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RESEARCH ARTICLE

In vitro antibacterial effect of Vernonia amygdalina leaves extract on *Escherichia coli* and *Staphylococcus aureus* in Kebbi State, Northern Nigeria

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ABSTRACT

In the developing world, sufficient access to conventional medicines has been arguably of immense challenge perhaps due to socioeconomic predicaments. This has consequently led to an increase in the use of ethnomedicinal regimens such as *Vernonia amygdalina*, commonly called bitter leaf. This study is aimed at investigating the *in vitro* antibacterial effect of *V. amygdalina* leaves extracts on *Escherichia coli* and *Staphylococcus aureus*. Conventional microbiological techniques were used to screen aqueous extracts of *V. amygdalina* for antibacterial sensitivity and phytochemical properties. Zones of inhibition produced by ethanolic extract ranged from 11.30 ± 0.30 mm at 25 mg/ml to 17.40 ± 2.88 mm at 100 mg/ml against *E. coli*; 12.63 ± 2.97 mm at 25 mg/ml to 14.5 ± 2.5 mm at 100 mg/ml against *S. aureus*; the most sensitive organisms on the ethanolic extract was *E. coli* while *S. aureus* was the least sensitive. The leaves extracts were positive for flavonoids, terpenoids, saponins, anthraquinones, alkaloids, and phenols. This outcome suggests the possibility of obtaining a safe and efficacious chemotherapeutic derivative from *V. amygdalina* as it already serves as an important food ingredient in Nigeria.

Keywords: Bitter leaf, Ethnomedicinal, Phytochemical, *Vernonia amygdalina*

INTRODUCTION

The use of medicinal herbs for the treatment of various diseases is very common among the human population since prehistoric times. Thousands of plant species including *Vernonia amygdalina* (bitter leaf) have been used worldwide for ethnomedicinal purposes. Despite the fact that there have been numerous studies on the development of various drugs in modern day, many plants with therapeutic promise have, however, limited assessment. This has encouraged many scientists all over the world in bringing about newer and safer antimicrobial agents by evaluating the therapeutic efficacy of plant extracts as a substitute for synthetic antimicrobial agents. The diversity of the species of these plants has also helped scientists in developing diverse drugs with different mechanisms of actions to help reduce antimicrobial resistance which has been a serious challenge to the science of medicine.

The use of plant extracts for therapeutic purposes has continued to play an essential role in traditional medicine for the treatment or management of various human diseases, especially in rural Africa where many infectious diseases are endemic due to socioeconomic factors. In many rural communities in Ghana, Mali, Nigeria, and Zambia, the primary means of treatment for many ailments involve the use of herbal medicines. Despite the ongoing rigorous pharmaceutical and microbiological researches, the trends of emerging and reemerging infectious diseases worldwide are a thing of

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concern. In an attempt to respond effectively to these challenges, drugs of plant origin must play an important role in drug development by pharmaceutical industries so as to curb the rise in antimicrobial resistance, mutations, and emergence.

Many drugs with good therapeutic potential are of limited use because of their toxicities. Hence, the need for more studies to be conducted so as to bring about diversity in medical drug regimens.

Plants are indispensable sources of medicinal preparations, both preventive and curative. China and India are the leading countries in using medicinal plants. Their traditions of plant remedies date back to at least 7000 years. According to the WHO, 80% of the World's population relies on traditional medicine to meet their daily health requirement.

V. amygdalina, a species in the family Asteraceae, is a tropical shrub with height of 1–3 mm, petiole leaf of about 6 mm in diameter. *V. amygdalina* commonly called bitter leaf in English. The leaves are consumed as vegetables and condiments in special African delicacies. The bitter taste in *V. amygdalina* is due to the presence of alkaloids, saponins, tannins, and glycosides. Some studies have reported the antihelminthic, antimalarial, hypoglycemic, and hypolipidemic properties of *V. amygdalina*. Other studies have also reported the phytochemical and antibacterial activity of the plant extracts against food-borne pathogen (Ibrahim *et al*., 2009), urinary tract pathogens (Uzoigwe and Agwa, 2011), and other clinical isolates.

Statement of the problem and justification of the study

The development of antibiotic resistance has become a global public health challenge. This has decreased the efficacies of several antibacterial agents leading to increase in diseases and death outbreaks. As a result of the emergence of resistance in human pathogens against commonly used antibiotics, this has necessitated a search for newer, efficacious, and safe antibiotics derived from sources such as plants.

Aim of the study

The aim of this study is to determine the antimicrobial effect of *V. amygdalina* (bitter leaves) against *E. coli* and *Staphylococcus aureus*.

Objectives of the study

The objectives of the study were to assess the antibacterial activity of *V. amygdalina* leaves extracts against pathogenic bacteria and to determine the minimum inhibitory concentration (MIC) on the minimum bacterial concentration (MBC) against each isolate.

MATERIALS AND METHODS

Study area

Aliero Local Government Area of Kebbi State, North Nigeria, was marked as the study area. It is located at 12^{o} 16'42''N4^{o} 27'6''E southeastern part of Kebbi State.

Sample collection

V. amygdalina was purchased at the Aliero market in Aliero Local Government, Kebbi State, Nigeria. The bitter leaves were identified botanically at the Department of Plant Science and Biotechnology, Kebbi State University of Science and Technology, Aliero. *V. amygdalina* leaves extracts were dried and pulverized into powder. The powder was subjected to aqueous and organic solvent extraction for analysis.

Test bacteria

Clinical isolates of *S. aureus* and *E. coli* were obtained from the Microbiology Laboratory, Department of microbiology, Kebbi State University of Science and Technology, Aliero. The isolates were preserved in nutrient agar and stored at 4°C until when it is needed.

Preparation of plant extract

Fifty grams of dried powdered leaves were dissolved in 150 ml sterile water at room temperature, allowed

to dissolve for 24 h, and finely filtered using sterile Whatman filter paper.

Phytochemical analysis

The tests for the presence of saponins, alkaloids, tannins, glycosides, flavonoids, volatile oil, steroid, anthraquinones, and cardiac glycoside were conducted as follows.

Test for saponins

Saponins were examined using the froth test. One gram of the sample was weighed into a conical flask in which 10 ml of sterile distilled water was added and boiled for 5 min. The mixture was filtered and 2.5 ml of the filtrate was added to 10 ml of sterile distilled water in a test tube. The test tube was stoppered and shaken vigorously for about 30 s. It was then allowed to stand for half an hour. Honeycomb froth indicated the presence of saponins.

Test for glycosides

2.5 ml of 50% H_2S0_4 was added to 5 cm^3 of the extract in a test tube. The mixture was heated in boiling water for 15 min. Cooled and neutralized with 10% NaOH, 5 ml of Fehling's solution was added and the mixture was boiled for 15 min. A brick red precipitate was observed which indicates the presence of glycoside.

Test for alkaloid

Two milliliters of the extract were stirred with 2 ml of 10% aqueous hydrochloric acid. One milliliter was treated with a few drops of Wagner's reagent and another 1 ml portion was treated similarly with Mayer's reagent. Turbidity or precipitation with either of these reagents was taken as preliminary evidence for the presence of alkaloid.[1-10]

Test for tannins

Four milliliters of the extract were diluted with water, 3–4 drops of 10% ferric chloride solution were added. A blue color is observed for gallic tannins and green color indicates for catecholic tannins.

Test for steroids

0.5 g of the extracts was dissolved in 2 ml of chloroform. Two milliliters of sulfuric acid were carefully added to form lower layer. A reddish-brown color at the interface indicates the presence of a steroidal ring.

Test for flavonoids

Four milliliters of the extract were added with 1.5 ml of methanol solution. The solution was slightly heated and metal magnesium was used to cover the flask containing the solution, 5–6 drops of concentrated hydrochloric acid were added, and red color was observed for flavonoids and orange color for flavones.[11-21]

Antibiotic susceptibility test with sample antibiotic

Antimicrobial disc tests of the isolates were carried out using the following antibiotic discs: Ciprofloxacin (10 ug) and chloramphenicol (25 ug). The antibiotic resistance was interpreted by diameter of zones of inhibition (Cheesbrough, 2006).

Antibacterial susceptibility test with extracts of *V. amygdalina*

The inoculum was prepared by inoculating the test organisms in nutrient broth and it was incubated for 24 h at 37°C. The cultures were diluted to 0.5 McFarland turbidity standards after the incubation. 0.2 ml of the culture was further diluted in normal saline and was inoculated into solidified nutrient agar using glass rod by spreading technique. The ability of the various extracts to inhibit the growth of the clinical test organisms was determined using the agar well technique. The inoculated nutrients agar plates were allowed to dry. After which, wells were bored on the surface of inoculated agar plates using 4 mm cork borer. 0.2 ml of the different concentration of each extracts were transferred into the well using Pasteur pipette. The wells were

sufficiently spaced to prevent the resulting zones of inhibition from overlapping. The plates were incubated at 37°C for 24 h.

MIC

The MIC of the methanol extracts was determined for each of the test organisms in triplicates at varying concentrations of 100, 50, and 25 mg/ml. One milliliter of nutrient broth was added and then a loopful of the test organism previously diluted to 0.5 McFarland turbidity standard was introduced to the tubes. A tube containing nutrient broth was seeded with the test organism to serve as control. All the tubes were then incubated at 37°C for 24 h and then examined for growth by observing for turbidity.

MBC

The MBC of the ginger extract on the clinical bacterial isolates was carried by briefly adding 1 ml of bacterial culture taken using a pipette from the mixture obtained in the determination of MIC tubes which did not show any growth and was sub-cultured onto nutrient agar and incubated at 37°C for 24 h. After incubation, the concentration at which there was no single colony of bacteria was taken as MBC.[22-30]

Statistical analysis

The data were presented as mean standard deviation to calculate zone of inhibition of the test organism in the plant extract.

RESULTS

Phytochemical analysis of *V. amygdalina* revealed the presence of phenols, flavonoids, tannins, saponins, and alkaloids in the extract, while steroids and terpenoids were found to be absent, as shown in Table 1. Zones of inhibitions were produced at 25 mg/ml, 50 mg/ml, and 100 mg/ml portrayed in Table 2. However, based on the zones of inhibition produced by the extracts, *E. coli* was found to be more sensitive than *S. aureus,* as shown in Table 3.

Table 1: Phytochemicals screening of *Vernonia amygdalina* leaves

Chemicals	Result
Flavonoids	+
Alkaloids	+
Tannin	+
Saponin	+
Terpenoids	–
Steroids	–
Phenols	+
Anthraquinones	+

Key: (+): Present, (–): Not detected

Table 2: Zone of inhibition of *Vernonia amygdalina* leaves extract against *Staphylococcus aureus* and *Escherichia coli*

Conc. (mg/ml)	Zone of inhibition (mm)	
	Escherichia coli	***Streptococcus aureus***
25 mg/ml	11.30±0.30	12.63±2.97
50 mg/ml	11.76±1.97	13.43±2.21
100 mg/ml	17.40±2.88	14.97±1.79
Ciprofloxacin (10 µg)	22.55±0.00	10.00±0.00

Table 3: Minimum inhibitory concentration and minimum bactericidal concentration

Test organisms	MIC (mg/ml)	MCB (mg/ml)
Escherichia coli	25	40
Streptococcus aureus	50	100

DISCUSSION

This study has shown a significant antibacterial promise in the leaf extracts of *V. amygdalina*. This outcome means that the leaf extracts could confer both antibacterial activity and a possible wide margin of safety as it is traditionally consumed as a meal in many countries such as Nigeria. *E. coli* and *S. aureus* were tested due to fact that they have been implicated in bacterial food toxicities worldwide probably due to their ubiquitous nature. Furthermore, phytochemicals detected in *V. amygdalina* included flavonoids, terpenoids, saponins, anthraquinones, alkaloids, and phenols, as shown in Table 1. These phytochemicals were present in ethanol extract. Terpenoids and steroids were not detected on the ethanolic extract of *V. amygdalina*.[31-34]

The antibacterial activity of *V. amygdalina* was found to be dependent on the nature of the solvent used for extraction and the concentration of the extract. Ethanolic extract was observed to possess more antibacterial activities. This is attributable to the fact that the ethanol extracted more of the bioactive component of the plant (Alara *et al*., 2017). Zones of inhibition produced by ethanolic extract ranged from 11.30 ± 0.30 mm at 25 mg/ml to 17.40 ± 2.88 mm at 100 mg/ml against *E. coli*; 12.63 ± 2.97 mm at 25 mg/ml to 14.5 ± 2.5 mm at 100 mg/ml against *S. aureus*; the most sensitive organisms on the ethanolic extract were *E. coli* while *S. aureus* was the least sensitive. The leaf extracts were found to possess inhibitory activities against the test bacterial species. This finding is in agreement with studies by Udochukwu *et al*. (2015) who reported the phytochemical and antibacterial activity of *V. amygdalina.*

The susceptibility of these organisms to these extracts explains their use in native medicine for the treatment of bacterial infections such as dysentery, sore throat, cough, and wound infections. The extracts were shown to exhibit a broad spectrum of antimicrobial property against the tested organisms. A study conducted by Iwalokun (2003), showed *V. amygdalina* leaf extracts to have a higher degree of antimicrobial activity on the organisms tested. Even at considerably lower concentration, bitter leaf extracts still exhibited a moderately antimicrobial effect on the clinical isolates. The result of this study also agrees with the report of Tula *et al.* (2012) who reported higher activity of ethanolic extract of the leaves of *V. amygdalina* against the organism tested in this study. It can, however, be deduced from this research that the test bacterial isolates were differentially affected by the ethanolic extracts. This is due to the variations in the dissolution capacity of the different solvents which consequently affected the degree of phytochemicals extracted. These irregularities may have risen from drug-inactivating enzymes present in *E. coli.* Furthermore, variations of the susceptibility of Gram-positive and -negative bacteria could have resulted from their relative composition of cell wall components. Gram-positive bacteria have thick peptidoglycan layer, while Gram-negative bacteria have thick lipopolysaccharide layer.

The results of this study revealed that Gram-positive bacteria are susceptible to *V. amygdalina* extracts. This finding agrees with reports. It has also been reported that bitter leaf could be effectively used against drug-resistant microorganisms. This observation agrees with the study of Iwalokun *et al.* (2003) who reported on the effectiveness of *V. amygdalina* leaf extract. Uzoigwe and Agwa (2011) observed in their study that leaf extracts of *V. amygdalina* were more effective against *Klebsiella* species. The varying degree of sensitivity of the bacterial species may be due to the intrinsic susceptibility of the bacteria and the nature and combinations of phytocompounds present in the extracts as observed by Suree and Pana (2005). It could also be attributed to physical factors, extracting solvents, and method of extraction. More experiments should be carried out at higher concentrations of the aqueous and ethanol extracts to assess their activity on these enterotoxin-producing microorganisms such as *S. aureus* and *E. coli*. The extracts should also be tested on other microorganisms to ascertain their activity on other disease-causing agents. Further studies could be experimented *in vivo* using laboratory animals to test the efficacy of the extracts and to address the limiting issue of dosage.

CONCLUSION

This research work has shown that *Vernonia amygdalina* has potential bioactive phytochemicals that are responsible for its antibacterial activities. It has also proven that bitter leaf extract is a more antibacterial substance than conventionally used antibiotics. Therefore, more research should be carried out to enable the purification of the specific biopotential chemicals and their subsequent processing into chemotherapeutic agents.

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