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Phytochemical Screening and In-Vitro Antibacterial Activities of the Ethanol Extract of *Vernonia Amygdalina* (Bitter Leaf) *on Staphylococcus Aureus*

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ABSTRACT

Natural products, including medicinal plants, have drawn a lot of interest because of their ability to fight infections that are resistant to drugs. The present study was aimed at investigating the phytochemical and in vitro antimicrobial activities of the ethanolic extract of Vernonia amygdalina (bitter leaf) on Staphylococcus aureus. Standard procedures were used to carry out the phytochemical screening. The agar-well diffusion method was employed to ascertain the plant extract's antibacterial activity. Using a transparent metre rule, the diameter zone of inhibition of the extract against the bacterial isolates was found. Flavonoids, alkaloids, tannins, saponins, and cardiac glycoside were found in the extract as determined by the qualitative and quantitative phytochemical screening. Alkaloids made up the majority of the constituents, accounting for about 11.00%, while flavonoids, saponins, and tannins made up 10.00, 6.00, and 2.90%, respectively, and cardiac glycoside was the least common, making up 1.67%. The result shows the extract's effectiveness against S. aureus. At the highest extract concentration level of 200 mg/ml, the highest activity (11.3 ± 1.14 mm) was seen, whereas the lowest activity was recorded at the lowest concentration (12.25 mg/ml). It is determined that the antibacterial properties of V. amygdalina may be attributed to its potential bioactive phytochemicals. It is concluded that V. amygdalina has potential bioactive phytochemicals that are responsible for its antibacterial activities.

KEYWORDS: *antibacterial activities, extract, phytochemical, Staphylococcus aureus, Vernonia amygdalina, zone of inhibition*

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1. INTRODUCTION

The development of germs that are resistant to several drugs has become a major worldwide health issue in recent times, hence requiring the exploration of substitute antimicrobial medicines. Natural products including medicinal plants have drawn a lot of interest because of their ability to fight infections that are resistant to drugs (Subramani *et al*., 2017). The human pathogen Staphylococcus aureus has become a central focus of this epidemic, highlighting the urgent need for innovative therapeutic strategies (Hussein *et al*., 2023). In response to this challenge, exploration into the realm of natural products, particularly plant-derived compounds, has gained prominence for their inherent diversity of bioactive molecules with potential antibacterial properties. The shrub *Vernonia amygdalina Del.* is medium-sized and has a lot of bitter elements throughout. It is a member of the Asteraceae family. In Yoruba, Western; Igbo, Southern; and Hausa, Northern Nigeria, the plant is referred to as "Ewuro," "olugbu," and "shuwakaa," respectively (Okeke *et al.,* 2015). In Southern Nigeria, it is a commonly utilised native plant for both nutritional and medicinal purposes (Oladunmoye and Kehinde, 2011). As reported by Ruangchawalee 2018, *V. amydalina* is a widely consumed vegetable in many African countries. Besides its culinary uses, it possesses notable medicinal properties and has been traditionally used to manage various diseases (Ogidi *et al*., 2019). Studies have shown that bitter leaf extracts possess antimicrobial activities against both

bacterial and fungal pathogens (Ali *et al.,* 2019). The active compounds responsible for these activities include alkaloids, flavonoids, and terpenoids (Kaushik *et al.,* 2021). Bitter leaf extracts have also exhibited antiviral properties against certain viral strains, although this area requires further investigation (Ikwebe *et al.,* 2023). Although plant secondary metabolites like alkaloids, flavonoids, terpenoids, and phenolic compounds frequently have strong bioactive properties, the main focus of this study is on the phytochemical composition and antibacterial effects of extracts derived from these botanical entities against Staphylococcus aureus. The goal of the research is to provide significant knowledge to the current discussion on alternate treatment approaches by examining the antibacterial activity of bitter leaf extracts against Staphylococcus aureus.

2. MATERIALS AND METHODS

2.1. Sample Collection

The leaves of *Vernonia amygdalina* were purchased from the Kazaure market in Jigawa State, Nigeria, while the preparation was done at the biology laboratory of Hussaini Adamu Federal Polytechnic Kazaure. The leaves were washed and placed in a neatly washed and dried tray. The leaves were then shaded, dried, and crushed to a coarse powder using a neatly washed local mortar and pestle. The powdered form of the leaves was placed in a sterilised container, labelled, and properly stored. The plant materials were authenticated by the Botanist of Science Laboratory Technology Department, Hussaini Adamu Federal Polytechnic Kazaure.

2.2. Test Organism

The bacterial culture of *Staphylococcus aureus* used in this study was obtained from the laboratory section of the Department of Microbiology, Bayero University, Kano State, Nigeria, and used as an antimicrobial test organism. Their identity was confirmed using cultural, morphological, and biochemical tests as previously described by NCCLS 2000.

The bacterial isolates were maintained on nutrient agar slants at 40 °C.

2.3. Biochemical Identification of the test organism (*S. aureus*)

The *S. aureus* was placed on Mannitol Salt Agar (MSA) for 18 hours. Smooth circular colonies with a yellow colour indicate a positive result for *S. aureus* (Unegbu *et al*., 2020).

2.4. Standardization of the Test Organism

The test organism (*S. aureus*) was standardised by the use of 24-hour-old broth cultures prepared by inoculating the test organism into 5ml of nutrient broth, and the culture was adjusted to obtain 0.5 McFarland turbidity equivalent standards (NCCLS 2000)

2.5. Preparation of Ethanolic Extract

Ten grams of dried grinded leave powder was dissolved in 100 ml of 95% ethanol for 24 hours. The mixture was filtered using Whatman's filter paper No. 1 to obtain solution free of solids. The filtrate was placed into evaporator to drive-off the solvent and stored at 4°C.

2.6. Extract Dilution

After preparation of the extract as described, the hot and cold aqueous and the ethanolic extract were reconstituted using sterile distilled H20 to obtain concentrations of 200, 100, 50, 25, and 12.5 mg/ml

2.7. Sterility Test of leave Extract

The leave extract was tested for growth of contaminants. Three plates of Nutrient Agar were prepared, on each of the plates, 1 ml of the plant extract were inoculated aseptically and incubated at 37^0C respectively for 24hrs. The plates were observed for any sign of visible growth. No growth on the plates signified that the extracts were sterile.

2.8. Qualitative Phytochemical Screening of *V. amygdalina*

The analysis determines the bio-active compounds that are present in the leaves of *V. amygdalina: examples* Carbohydrate, Flavonoid, Tannins, Cardiac glycoside, Anthraquinones, Steroid, Terpenoids and Alkaloid respectively. This was done following the method described by Trease and Evans, 2002.

Test for Carbohydrate (Molisch's Test)

The crude extract of the plant leaves was treated with Molisch reagent and concentrated Sulphuric Acid Corrosion. The reddish violet ring shows the presence of carbohydrates.

Test for Flavonoids (Shinoda's Test)

1 ml of the crude extract, was transferred into a test tube, then few drops of concentrated HCl acid added, followed by 0.5mg of magnesium ribbon and shaken. Emergence of pink coloration indicates the presence of flavonoids.

Test for Tannins (Ferric Chloride Test)

In a 1 ml solution of each crude extract in a test tube, a 1% gelatin solution containing ferric chloride was added and shaken. The formation of a bluish-black colour showed the presence of phenols.

Test for Cardiac glycoside (Keller Killian's Test)

10 ml of *V. amygdalina* extract was transferred separately into a test tube. Then 4 ml of glacial acetic acid was added, followed by 1 drop of 2% FeCl3

solution, and shaken. Then 1 ml of concentrated H_2SO_4 was added to the mixture. The procedure was carried out separately for each extract. A brown ring formed between the two layers indicates the existence of cardiac glycosides.

Test for Antraquinones (Bontrager's Test)
10 ml of benzene was added to 6g of the crude extract in a conical flask and soaked for 10 minutes, then filtered. A 10 ml of 10% ammonia solution was added to the filtrate and shaken vigorously for 30 seconds. The appearance of pink in the ammonia phase showed the occurrence of anthraquinone

Test for Steroids (Libermann Bruchard's Test)
5 ml of crude extract of *V. amygdalina* was transferred into a test tube. 2 ml of chloroform and concentrated H_2SO_4 were added. The appearance of a red colour at the lower chloroform layer signified the presence of steroids.

Test for Terpenoids (Salkowski test)
About 0.5 ml of the extract was evaporated to dryness in a water bath and heated with 3 ml of concentrated sulfuric acid for 10 minutes in a water bath. The grey colour indicates the presence of terpenoids.

Test for Alkaloid (Dragendoff's Test)
In this test, 1 ml of extract was added to 1 ml of potassium bismuth iodide solution (Dragendoff's reagent) and shaken. An orange-red precipitate formed, indicating the presence of alkaloids.

2.9. Quantitative Phytochemical Analysis

Total Flavoniods
In this test, powdered sample of *V. amygdalina* crude extract, were mixed with 50ml of 80% aqueous methanol in 250ml beaker, and allowed to stand for 24 hours at room temperature. The supernatant layer was discarded, and the residue was re-extracted three times with 50ml of ethanol. The solution produced was filtered using Whatman filter paper number 42 (125mm). The filtrates were later evaporated to dryness over a water bath. The content was cooled in a desiccator and weighed until constant weight was obtained (Boham and Koupai-Abyazan, 1974). The percentage yield for flavonoid was calculated as follows: % Yield = Weight of flavonoid / Weight of sample ×100.

Total Alkaloids
The sample of V. amygdalina crude extract was taken into a 250ml beaker. Then 200 ml of 10% acetic acid in ethanol was transferred, covered and allowed to stand for 4 hrs. The mixture was filtered and then concentrated over water bath to ¼ of the initial volume. Later, few drops of concentrated HN_4OH were added to the extract until complete precipitate was formed. The whole mixture was allowed to settle and the precipitate was collected and washed with dilute HN_4OH and then filtered. The residue produced was dried and weighed as alkaloid (Harbone, 1973).

Total Tannins
In this test, the sample of *V. amygdalina* crude extract, 500 mg weighed into a 50 ml plastic bottle. About 50 ml of distilled water was added and shaken for 1 hour. This was filtered into a 50 ml volumetric flask and made up to the mark. Subsequently, 5 ml of the filtrate was pipetted into a test tube and mixed with 2 ml of 0.1 M FeCl3 in 0.I N HCl and 0.008 M potassium ferrocyanide. The absorbance of the mixture was measured at 120 nm within 10 min (Van-Buren and Robinson, 1969). The percentage yield of tannin was calculated using the formula below: Weight of sample (g): Absorbance of sample/Absorbance of standard x Concentration of standard. % Yield = Weight of saponin / Weight of sample ×100.

Total Saponins
V. amygdalina crude extract was placed in conical flask and 100ml of 20% aqueous ethanol was added. The mixture was heated on water bath at 55ºC for 4 hours with continuous stirring. It was filtered and the residue re-extracted with another 200 ml of 20% ethanol. The combined extract was reduced to 40 ml over water bath at about 90ºC. The concentrate was transferred into a 250 ml separation funnel and 20 ml of diethyl ether added and vigorously shaken. The aqueous layer was recovered while the ether layer was discarded. The aqueous layer was purified and 60 ml of n-butanol was added. The n-butanol extract washed twice with 10 ml of 5 % aqueous NaCl. The remaining solution was evaporated and dried in the oven to a constant weight (Obadoni and Ochuko, 2002). The saponin content was calculated using the formula below: % Yield = Weight of saponin / Weight of sample ×100.

Total Cardiac Glycosides
In this test, 10% of *V. amygdalina* extract, was mixed with 10 ml freshly prepared Baljet's reagent (95 mL of 1% picric acid + 5 mL of 10% NaOH). The mixture was allowed to stand for 1 hour. This is followed by dilution with 20 mL distilled water and the absorbance was measured at 495 nm using UV spectrophotometer (Solich et al., 1992). The percentage yield of cardiac glycosides was calculated as follows: Weight of sample (g): Absorbance of sample/Absorbance of standard x Concentration of standard. % Yield = Weight of saponin / Weight of sample ×100.

2.10. Antimicrobial Assay

Minimum inhibitory Concentration (MIC) The antimicrobial assay of the plant leaf Extracts test

was carried out on the test isolates using the agar-well diffusion technique. The isolates were inoculated on the surface of freshly gelled nutrient agar plates by streaking using a sterilised wire loop. Wells were aseptically bored on each agar plate using a sterile Cork borer (6 mm), and wells were properly labelled. Fixed volumes (0.1 ml) of the different concentrations of the extracts were then introduced into the wells in the plates, respectively. The last well was used as negative control well (filled with sterile water). The plates were allowed on the bench for 1 hour for pre-dilution of the extract to occur. The plates were then incubated at 37 °C for 24 hours (Oloyede *et al., 2010).* The resulting zone diameter of inhibition was measured using a transparent ruler calibrated in millimetres. The readings were taken to be the zone diameter of inhibition of the bacterial isolate in question at that particular concentration (Unegbu *et al.,* 2020).

2.11. Mode of Action of the Extracts

All plates showing no visible growth on the nutrient agar (NA) indicated the bactericidal effect of the concentration of the extract used. Plates showing light growth indicated the bacteriostatic effects of the extract concentration. Concentrations of the extracts showing moderate and heavy growth were considered to have no inhibitory effect on the organism (Tula *et al.,* 2012).

3. RESULTS

3.1. Phytochemical Screening

The qualitative phytochemical analysis in Table 1 shows that carbohydrates, flavonoids, tannins, cardiac glycosides, antraquinones, steroids, terpenoids, and alkaloids were present in the leaf extract of *V. amygdalina.*

Table 1. The result of the qualitative phytochemical screening of the ethanol leaf extracts of *V. amygdalina*

S/N	Phytochemical Contituents	Inference
1	Carbohydrate	+
2	Saponin	+
3	Flavonoid	+
4	Tannins	+
5	Cardiac glycoside	+
6	Antraquinones	-
7	Steroid	+
8	Terpenoids	+
9	Alkaloid	+

Keys: + = Presence of phytochemical, - = Absence of phytochemical.

The result of the quantitative analysis of the secondary metabolites in Table 2 revealed that alkaloids were found to be the most abundant constituents in *V. amygdalina* leaf, making up about 11.0%, followed by flavonoids, saponins, and tannins, constituting 10.0, 6.0, and 2.9%, respectively.

Table 1. The result of the quantitative phytochemical screening of the ethanol leaf extracts of *V. amygdalina*

S/N	Phytochemical	(%) yield
1	Flavonoid	10.00
2	Alkaloid	11.00
3	Tannins	2.90
4	Saponins	6.00
5	Cardiac glycoside	1.67

3.2. Antibacterial activities of the Extracts

The average antibacterial activity of *V. amygdalina* ethanol leaf extract is presented in Table 3. The results showed that the zones of inhibition recorded by the isolates depended on the concentration of the extracts. The highest zone of inhibition (11.3 mm) was recoded at the 200 mg/ml level of concentration, followed by 5.2 mm under the 50 and 100 mg/ml concentrations, with 12.5 mg/ml having the lowest diameter of inhibition of 2.0 mm. However, the sterile water recorded a zero diameter of inhibition.

Table 3. Antimicrobial Activities of *V. amygdalina*

Isolate	Concentration (mg/ml) zone of inhibition (mm)					
	12.5	25	50	100	200	Control
S. aureus	2.0 ± 0.15	3.6 ± 0.40	5.2 ± 0.32	5.2 ± 0.31	11.3 ± 1.14	0.00

DISCUSSION

The phytochemical screening of the leaf extract of V. amygdalina revealed the presence of carbohydrates, flavonoids, tannins, cardiac glycosides, antraquinones, steroids, terpenoids, and alkaloids. These phytochemicals have been demonstrated to have a range of pharmacological and biochemical effects, be beneficial to human health, and have antioxidant activity (Yu *et al.,* 2021). Numerous investigations have been carried out to identify and evaluate specific bioactive elements found in the leaf extract of *V. amygdalina,* among which are Usunobun & 2015., Ojimelukwe & Amaechi (2019), and Alara & Abdurahman (2019). Flavonoids, saponins, alkanoids, terpenes, tannins, steroids, glycosides, triterpenoids, and other forms of sesquiterpene lactons had been isolated as a consequence of the phytochemical investigations (Kaushik *et al*., 2021).

The findings of these studies were in conformity with the findings of Unegbu *et al.,* 2020. Ali *et al (* 2019 reported that V. amygdalina leaves contain 4.6% Aalkaloids 12.20% flavonoids, 2.7% saponin, 4.8%

steroids, 1.7% terpenoid, 3.6% phenol, and 1.2% tannin. Ndukwe *et al. (*2013 reported 0.47% flavonoids, 2.78% alkaloids, 0.6% saponins, and 0.74% tannins.

Alkaloids are known to play some metabolic roles and control development in the living system (Salam *et al.,* 2023). It also interferes with cell division; hence, the presence of alkaloids in *V. amygdalina* could account for their use as antimicrobial agents.

Flavonoids are also expressed in plants in response to microbial infection, suggesting their antimicrobial activity (Ahmed *et al.,* 2015). The majority of biological actions associated with human cell division and proliferation are caused by saponins found in medicinal plants, which also have unpleasant effects on inflammation. (Ali *et al.,* 2020). The presence of saponin in the leaves of V. amygdalina lends credence to the plant's potential for reducing inflammation. Tannin is known to havpotential2020),ls antiviral activity (Kaczmarek, 2020) As well as Potential prophylactic and therapeutic effect against cancer cells (Ali et al., 2020). The present study showed that the leaves of V. amygdalina possesses antimicrobial potentials against Staphylococcus aureus. Several studies were conducted on antibacterial activity *V. amygdalina* leaf extract (Unegbu *et al.,* 2020 and Ali *et al.,* 2020). Their results show a varying degree of antibacterial activities of the extracts. Based on the finding of this study, the diameter zone of the inhibition zone increases with an increase in the concentration of the extracts. With 11.3 mm recoded at the 200 mg/ml level of concentration and lowest diameter of 2.0 mm recorded at 12.5 mg/ml,. This finding agrees with the work of Unegbo *et al*. (2020), who also reported that a higher concentration produces a higher zone of inhibition. Okeke *et al.* (2023) also reported a similar trend in their work. The antimicrobial substance appears to exert antimicrobial activity by inhibiting the growth of *S. aureus*.

CONCLUSION

The results of this study have demonstrated that the leaf extract of V. amygdalina exhibits antibacterial activity against S. aureus. The activity of the extract against the isolates is attributed to the presence of bioactive compounds in the extract, such as flavonoids, alkaloids, tannins, saponins, and cardiac glycosides. Consequently, the extracts can be used to create new herbal formulations that prevent bacterial infections. Consequently, further research should be conducted to enable the purification of the particular bio-potential chemicals and their subsequent conversion into chemotherapeutic agents.

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