BIOLOGY, MEDICINE, & NATURAL PRODUCT CHEMISTRY ISSN 2089-6514 (paper)
Volume 14, Number 1, April 2025 | Pages: 519-527 | DOI: 10.14421/biomedich.2025.141.519-527 ISSN 2540-9328 (online)

Phytochemicals Composition and Anti-bacterial Activity of Methanol Leaves Extract of *Vernonia amygdalina*

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Manuscript received: 23 Maret, 2025. Revision accepted: 23 July, 2025. Published: 01 August, 2025.

Abstract

Bacterial infections have been a major health challenge associated with high morbidity and mortality rates. Plants and their metabolite constituents are important in local therapies and drug synthesis. This study aimed to evaluate the phytochemical composition and anti-bacterial activity of the methanol leaves extract of *Vernonia amygdalina*. Phytochemicals in the extract were estimated using standard analytical methods. The antibacterial activity test of the plant extract was carried out using the agar diffusion method. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined using the tube dilution method and sub-culturing technique, respectively. The extract contains significant amount of alkaloids (47.44 %), flavonoids (16.60 %), tannins (4.35 %), saponins (12.28 %), steroids (0.86%), and glycosides (0.18%). The extract exhibited significant ($p < 0.005$) inhibitory effect against *Staphylococcus aureus, Streptococcus agalactiae,* and *Lactobacillus rhamnosus* isolates with MIC and MBC values of 12.45 and 25.51 mg/mL, 22.03 and 44.84 mg/mL, and 31.64 and 63.95 mg/mL, respectively. The methanol leaves extract of *Vernonia amygdalina* demonstrated an inhibitory effect against *Staphylococcus aureus, Streptococcus agalactiae,* and *Lactobacillus rhamnosus* isolates with low minimum inhibitory concentration values.

Keywords: Antibacterial activity; Bacterial infection; Phytochemicals; Vernonia amygdalina.

INTRODUCTION

Bacterial infections remain a public health problem associated with high morbidity and mortality rates in the world. In general, microbial infections cause over 13 million deaths every year worldwide (Abreu *et al.,* 2017). It has been predicted that by 2050 bacterial infections alone will cause 10 million deaths each year worldwide (Luo *et al.,* 2024; Halawa *et al.,* 2024). The emergence of antibacterial drug resistance remains a major challenge in the treatment of bacterial infections. Several bacteria demonstrate rapid and high resistance development to various antibiotics (Pacheco *et al.,* 2022; Tiseo *et al.,* 2022). It was predicted that almost all bacteria will be resistant to the majority of the current antibiotics within 25 years (Decker *et al.,* 2024; Luo *et al.,* 2024). This will increase the high mortality rates associated with bacterial infections. However, conventional antibiotics have serious side effects and are expensive to purchase by the majority of the communities worldwide. Hence, there is need for finding highly effective, accessible, safe, and affordable treatments to address these challenges.

Plants and herbs have been used in the traditional treatment of several diseases for many years ago. Plants and herbs have been reported to demonstrate various medicinal properties and pharmacological activities, including antioxidant activity (Ibrahim *et al.,* 2024), antimicrobial activity (Dagne *et al.,* 2023), antiulcer activity (Abubakar *et al.,* 2020a; 2020b; 2021), antidiabetic activity (Shubham *et al.,* 2021), antihypertensive activity (Sultana & Muhammad, 2017), antitrypanosomal activity (Goronyo *et al.,* 2022), and analgesic activity (Abubakar *et al.,* 2024). Many medicinal plants have been reported to demonstrate inhibitory effects against bacteria and modulate microbial antibiotic resistance (Dagne *et al.,* 2023; Gonfa *et al.,* 2022; Degu *et al.,* 2021). Medicinal plants constitute various phytochemicals that are important in drug synthesis and development (Abubakar *et al.,* 2021). The medicinal properties and pharmacological activities of plants and herbs are attributed to their phytoconstituents. Identification and quantification of plants phytochemicals are important to ensure the

effectiveness of phytomedicines (Gupta *et al.,* 2023; Adhikari PP, Paul, 2018). Natural products particularly plants and herbs are easily access by most of communities in the world, less expensive to purchase and have less side effects than conventional treatments.

Vernonia amygdalina is a perennial plant and a member of the family Asteraceae and the genus Vernonia (Ijeh & Ejike, 2011). The plant is commonly called 'bitter leaf' due to its bitter to taste. *Vernonia amygdalina* is an Africa native plant that is abundantly found in sub-Saharan African countries (Echem & Kabari, 2013). *Vernonia amygdalina* has been used by many communities around the world as a chewing stick for promoting oral hygiene. The plant has also been used as a bittering agent and antimicrobial agent in food industries. *Vernonia amygdalina* is locally used in the management of many diseases including infections, malaria, diabetes, dyspepsia, pains, inflammation, gout, tonsillitis, dysentery, constipation, and gastrointestinal diseases (Girma *et al.,* 2022; Mekonnen *et al.,* 2022; Habtamu & Melaku, 2018). However, the plant has been reported to demonstrate anti-sickling activity, plaque development inhibitory activity, and regulated hemostasis activities (Satria *et al.,* 2023). In Nigeria, *Vernonia amygdalina* has been cultivated in many local communities and is locally known as 'Shuwaka' (Hausa), 'Onugbu' (Igbo) and 'Ewuro' (Yoruba). Leaves of the plant have been used in preparation of bitter leaf soup in the country especially in the local communities where the plant is mostly available. Also, aqueous extracts of the plant have been consumed by the communities as tonics for the management of several diseases. This study was conducted to evaluate the phytochemical composition and anti-bacterial activity of methanol leaves extract of *Vernonia amygdalina*.

MATERIALS AND METHODS

Drugs and Chemicals

Amoxicillin manufactured by Amagesan (Ritsert, Ger. Germany) was purchased from the pharmacy unit of Federal Medical Center Owerri, Imo, Nigeria. The chemicals manufactured by Cormart Nigeria Limited (Lagos, Nigeria) and Maoming Xiongda Chemical Co., Limited (Guangdang, China) were used in the current study. All the chemicals were of analytical grade.

Plant Material

Fresh leaves of *Vernonia amygdalina* were obtained from Akabo, Ahiazu-Mbaise, in Ikeduru Local Government Area, Imo State, Nigeria. The plant materials were identified by a Botanist at Farm Unit, Centre for Agricultural Research and Extension, Federal University of Technology, Owerri, Imo, Nigeria.

Extracts Preparation

The plant samples were washed thoroughly and then crushed into pieces using mortar and pestle. The samples were dried at room temperature for twenty one days and then grinded to fine powder. The preparation of the extract was done using the method of Abubakar et al. (2021). The powdered leaves (500 g) were soaked in 1.5 L of methanol for three days with constant shaking at two hours intervals. The extract was filtered using bacterial membrane filter and the filtrate was concentrated to dryness. The weight and percentage yield of the extract obtained was 21.2 g and 4.24 %, respectively.

Qualitative Phytochemicals Analysis

Alkaloids Test

Alkaloids presence in the extract was evaluated using Wagner's test as described by Trease and Evans (1989) and Abubakar et al. (2022; 2020). Two miles of the extract was transferred into the test tubes followed by addition of three miles of one percent hydrochloric acid. The contents were incubated at 60 ºC for twenty minutes and then cold at room temperature. A few drops of the Wagner's reagent were added into the test tube. A reddish-brown precipitate formed which indicates the presence of alkaloids.

Flavonoids Test

The sodium hydroxide test was used to identify flavonoids in the extract using the methods of Mosa et al. (2012) and Ibrahim et al. (2024). The extract (2 mL) was treated with one mile of ten percent sodium hydroxide solution. The development of an intense yellow colour later colourless following the addition of HCl solution showed the presence of Flavonoids in the extract.

Glycosides Test

The assessment of glycosides in the extract was done using Salkowski's test as stated by Mosa et al. (2012) and Ibrahim et al. (2024). An equal volume of the extract and one percent sulphuric acid (5 mL) was taken into a test tube, boiled for fifteen minutes and then cold. Five miles of Fehling's solution A and B were added into the mixture after the addition of ten percent sodium hydroxide solution. Glycosides were detected by the development of a brick red precipitate.

Tannins Test

Tannins in the extract were detected using Ferric chloride test described by Trease and Evans (1989) and Ibrahim et al. (2024). The extract (2 mL) was transferred into the test tube containing two miles of five percent ferric chloride solution. Blue-green colour was formed which showed the presence of tannins in the extract.

Saponins Test

Identification test of saponins in the extract was carried out using the Froth test recorded by Mosa et al. (2012) and Trease and Evans (1989). The extract (2 mL) was taken into the test tube and then diluted with two miles of

distilled water. The contents were thoroughly mixed and settled for half an hour. Saponins in the extract were identified by observing stable persistent froth.

Steroids Test
Steroids in the extract were identified using the method of Trease and Evans (1989) and Ibrahim et al. (2024). An equal volume (5 mL) of chloroform and sulphuric acid solution was added into the test tube containing one mile of the extract. The violet colour later blue-green indicated the presence of steroids in the extract.

Quantitative Phytochemicals Analysis
Alkaloids Test
The alkaloid content in the extract was estimated using the method of Trease and Evans (1989) and Ibrahim et al. (2024). Twenty miles of 2 mM H_2SO_4 was added into the test tube containing the extract. The mixture was shaken and then partitioned with ether. The upper phase liquid was treated with NH_3 solution followed by extraction with chloroform solvent. The alkaloid residue obtained after drying the extract was weighed and the alkaloid content was obtained.

Flavonoids Test
The total flavonoids in the extract were determined using the method described by Harborne (1973) and Ibrahim et al. (2024). Five milligrams of the extract was treated with fifty miles of 2 M hydrochloric acid, boiled for thirty minutes and then cooled. The mixture was filtered and the filtrate was treated with fifty of ethyl*acetate solution. The mixture was filtered and then concentrated to dryness. The dried flavonoid residue was weighed and the flavonoid content was obtained.

Glycosides Test
Ten miles of the extract was mixed with fifty miles of chloroform in a conical flask. The contents were mixed, filtered, and the filtrate was treated with ten mile of pyridine and two mile of two percent sodium nitroprusside. The mixture was shaken for ten minutes and then treated with 3mL of twenty percent NaOH. The absorbance was measured spectrophotometerically at 510 nm wavelength and the glycosides content was obtained (Ibrahim *et al.,* 2024).

Tannins Test
AOAC (1999) was employed to quantitative estimate of tannins in the extract. Two miles of Folin-Denis reagent and one mile of sodium carbonate solution were added into the flask containing the extract and the prepared standard solution. The contents were allowed to stand at 25 ^oC for thirty minutes. The absorbance was read using spectrophotometerically at 760 nm wavelength. The amount of tannin in the extract was obtained from the prepared standard curve.

Saponins Test
The saponins content in the extract was estimated using the method of El-Olemyl et al. (1994). One hundred and fifty miles of fifty percent ethanol was added into the flask containing five grams of the extract. The contents were boiled for half hour, cooled and then filtered. The filtrate was treated with charcoal, heated for half hour, and then filtered. One hundred and fifty miles of acetone was added into the filtrate and the mixture was filtered. The filter paper was quickly taken into the desiccator containing anhydrous $CaCl_2$ solution. The saponins residue was weighed and saponins content was obtained.

Steroids Test
Quantitative analysis of steroids in the extract was performed using the method of Trease and Evans (1989) and Ibrahim et al. (2024). Two miles of sulphuric acid and ferric chloride were added into the test tube containing one mile of the extract. Two miles of potassium hexacynoferrate (III) was added into the test tube and heated at 70 °C for thirty minutes. The absorbance was measured spectrophotometerically at 780 nm wavelength and the steroid content was obtained.

Bacterial Isolates
The bacterial isolates used in this study were *Staphylococcus aureus, Streptococcus agalactiae,* and *Lactobacillus rhamnosus*. The pure bacterial isolates were obtained from the Microbiology Laboratory, of Federal Medical Center Owerri, Imo, Nigeria. The isolates were sub-cultured from the original agar plates and transferred to MacConkey agar and Selenite F broth media plates. The isolates were re-identified and confirmed using the Gram staining technique and standard biochemical tests.

Antibacterial Activity Test
The antibacterial susceptibility test of the plant extract was carried out using the agar diffusion method as described by Kirby-Bauer (1996) with some modifications. The inoculums of the bacterial isolates were standardized by taking loopful colonies of the bacterial isolates into nutrient agar plates. The plates were incubated overnight at 37 °C and then the turbidity developed was equally adjusted to Mac Farland standard (0.5) value. The agar discs of diameter 6 mm were aseptically dipped in 2 mL of 25, 50, 75 and 100 mg/ml extract for sixty seconds and then placed over the separate nutrient agar plates each containing the labeled test isolates. The plates were incubated at ambient temperature for half an hour and then at 37 °C overnight. The plates were observed for the development of the zone of inhibition which was measured in millimetres using the meter rule. Amoxicillin (30 µg) (Udensi *et al.,* 2025) was used as a reference standard control. The experiment was carried out three times and the mean results were calculated.

Determination of Minimum Inhibitory Concentration
The minimum inhibitory concentration (MIC) of the extract against the isolates was determined using the tube dilution method. A loopful of the bacterial isolates was transferred into the test tubes containing 5 mL of nutrient broth. One mile of the extract (100, 75, 50, and 25 mg/ml) was added into the test tubes. A test tube containing only broth and extract was used as control. The test tubes were incubated at 37°C for one day and the bacterial growth was observed. The lowest concentration of the extract that inhibited the observed bacterial growth was considered the MIC of the extract.

Determination of Minimum Bactericidal Concentration
The minimum bactericidal concentration (MBC) of the extract was evaluated using the sub-culturing method. The MIC broth culture tubes with no growth observed were sub-cultured onto fresh nutrient agar plates. The plates were incubated at 37 °C for two days and then observed for visible growth. The lowest extract concentration with no visible growth was considered the MBC of the extract.

Statistical Analysis
All the analyses were performed in triplicate. The results were statistically analyzed using Statistical Package for Social Sciences (SPSS) version 22 software and expressed as mean ± SEM. Significant differences between the mean results were computed at a 95 % confidence level by One-way analysis of variance (ANOVA). Significance was considered by two-tailed ($p < 0.05$) values.

RESULTS

Phytochemicals Composition of Methanol Leaves Extract of *Vernonia amygdalina*
Table 1 shows the phytochemical screening of the methanol leaves extract of *Vernonia amygdalina*. The result showed that methanol leaves extract of *Vernonia amygdalina* contain high amount of alkaloids and flavonoids. A moderate amount of tannins and saponins was observed in the extract. The extract exhibited a low amount of steroids and glycosides (Table 1).

Table 1. Qualitative Phytochemicals Screening of Methanol Leaves Extract of *Vernonia amygdalina.*

Phytochemical	Va Extract
Alkaloids	+++
Flavonoids	+++
Tannins	++
Saponins	++
Steroids	+
Glycosides	+

+++ (High amount), ++ (Moderate amount), + (Low amount)

Figure 1 shows the quantitative phytochemicals constituents of methanol leaves extract of *Vernonia amygdalina*. The methanol leaves extract of *Vernonia amygdalina* contains a significant amount of alkaloids (47.44 %), flavonoids (16.60 %), tannins (4.35 %), saponins (12.28 %), steroids (0.86%), and glycosides (0.18%) (Figure 1).

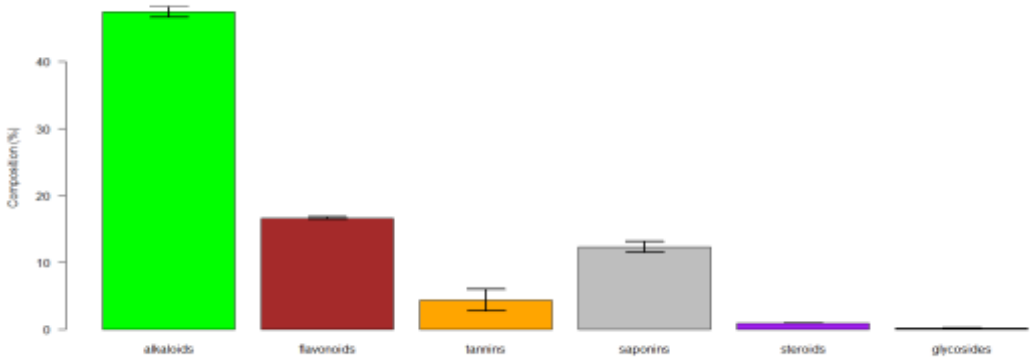

Figure 1. Quantitative Phytochemicals Constituents of Methanol Leaves Extract of *Vernonia amygdalina*
Values are expressed as mean ± SD (n = 3).

Inhibitory Effect of Methanol Leaves Extract of *Vernonia amygdalina* on Some Bacterial Isolates
Figure 2 shows the inhibitory effect of methanol leaves extract of *Vernonia amygdalina* on *Staphylococcus aureus* isolate. The methanol leaves extract of *Vernonia amygdalina* demonstrated inhibitory effect against *S. aureus* in dose dependent manner. At 25 mg/mL, 50 mg/mL, 75 mg/mL, and 100 mg/mL the extract demonstrated significant ($p < 0.05$) inhibition of 7.36 mm, 14.43 mm, 15.47 mm, and 17.16 mm against the isolate, respectively. However, the highest significant inhibition (17.16 mm) against the isolate was observed at 100 mg/mL dose of the extract which is more than the

zone of inhibition (16.12 mm) exhibited by the standard drug, amoxicillin (Figure 2).

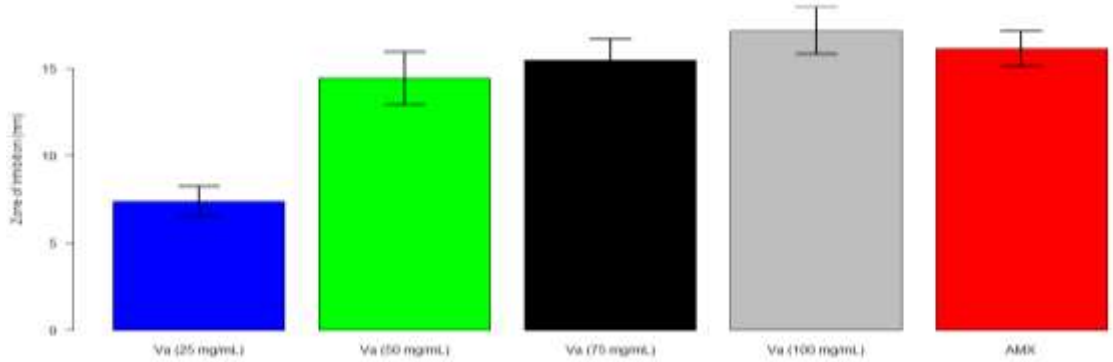

Figure 2. Inhibitory Effect of Methanol Leaves Extract of *Vernonia amydalina* on *Staphylococcus aureus* Isolate
Data are expressed as mean ± SD (n = 3), Va (*Vernonia amydalina*), AMX (Amoxicillin).

The inhibitory effect of methanol leaves extract of *Vernonia amydalina* on *Streptococcus agalactiae* isolate is shown in Figure 3. A dose dependent inhibitory effect of the methanol leaves extract of *Vernonia amydalina* against *Streptococcus agalactiae* isolate was observed. The zone of inhibition was not observed at 25 mg/mL dose of the extract. A significant ($p < 0.05$) inhibition of 6.76 mm, 12.47 mm, and 16.83 mm against the isolate was observed at 50 mg/mL, 75 mg/mL, and 100 mg/mL dose of the extract, respectively. Interestingly, at 100 mg/mL the extract demonstrated significant inhibition (16.83 mm) against the isolate more than the standard drug, amoxicillin (14.50 mm) (Figure 3).

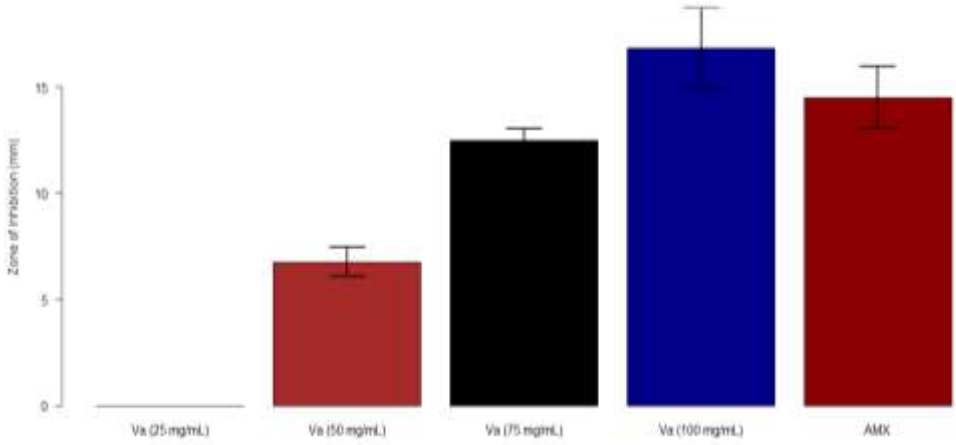

Figure 3. Inhibitory Effect of Methanol Leaves Extract of *Vernonia amydalina* on *Streptococcus agalactiae* Isolate
Values are given as mean ± SD (n = 3), Va (*Vernonia amydalina*), AMX (Amoxicillin).

Figure 4 indicates the inhibitory effect of methanol leaves extract of *Vernonia amydalina* on *Lactobacillus rhamnosus* isolate. The results showed that methanol leaves extract of *Vernonia amydalina* significantly ($p < 0.05$) inhibited *Lactobacillus rhamnosus* isolate in dose dependent manner. At 25 mg/mL, the extract did not showe any inhibitory effect against the isolate. At 50 mg/mL, 75 mg/mL, and 100 mg/mL, the extract exhibited a significant ($p < 0.05$) inhibition of 9.45 mm, 12.12 mm, and 17.19 mm against the isolate, respectively. However, at 100 mg/mL the inhibitory effect of the extract against the isolate (17.19 mm) was more than the inhibitory effect (15.27 mm) of the standard drug, amoxicillin (Figure 4).

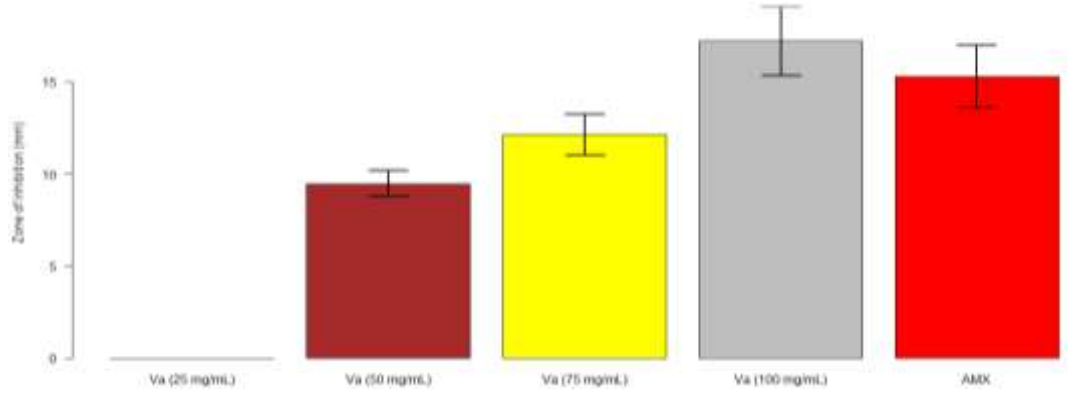

Figure 4. Inhibitory Effect of Methanol Leaves Extract of *Vernonia amydalina* on *Lactobacillus rhamnosus* Isolate
Results are expressed as mean ± SD (n = 3), Va (*Vernonia amydalina*), AMX (Amoxicillin).

MIC and MBC Value of the Methanol Leaves Extract of *Vernonia amydalina* Against the Bacterial Isolates

The MIC and MBC values of the methanol leaves extract of *Vernonia amydalina* against the bacterial isolates are shown in Figure 5. The extract demonstrated MIC and MBC values of 12.45 and 25.51 mg/mL, 22.03 and 44.84 mg/mL, and 31.64 and 63.95 mg/mL against the *Staphylococcus aureus, Streptococcus agalactiae,* and *Lactobacillus rhamnosus* isolates, respectively.

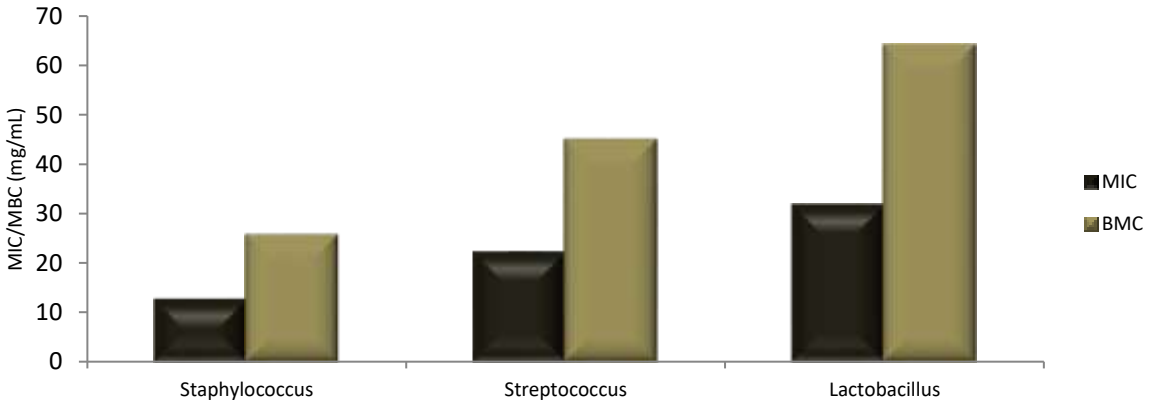

Figure 5. MIC and MBC Value of the Methanol Leaves Extract of *Vernonia amydalina* Against the Bacterial Isolates
Results are expressed as mean ± SD (n = 3).

DISCUSSION

In this study, the methanol leaves extract of *Vernonia amygdalina* demonstrated inhibitory effect against *Staphylococcus aureus, Streptococcus agalactiae,* and *Lactobacillus rhamnosus* isolates. This finding is in line with the findings of the relevant study which showed that ethanol leaf extract of *Vernonia amygdalina* demonstrated inhibitory effect against S. aureus and Shigella (Ogundare, 2011). A research study by Zubairu et al. (2019) showed that *Vernonia amygdalina* leaf extract exhibited an anti-bacterial effect against E. coli, S. aureus and S. typhi. This finding indicated that methanol leaves extract of *Vernonia amygdalina* contains a significant amount of secondary metabolites which include alkaloids, flavonoids, tannins, saponins, steroids, and glycosides. Results of this study are in agreement with the findings of similar studies by Ali et al. (2019) and Olumide et al. (2019), who found that *Vernonia amygdalina* contained significant amounts of phytochemicals such as saponins, alkaloids, flavonoids, terpenoids, tannins, steroids, and phenols. Significant amounts of alkaloids, glycosides, steroids, flavonoids, tannins, and terpenoids were reported in *Vernonia amygdalina* (Senthilkumar *et al.,* 2018; Tian *et al.,* 2023).

The anti-bacterial activity of the methanol leaves extract of *Vernonia amygdalina* could be attributed to the secondary metabolites present in the plant extract. Phytochemicals such as alkaloids, flavonoids, terpenoid, phenolics, and tannin have been shown to demonstrate antimicrobial activity (Usunomena & Ngozi, 2016). Alkaloids and their synthetic derivatives have the potential to inhibit bacterial growth (Atinga *et al.,* 2021). Alkaloids isolated from the plant's extract exhibited significant antibacterial activity due to its inhibitory effect on bacterial topoisomerase causing DNA damage (Khameneh *et al.,* 2029). Berberine, an alkaloid isolated

from different plants extracts demonstrated inhibitory effect against Gram-negative and Gram-positive microorganisms (Wu *et al.,* 2022; Xia *et al.,* 2022). The study showed that berberine exhibited MIC value of 51 µg/mL against S. aureus strain ATCC 25923 (Wu *et al.,* 2022). The antimicrobial effect of berberine was due to its efflux pump inhibitory potential (Li & Ge, 2023) or its potential to influence the activity of the shikimic acid pathway in bacteria causing oxidative damage and disrupting integrity of cell membranes (Wu *et al.,* 2022; Xia *et al.,* 2022). Phenolic compounds including phenols and flavonoids have been documented as potent antimicrobial agents (Ecevit *et al.,* 2022; Porras *et al.,* 2021). Panduratin A isolated from different plant extracts showed anti-bacterial activity against S. mutans, S. sobrinus, P. intermedia, P. loescheii, and P. gingivalis (Park *et al.,* 2005). Clinical studies showed that panduratin A exhibited anti-bacterial effect against many isolates of Staphylococcus strains (Rukayadi *et al.,* 2009).

In the present study, the extract exhibited low minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values against *Staphylococcus aureus, Streptococcus agalactiae,* and *Lactobacillus rhamnosus* isolates Antimicrobial activity of agent or substance is determined by its minimum inhibitory concentration values. Minimum inhibitory concentration (MIC) refers to the lowest concentration of tested antimicrobial agent that inhibits or kills the growth of tested microbe (Balouiri *et al.,* 2016). Plant extracts with MIC values below 100 µg/mL are considered to demonstrate high antibacterial activity, while from 100 to 625 µg/mL moderate activity, and above 625 µg/mL low activity (Famuyide *et al.,* 2019; Dzotam & Kuete, 2017; Voukeng *et al.,* 2016).

CONCLUSION

The methanol leaves extract of *Vernonia amygdalina* contains significant amounts of phytochemicals. The extract demonstrated an inhibitory effect against *Staphylococcus aureus, Streptococcus agalactiae,* and *Lactobacillus rhamnosus* isolates with low minimum inhibitory concentration values. The inhibitory effect of the extract against the *Staphylococcus aureus, Streptococcus agalactiae,* and *Lactobacillus rhamnosus* isolates could be attributed to the various phytochemicals present in the extract.

Conflict of Interest: The authors declared that there was no conflict of interest.

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