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Phytochemical Analysis and Assessment of Antibacterial Efficacy of *Vernonia amygdalina* (Bitter Leaf) against Some Selected Clinical Bacterial Isolates

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Abstract

Vernonia amygdalina is a plant known to contain substances with reported antimicrobial properties against various microorganisms. This study therefore, aimed at assessing the antibacterial properties of Vernonia amygdalina extract against some clinical bacterial isolates. The bacterial isolates were obtained from General hospital Azare, and the Vernonia amygdalina leaves were purchased at Azare central market. Various compounds, including saponins, glycosides, tannins, flavonoids, steroids, phenolic compounds, and alkaloids, were identified in the plant extract via phytochemical screening. Different concentrations of the V. amygdalina extract (25mg/mL, 50mg/mL, and 100mg/mL) were prepared and screened for antibacterial using disc diffusion method, revealing zones of inhibition of 7.00 mm, 9.00 mm and 4.00 mm against Escherichia coli, Staphylococcus aureus, and Klebsiella pneumoniae respectively, at 100mg/ml of the extract. The MIC of the extract against Escherichia coli and Staphylococcus aureus was 25 mg/mL, whereas 50 mg/mL of the extract was found to be the minimum concentration active against Klebsiella pneumoniae. The study found that the extract exhibited modest antibacterial activity, with different minimum inhibitory concentrations for the bacterial strains.

Keywords: Antibacterial, Phytochemistry, Susceptibility, Vernonia amygdalina, Extract

INTRODUCTION

Vernonia amygdalina, commonly known as bitter leaf, belongs to the *Asteraceae* family and is a local vegetable widely used in African countries. It goes by different names, such as Onugbo or Olubu (in Igbo), Ewuro (in Yoruba) and Shuwaka (in Hausa) (Kokwaro, 2009; Egedigwe, 2010). This shrub, reaching heights of 2-5 meters, features leaves with a width of approximately 6.0mm and is utilized in various culinary dishes and traditional medicine for treating various infections (Nwanjo and Ojiako, 2005). Several studies have highlighted its antimicrobial, antiplasmodial, antitumor, antioxidant, and antihelminthic properties (Izevbigie *et al.*, 2004).

Earlier research by Huffman in 2003 indicated that the roots of *V. amygdalina* were employed to treat conditions like gingivitis and toothache. In various African nations, including Nigeria, *V. amygdalina* has been traditionally utilized for diverse purposes such as addressing ailments like diabetes, fever (Magadula and Erasto, 2009), and fostering wound healing (Adetutu *et al.*, 2011; Noumedem *et al.*, 2013). Additionally, the Hausa tribe in northern Nigeria employs the root and twig of *V. amygdalina* for alleviating stomach-ache and gastrointestinal issues. *V. amygdalina* is also recommended to nursing mothers due to its positive impact on lactation (Anibijuwon *et al.*, 2012).

Pharmacological studies on *V. amygdalina* have revealed its antihelminthic and antimalarial properties (Abosi and Raseroka, 2003), antitumorigenic effects (Izevbigie *et al.*, 2004), as well as analgesic and antipyretic activities (Tijjani *et al.*, 2017). Furthermore, experimental findings indicate hypoglycemic and hypolipidaemic effects in animals (Nwanjo, 2005). The plant is rich in biologically active phytoconstituents such as Alkaloids, Flavonoids, Terpenes, Saponins, Coumarins, Xanthones, Phenolic acids, Lignans, Steroids, Anthraquinones (Tona *et al.*, 2004), and Edotides (Izevbigie, 2003).

The *V. amygdalina* stands out as the most frequently utilized medicinal plant within the *Vernonia* genus, garnering attention from the phytomedicine community due to its effectiveness in managing various health issues. Traditional uses include laxative properties, digestive tonic, febrifuge, wound treatment, and oral hygiene. Scientific scrutiny has revealed its antibiotic, antifungal, and amoebicidal properties (Kamatenesi-Mugisha, 2004; Erasto *et al.*, 2006).

Due to its richness in flavonoids, *V. amygdalina* demonstrates potent antioxidant properties. Its extracts exhibit abilities to combat oxidative stress, showcasing potential applications in preserving food and developing natural antioxidants, crucial for avoiding synthetic alternatives' potential health risks (Nwanjo, 2005; Owolabi *et al.*, 2008).

V. amygdalina contributes to cardiovascular health by modulating serum lipid levels. Studies indicate its ability to lower triacylglycerol and LDL-cholesterol while increasing HDL-cholesterol, making it a promising natural intervention against dyslipidemia (Egedigwe, 2010; Ugwu *et al.*, 2010).

Traditionally *V. amygdalina* is used to induce uterine motility, recent studies affirm *V. amygdalina's* oxytocic potential, supporting its application in aiding childbirth and controlling post-partum hemorrhage (Kamatenesi-Mugisha *et al.*, 2005).

The plant exhibits hepatoprotective effects against liver damage, attributed to sesquiterpene compounds. Additionally, it shows potential nephroprotective properties, emphasizing its role in safeguarding vital organs (Arhoghro *et al.*, 2009).

The growing threat of antimicrobial resistance leads to increased illness, healthcare costs, and fatalities. Consequently, there is a pressing need to discover new antimicrobial agents that can combat drug-resistant microorganisms (Niccoli *et al.*, 2001). The emergence of multi-drug-resistant pathogens renders conventional antibiotics ineffective, necessitating the search for substances from alternative sources with proven antimicrobial properties, especially plant materials. This pursuit aims to uncover potentially valuable active components that could inspire the development of new antimicrobial drugs (Okwori *et al.*, 2007; Oshilim, 2017). Therefore, this study aimed to evaluate the phytochemical composition and antibacterial effectiveness of *V. amygdalina* leaf extracts against some clinical bacterial isolates obtained from General Hospital Azare, Bauchi State, Nigeria.

MATERIALS AND METHODS

Collection of *Vernonia amygdalina* leaf samples

Leaves of *Vernonia amygdalina* were purchased from Azare main market. Bauchi State, Nigeria. The samples were collected in a clean container and transported to Microbiology laboratory of the Department of Microbiology for processing and further analysis. While, the authentication of the leaves was conducted at the Department of Biological Sciences Bauchi State University Gadau. The specimen voucher number at the herbarium unit was 324a.

Collection of Clinical Bacterial Isolates

Bacterial isolates previously recovered from clinical specimens were obtained from the General Hospital in Azare, Bauchi State, Nigeria, and transported to the Microbiology laboratory at Bauchi State University Gadau for identification and processing.

Bitter Leaf Extraction

The extraction procedure followed the methods outlined by Hussain *et al.*, (2011) Edeoga *et al.*, (2005) and Roopashree *et al.* (2008). Bitter leaf leaves were cleaned, dried at room temperature, and ground into a powder. A 10g portion of the powder was mixed with 100 mL of distilled water. The resulting water extract was filtered through Whatsman No.1 filter paper and assessed for antimicrobial properties.

Phytochemical Analysis of the Crude Extract

The presence of Alkaloids, Saponins, Flavonoids, Glycosides, Tannins, and Phenolic compounds in the crude extract was determined using methods described by Hussain *et al.*, (2011) Edeoga *et al.*, (2005) and Roopashree *et al.* (2008).

Test for steroids

One milliliter of the sample solution was mixed with two milliliters of chloroform, followed by two milliliters of concentrated Sulfuric acid to create a lower layer. The presence of steroids was confirmed by the appearance of reddish-brown color.

Terpenes Test:

To detect terpenes, 200mg of leaf powder was dissolved in ethanol, followed by the addition of 1mL of acetic acid and concentrated H_2SO_4. The change in color from pink to violet served as an indicator of Terpenes presence.

Glycosides Test:

For identifying glycosides, 500 mg of leaf powder was dissolved in 2 mL of chloroform, with the careful addition of a small amount of sulfuric acid to form a lower layer. The appearance of a reddish-brown color signified the presence of the glycone portion of glycosides.

Tannins Test:

To determine tannin presence, 100mg of leaf powder was extracted in 10mL of distilled water. The solution was heated and filtered, and the formation of a green to blue-black precipitate indicated the presence of tannins.

Alkaloids Test:

For detecting alkaloids, 2g of the sample was mixed with 20mL of 5% sulfuric acid in 50% ethanol, heated, cooled, and filtered. Dragendoff's reagent was added, and the development of a brick red precipitate indicated the presence of alkaloids.

Saponins Test:
To test for saponins, 0.25g of the sample was boiled in 20mL of distilled water, filtered, and then mixed with dilute sulfuric acid, Fehling's solution, and sodium hydroxide. The formation of a brick red precipitate confirmed the presence of reducing sugars obtained from saponins hydrolysis.

Flavonoids Test:
For the identification of flavonoids, 100mg of the dry sample was dissolved in 10% sodium hydroxide solution (NaOH) and diluted HCl. A yellow solution that later became colorless indicated the presence of flavonoids.

Phenolic Compounds Test:
To detect phenolic compounds, 0.1g of the extract was mixed with 5mL of distilled water and filtered. The addition of 5% ferric chloride ($FeCl_3$) resulted in the formation of a blue-black precipitate, confirming the presence of phenolic compounds.

Confirmation of Bacterial Isolates
Confirmation of the bacterial isolates was carried out using Gram staining techniques and biochemical tests, as per Cheesebrough (2006). Biochemical tests used were catalase, nitrate, oxidase, urease, citrate utilization, indole, and coagulase tests.

Preparation of Discs
Sterile Whatman No. 1 filter paper was used to create 5mm diameter discs, following the procedures of Oboh and Masodje (2009). These discs were sterilized and impregnated with different concentrations (25mg/mL, 50mg/mL and 100mg/mL) of the bitter leaf extract, which were obtained through serial dilution of the stocked extract (100mg/mL). One milliliter of distilled water was added to a test-tube containing 1mL of the stocked extract, resulting new concentration (50mg/mL). This was repeated using 50mg/mL to obtain 25mg/mL of the *V amygdelina* extract.

Antibacterial Assay of Bitter Leaf Crude Extract
The antibacterial assay was performed in accordance with the method described by Okwu and Iruabuchi (2004). Discs containing different concentrations of bitter leaf extract were placed in Petri dishes inoculated with selected organisms and incubated at 37°C for 24 hours. The zones of inhibition were observed and measured, with discs impregnated with chloramphenicol serving as a control.

Determination of Minimum Inhibitory Concentration
The minimum inhibitory concentration (MIC) of *Vernonia aamygdalina* extracts was assessed in triplicates for each of the bacterial isolates at varying concentrations (25mg/mL, 50 mg/mL and 100mg/mL). Using a standard wire loop, a loopful of each of the *Klebsiella pneumoniae*, *Escherichia coli* and *Staphylococcus aureus* at 0.5 McFarland standard was inoculated into test tubes containing nutrient broth and 1mL of the various concentrations of the extract. A test-tube containing nutrient broth only serving as control, was inoculated with the each of the test organism. The tubes were then incubated for 24 hours at 37°C. The bacterial growth was then examined by noticing the turbidity (Eucast, 2003).

Determination of Minimum bactericidal concentration
The minimum bactericidal concentration (MBC) of *Vernonia aamygdalina* extracts against the bacterial isolates (*Klebsiella pneumoniae, Escherichia coli* and *Staphylococcus aureus*) was assessed according to the method described by CLSI (2012), One milliliter of each of the bacterial culture was withdrawn from the mixture obtained from the determination of minimum inhibitory concentration, that was contained in the tubes which did not show any growth, and were inoculated onto nutrient agar plates, and incubated at 37°C for 24 hours. The plates were then examined for growth. The concentration at which there was no single bacterial colony was recorded as minimum bactericidal concentration of the extract.

RESULTS

The phytochemical analysis of the extract revealed the presence of Saponins, Glycosides, Tannins Flavonoids, Steroids, Phenolic compounds and Alkaloids (Table 1).

The study revealed that the extract had no antibacterial effect on *Klebsiella pneumoniae* at 25 mg/mL, however, it showed zones of inhibition of 3.00 ± 0.00 mm and 4.00 ± 0.33 mm against *Escherichia coli* and *Staphylococcus aureus*, respectively at 25mg/mL. At 50 mg/mL, the zones of inhibition of 6.00 ± 0.67mm, 8.00 ± 0.00mm and 2.00 ±0.00mm were produced against *Escherichia coli, Staphylococcus aureus* and *Klebsiella pneumonia*, respectively. While at 100 mg/mL, the zones of inhibition of 7.00 ± 0.67mm, 9.00 ± 0.00mm and 4.00 ± 0.67mm were produced against *Escherichia coli, Staphylococcus aureus* and *Klebsiella pneumonia*, respectively (Table 2)

Moreover, a minimum inhibitory concentration (MIC) of the extract of 25mg/mL was observed against *Escherichia coli* and *Staphylococcus aureus*, while for *Klebsiella pneumoniae* it was 50mg/mL (Table 3). However, it was noteworthy that, none of the concentrations employed in the study demonstrated ability to completely eradicate the bacterial isolates, when the extracts were assayed for minimum bacteriacidal concentration (MBC) (Table 4).

Table 1: Phytochemical Constituents of *Vernonia amygdalina* Leaf Extracts

Compounds	Status
Saponins	+
Glycosides	+
Flavonoids	+
Steroids	+
Alkaloids	+
Tannins	+
Phenolic compounds	+

Key:- absent; + present

Table 2: Antibacterial Activity of *Vernonia amygdalina* Crude Extracts Against the Selected Bacterial Isolates

Isolates	Zones of inhibition(mm)			
	Chloramphenicol	25mg/mL	50mg/mL	100mg/mL
Escherichia coli	20.00	3.00 ± 0.00	6.00 ± 0.67	7.00 ± 0.67
Staphylococcus aureus	25.00	4.00 ± 0.33	8.00 ± 0.00	9.00 ± 0.00
Klebsiella pneumonia	18.50	0.00 ± 0.00	2.00 ± 0.00	4.00 ± 0.67

Key:(±) Standard deviation

Table 3: Minimum Inhibitory Concentration (MIC)of the *V. amygdalina* Leaf Extracts

Bacterial isolates	25mg/ml	50mg/ml	100mg/ml
Escherichia coli	+	-	-
Staphylococcus aureus	+	-	-
Klebsiella pneumoniae	-	+	-

Key: + = MIC, - = Nil

Table 4: Minimum Bacteriacidal Concentration (MBC) of the *V. amygdalina* Leaf Extracts

Bacterial isolates	25mg/ml	50mg/ml	100mg/ml
Escherichia coli	-	-	-
Staphylococcus aureus	-	-	-
Klebsiella pneumoniae	-	-	-

Key: + = MBC, - = Nil

DISCUSSION

The present study revealed the presence of Saponins, Glycosides, Tannins Flavonoids, Steroids, Phenolic compounds and Alkaloids. This is in line with the finding of Akinjogunla *et al.* (2011), who reported the presence of the compounds. The study is however contrary to the finding of Evbuomwan *et al.* (2018) who reported the absence of Tannins in the *V amygdelina* leaf extract. This might be due to difference in the geography and the climatic conditions of the study areas and the solvents used to extract the plant's materials.

Moreover, the study revealed that, *Klebsiella pneumoniae* was found to resist 25mg/mL of the extract, while *Escherichia coli* and *Staphylococcus aureus* were slightly sensitive to this concentration. Evbuomwan *et al.*, (2018) reported the antibacterial activity of the plant extract against *Klebsiella pneumoniae* at 25mg/ml, where zone of 7.50 ±1.50mm was recorded. They also reported the resistance of *Escherichia coli* to all of the concentrations used for their study. This might be as a result of the variations in the methods and solvents used in the studies, where they used ethanol as solvent for extraction while, this study used water as solvent. Moreover Inusa *et al.*, (2018), reported that *Staphylococcus aureus* was found to be resistant to the plant extract. On the other hand, Karfi *et al.*, (2021) reported that, the plant extract produced zones of inhibition of 18mm and 22mm against *Staphylococcus aureus* and *Escherichia coli* respectively at 100mg/ml. Additionally, the previous studies conducted by Adetunji *et al.* (2013), Ibekwe *et al.* (2001), Moreno *et al.* (2006), Adetutu *et al*, (2011) and Koduru *et al.* (2006) demonstrated less antimicrobial activity of the aqueous extract of the plant. This might be attributed to the difference in the strain of the microorganisms used, the presence or absence of some phytochemical compounds in the plant, or the methods used in each of the studies. Additionally, the emergence of drug resistance in many bacterial strains might have also contributed to the variations. On the other hand, Habtamu and Melaku (2018) reported the antibacterial activity of *Vernonia amygdalina* against the isolates (*Klebsiella pneumoniae, Escherichia coli* and *Staphylococcus aureus*).

The study also indicated that, 25mg/mL of the extracts inhibited the growth of *Escherichia coli* and *Staphylococcus aureuss*, however *Klebsiella pneumoniae* resisted the concentration, but sensitive to 50mg/mL of the extracts. This is contrary to the results of Evbuomwan *et al* (2018), who reported 50mg/mL as the minimum inhibitory concentration of the extract against *Staphylococcus aureus,* 100mg/mL against *Klebsiella pneumoniae, and Escherichia coli* was reported to be resistant to all of the concentrations used. Furthermore, Karfi *et al*. (2021), reported that, the minimum inhibitory concentrations of the extract against *Escherichia coli* and *Staphylococcus aureus* were found to be 12.5mg/mL and 10mg/mL respectively, which is in disagreement with the findings of the present study. On the other hand, the present study revealed that, when compared with chloramphenicol, all of the isolates used in the study were resistant to the plant extract at all of the concentrations used. This is also contrary to the findings of Karfi *et al*. (2021) who highlighted that in comparison with gentamycin, the plant extract showed reasonable antimicrobial activity.

Additionally, the study highlighted that, all the concentrations used for the study were lacking bacteriacidal activity against all the bacterial isolates used, however Evbuomwan *et al* (2018) reported 50mg/mL, 100mg/mL and 200mg/mL to be minimum bacteriacidal concentrations of the ethanolic extracts of *V amygdelina* on *E coli, S aureus* and *K pneumoniae* respectively. Moreover, they reported that, the MBC of the aqueous extract of *V amygdelina* on *S aureus* was 200mg/mL. These variations might be due to the difference in the concentrations of the extract and the solvents used in the studies.

CONCLUSION

The study demonstrated that the *Vernonia amygdalina* extract contains medicinally important compounds capable of inhibiting the bacterial growth. The plant showed slight antibacterial activity against the bacterial isolates used for the study.

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