJANS is a multidisciplinary science journal and the official journal of the Faculty of Science, Nigeria Police Academy, Kano, Nigeria

1.0 Introduction

Traditional means of treating diseases involved using concoctions from plants, either in single form or in mixtures. This they did without knowing that these agents were used against some pathogenic microorganisms. This plant has been reportedly used in the traditional treatment of ailments such as diabetes, malaria, stomach disorder, fever symptoms and cough etc (Agbogidi and Akpomorine 2013).

Several studies carried out on this plant by researchers had shown that it contains different bioactive compounds, including, flavonoids, saponins, alkaloids, tannins, phenolics, terpenes, steroidal glycosides, triterpenoids, and several types of sesquiterpene lactones (Erasto *et al.*, 2006; Farombi and Owoeye, 2011; Kiplimo *et al.*, 2011; Toyang and Verpoorte, 2013; Adedapo *et al.*, 2014; Quasie *et al.*, 2016; Luo *et al.*, 2017). These bioactive compounds made them possess different

pharmacological properties like antimicrobial, anti-malarial, antithrombotic, antioxidant, anti-diabetic, laxative, hypoglycemic, anti-helminthic, anti-inflammatory, cathartic, anticancer, anti-fertility, anti-fungi, antibacterial, and among others (Ezeadila *et al.*, 2015; Udochukwu *et al.*, 2015 and Alara 2017c).

2.0 MATERIALS AND METHODS

2.1 Collection of Plant and Identification

Fresh stems of *Vernonia amygdalina* were collected from Wurukum market, Makurdi, Benue State. The stems were taken to Charis Rhema Research and Diagnostic Laboratories for anti-bacterial and qualitative phytochemical Analysis. The plant was authenticated by a botanist in the Department of Biological Sciences, Benue State University Makurdi. .

The Stems of *Vernonia amygdalina* collected were air dried at room temperature for two weeks. The dried stems were pounded with wooden mortar and pestle and were further blended to fine powder using an electric blender.



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The powdered material was then stored in a closed container in a cool, dry and dark place for analysis.

2.2 Preparation of Methanolic Extract

The powdered sample (100g) was placed in 1000ml capacity conical flask and 700ml methanol was added to it to make a suspension. The suspension was allowed to stand for 96hrs with intermittent agitation of the flask. The extract was obtained by filtering the suspension using Whatman's filter paper. The filtrate was exposed to dryness at room temperature for the solvent to evaporate and was stored for further use.

2.3 Separation of the Active Components

Separation of active components of the concentrated extract was done using chromatographic technique (column chromatography). The column was packed using silica-gel and N-hexene in an appropriate proportion. The concentrated extract was reconstituted using n-hexene and then poured on the packed column. 100ml of n-hexene was measured and poured on the extract in the packed column and allowed to elute (run) through the column which was then collected into a conical flask as the first eluent, labeled E1. After the elution of the 100ml n-hexene, 80ml n-hexene and 20ml ethanol were measured altogether and poured into the column. The same was carried out with 60ml n-hexene + 40ml ethanol, 40ml n-hexene + 60ml ethanol, 20ml n-hexene + 80ml ethanol and finally, 0ml n-hexene and 100ml ethanol was measured and poured sequentially. Each of these variable concentrations of n-hexene and ethanol was collected into separate conical flask labeled E1, E2, E3, E4, E5, E6 respectively. The eluents were then left undisturbed until the solvent was completely evaporated.

2.4 Qualitative Phytochemical Screening

The extract was subjected to phytochemical screening to test for the presence of the following bioactive compounds:

2.4.1 Tests for Phenol

2ml of the extract was measured and dispensed into a test tube, and then 3 drops of ferric

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chloride was added to it. Dark blue or dark green colouration indicates the presence of phenol.

2.4.2 Test for Saponins

Frothing method was used to determine the presence of saponins in the extract. 3ml of distilled water was measured and added to 1ml of the extract in a test tube and agitated vigorously for stable persistent froth. Formation of froth that is stable for up to 10min is an indicative of the presence of saponins.

2.4.3 Test for Flavonoids

2ml of the extract was measured and dispensed into a test tube then few drops of sodium hydroxide were added. Yellow colouration indicate the presence of flavonoids.

2.4.4 Test for Tannins

2ml of the extract was measured and dispensed into a test tube, and then 3 drops of ferric chloride was added to it. Dark blue or dark green colouration indicates the presence of tannins.

2.4.5 Test for Alkaloids

Three (3) drops of Dragendorff's reagents was added to 1ml of the plant extract in a test tube. An orange red precipitate turbidity indicates the presence of alkaloids.

2.5 Confirmatory Test for the Test Isolates

The isolates used in this study were obtained at Charis Rhema Research and Diagnostic Laboratories, and their identity were confirmed using cultural and appropriate biochemical tests. The isolates were sub-cultured onto Eosin Methylene Blue agar, Mannitol Salt agar, Xylose Lysin Deoxycholate agar and MacConkey agar. The inoculated plates were incubated at 37°C for 24h. The overnight plates were observed and recorded.

2.6 Biochemical Test

2.6.1 Coagulase Test

The slide test method as described by Cheesbrough (2006) was used to carry out the test. A drop of distilled water was placed on a slide and colonies of the test organism was emulsified to make a thick suspension, and a loopful of plasma was added to the suspension and mixed gently and observed for clumping (coagulation) within 10s indicate positive result.



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2.6.2 Indole Test

The test organism was inoculated in a test tube containing 5ml of sterile peptone water and incubated at 37°C for 48h and 0.5ml of Kovac's reagent was added and shaken gently and examined for red colour formation on the surface layer within 10m. The formations of red colour on the surface layer indicate positive result for Indole production.

2.6.3 Catalase Test

Small amount of colony from a 24h culture was picked with a wooden applicator and placed in a test tube containing 5-drops of hydrogen peroxide. The tube was observed for immediate bubble formation against a dark background. Production of gas bubble indicated catalase positive reaction.

2.6.4 Motility Test

A loopful of broth previously inoculated with the test organism was placed in the centre of a cover slip. A glass slide was carefully placed over the drop on the cover-slip. The slide was quickly turned over and viewed with light microscope under low and high power objectives.

2.6.5 Gram Reaction

The colony of each isolate was emulsified using wire loop, in a drop of normal saline on a clean microscope slide (smear). It was air dried and was heat fixed by passing the slide across a flame three times. The slide was placed on the staining rack and was flooded with crystal violet for 1 minute and was rinsed with clean water. It was then covered with Lugol's iodine (mordant) for 1 minute and rinsed with clean water. It was decolorized with acetone for few seconds and was rinsed immediately with clean water. It was lastly stained with safranin (secondary dye) for 30 seconds. Back of the slide was wiped with cotton wool and was allowed to air dry; the slide was mounted on the microscope stage and a drop of immersion oil was added. The specimen was brought into focus using 100x objective with the iris diaphragm completely opened to increase the amount of light that enters the objective. Gram reaction was determined on the basis of the colour of dye retained; Gram positive organisms retained the colour of the primary dye (crystal violet) and appear purple while Gram negative

organisms lost the colour of the primary dye after decolorization and pick up the colour of the secondary dye (safranin) and appear red in colour.

2.7 Antimicrobial activities of the Extract

The antibacterial screening of the extract was carried out using the agar well diffusion method as described by Ochei and Kohakter (2008). The isolates were prepared in PBS and adjusted to 0.5 McFarland turbidity standards (1.5×10^8 cfu/ml). The concentration of the extract was prepared by reconstituting 1.0g of the extract in 10ml of dimethylsulfoxide (DMSO) to make a stock solution of the concentration of 100mg/ml as initial concentration. Using sterile swab stick, the inoculums were collected and spread evenly over the surface of solidified Mueller Hinton agar. A sterile cork borer of 6mm in diameter was used to cut wells on the inoculated medium. 50µl of the extract of the concentration of 100mg/ml was introduced into each of the wells on the inoculated medium and incubated at 37°C for 24hrs and the plates were observed for zones of inhibition and the zones were measured with a transparent ruler in millimeter and recorded.

2.8 Minimum Inhibition Concentration (MIC)

The minimum inhibition concentration (MIC) of the extract was determined using broth dilution method. Two-fold serial dilution method. 50µl (0.5ml) isolate suspension was inoculated into different concentrations in 5ml peptone water and was incubated at 37°C for 24hrs. The tubes were observed for turbidity (growth). The lowest concentration of the extract in the sterile broth, which shows no turbidity, was recorded as the minimum inhibition concentration (MIC).

2.9 Minimum Bactericidal Concentration (MBC)

Minimum bactericidal concentration (MBC) was performed to determine whether the test microorganisms were killed or inhibited. Tubes at different concentrations that showed no turbidity were sub-cultured onto nutrient agar medium and incubated at 37°C for 24hrs. The plates with lowest concentration of the extract that showed no growth were recorded as the minimum bactericidal concentration (MBC).



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3.0 Results

This study was carried out to determine the phytochemical composition of *Vernonia amygdalina* stem extract and evaluate its potential against selected clinical bacteria strains. Table 1, shows the phytochemical composition of *Vernonia amygdalina*. The result shows the presence of phenols, tannins, saponins, flavonoids and alkaloids in the extract.

Table1. Phytochemical Composition of *vernonia amygdalina*.

Phytochemical	Reaction
Phenols	+
Tannins	+
Saponins	+
Flavonoids	+
Alkaloids	+

Key: + = Present

Table 2 shows the evaluation of the antimicrobial activity of *Vernonia amygdalina* on selected bacteria. The potential of the extract was evaluated by comparing its activity with that of an antibiotic (Gentamycin). Furthermore, the extract was reconstituted with Dimethylsulfoxide (DMSO) and as such, it was also tested on the organisms to investigate if it had an effect on them. The comparative effect of the extract and the antibiotic showed that, the extract had no effect *Salmonella typhi* (0.00 ± 0.00 mm). The antibiotic however showed a considerable inhibitory activity with a zone of inhibition of 12.00 ± 1.14 mm.

The effect of the extract on *E.coli* was also evaluated. The result showed higher effect of the antibiotic compared with the extract with a significant difference observed ($P < 0.05$).

Furthermore, the plant extract was observed to have inhibitory activity against *Klebsiella sp.* However, activity of the antibiotic was significantly higher ($P < 0.05$).

The extract also had considerable effect on *E.coli* and *Candida albicans*. It was observed that the DMSO showed no activity on any of the organism tested.

Table 2. Evaluation of the antimicrobial activity of *Vernonia amygdalina* on the selected micro-organism.

	Zone of Inhibition			
	<i>E.coli</i>	<i>S.typhi</i>	<i>Klebsiella sp</i>	<i>Candida</i>
<i>albican</i>				
Plant extract	0.00 ± 0.00	14.33 ± 0.58	12.67 ± 0.58	8.67 ± 0.58
Antibiotic (Gentamycin)	12.00 ± 1.41	16.00 ± 0.00	15.00 ± 1.00	0.00 ± 0.00
Dimethylsulfoxide (DMSO)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
FLSD (0.05)		1.450	1.330	

Table 3 shows the minimum inhibitory concentration and minimum bactericidal concentration of the stem extract of *Vernonia amygdalina*. The MIC for all the bacteria species was 25mg/ml, while that of *C. albicans* was 50mg/ml. The MBC on the other hand was 100mg/ml for *S.typhi* and *E.coli* and 50mg/ml for *Klebsiella sp.* While that of *C. albican* could not be determined.

Table3. Minimum Inhibition Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the stem extract of *Vernonia amygdalina*.

Microbial Isolate	MIC	MBC
<i>Salmonella typhi</i>	25mg/ml	100mg/ml
<i>Escherichia coli</i>	25mg/ml	100mg/ml
<i>Klebsiella sp</i>	25mg/ml	50mg/ml
<i>Candida albicans</i>	50mg/ml	Not determined

4.0 Discussion

4.1 Phytochemical Composition of *Vernonia amygdalina*

The results provide an overview of the phytochemical composition of *Vernonia amygdalina* (Bitter Leaf) extract. The presence of various phytochemicals is denoted by a plus sign (+). These phytochemicals play a significant role in the potential medicinal properties of the plant. The presence of phenols in the extract suggests its potential antioxidant properties. Phenolic compounds are known for their ability to scavenge free radicals, which can help protect cells from oxidative damage (Djeu et al., 2023). This antioxidant activity can contribute to the plant's overall health benefits. Tannins are a



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group of compounds with astringent properties. They are often associated with antimicrobial and anti-inflammatory effects. The presence of tannins in *Vernonia amygdalina* may explain its traditional use in treating various infections and inflammatory conditions. Saponins are amphiphilic molecules that can disrupt cell membranes. In the context of medicinal plants, saponins are often associated with their potential to exert antimicrobial effects. The presence of saponins in Bitter Leaf suggests that it may have the ability to disrupt bacterial cell membranes, leading to cell death (Nguemfo *et al.*,2023). Flavonoids are well-known for their antioxidant and anti-inflammatory properties. They are associated with various health benefits, including immune support. The presence of flavonoids in Phytochemical Composition of *Vernonia amygdalina* suggests its potential to reduce inflammation and potentially inhibit bacterial growth. Alkaloids are a diverse group of organic compounds with various pharmacological effects. Some alkaloids have been found to exhibit antibacterial properties. The presence of alkaloids in Bitter Leaf suggests they may contribute to its antibacterial activity (Diyaolu *et al.*,2022).

4.2 Evaluation of the Antimicrobial Activity of *Vernonia amygdalina*

the results of antimicrobial activity testing of *Vernonia amygdalina* extract against selected microorganisms, including *Salmonella typhi*, *Escherichia coli*, *Klebsiella sp*, and *Candida albicans*. The zone of inhibition (measured in millimeters) indicates the effectiveness of the plant extract in inhibiting the growth of these microorganisms compared to a standard antibiotic (Gentamycin) and a negative control (Dimethylsulfoxide, DMSO). The plant extract did not show any zone of inhibition against *Salmonella typhi*, suggesting that it was ineffective in inhibiting the growth of this bacterium as similarly reported by Usin *et al.*,(2023). In contrast, Gentamycin, the standard antibiotic, exhibited a significant zone of inhibition, indicating its effectiveness. The plant extract demonstrated a moderate zone of inhibition (14.33 mm) against *Escherichia coli*. However, Gentamycin exhibited a larger zone of inhibition, indicating superior antibacterial activity against *E. coli*.(Nagendra *et al.*,2022)

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The plant extract also showed moderate antibacterial activity against *Klebsiella sp*, with a zone of inhibition of 12.67 mm. Gentamycin, once again, exhibited a larger zone of inhibition against this bacterium. The plant extract displayed a zone of inhibition of 8.67 mm against *Candida albicans*, which is a fungus. While the effect was relatively modest, it suggests some antifungal activity. It's worth noting that Gentamycin is not effective against fungi, which explains the absence of a zone of inhibition (Zegeye *et al.*,2022 and Gitirana *et al.*,2023).

4.3 Minimum Inhibition Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *Vernonia amygdalina* Stem Extract

The results also provide information on the Minimum Inhibition Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *Vernonia amygdalina* stem extract against the tested microorganisms. The MIC for both *Salmonella typhi* and *Escherichia coli* was determined to be 25 mg/ml. This concentration effectively inhibits the growth of these bacteria. The MBC for both organisms was found to be 100 mg/ml, indicating that a higher concentration is required to kill the bacteria rather than just inhibiting their growth. The MIC for *Klebsiella sp* was also 25 mg/ml, similar to *S. typhi* and *E. coli* (Gitirana *et al.*,2023). However, the MBC for *Klebsiella sp* was lower, at 50 mg/ml, indicating that a lower concentration is needed to kill this bacterium compared to *S. typhi* and *E. coli*. The MIC for *Candida albicans* was 50 mg/ml, indicating that this concentration inhibits fungal growth. The MBC was not determined, which suggests that the extract may not have a fungicidal effect within the tested concentration range (Zegeye *et al.*,2022).

In conclusion, the results from suggest that *Vernonia amygdalina* stem extract has some antimicrobial activity, particularly against bacterial species like *S. typhi*, *E. coli*, and *Klebsiella sp*, with varying degrees of effectiveness. However, it appears to be less effective against *Candida albicans*. The MIC and MBC values provide insights into the concentration levels required to inhibit or kill these microorganisms. These findings underscore the potential of *Vernonia amygdalina*



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as a source of natural antimicrobial agents, although further studies are needed to understand its mechanisms of action and potential applications in medicine and healthcare.

5.0 Conclusion and Recommendation

In conclusion, the results from this study suggest that *Vernonia amygdalina* stem extract has some antimicrobial activity, particularly against bacterial species like *S. typhi*, *E. coli*, and *Klebsiella* sp, with varying degrees of effectiveness. However, it appears to be less effective against *Candida albicans*. The MIC and MBC values provide insights into the concentration levels required to inhibit or kill these microorganisms. These findings underscore the potential of *Vernonia amygdalina* as a source of natural antimicrobial agents. Although further studies are needed to understand its mechanisms of action and potential applications in medicine and healthcare.

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Standard And Clinically Isolated
pathogenic Bacteria Isolated From
University Of Gondar
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