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Exploring the Antibacterial Properties of *Vernonia amygdalina* (Bitter Leaf) Extract: A Potential Alternative to Conventional Antibiotics

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ABSTRACT

As part of the ongoing search for potent and resistance-free antibacterial medicinal plants, this study aimed to evaluate the antibacterial properties of the plant extract of *Vernonia amygdalina*, commonly known as bitter leaf. Standard procedures were used to provide a potential cheap alternative to conventional medication for treating bacterial infections. The aqueous extract of *V. amygdalina* leaves was prepared and subjected to phytochemical screening, which revealed the presence of tannins, phlobatannins, saponins, terpenoids, cardiac glycosides and alkaloids. The antibacterial activity of the extract was tested against the gram-positive bacterium *Staphylococcus aureus* and the gram-negative bacteria *Pseudomonas aeruginosa* and *Escherichia coli* using the agar well diffusion method. The extract showed moderate antibacterial activity, exhibiting 10 mm and 8 mm zones of inhibition against *S. aureus* and *P. aeruginosa* respectively at a concentration of 20 mg/ml. However, it displayed no activity against *E. coli*. In comparison, the standard antibiotic gentamicin produced larger zones of inhibition of 33.5 mm, 27 mm, and 23.5 mm against the respective test organisms. The results suggest that *V. amygdalina* extract had greater antibacterial activity on the gram-positive *S. aureus* than on the gram-negative microorganisms tested. The presence of phytochemicals like tannins, saponins and alkaloids in the extract may contribute to its antibacterial properties. Further research is warranted to fully elucidate the medicinal potential of *V. amygdalina* and isolate the active compounds responsible for the observed antimicrobial effects. Overall, the findings provide a scientific basis for the traditional use of this plant in treating bacterial infections.

Keywords: Vernonia amygdalina, Antibacterial activity, Phytochemicals, Antimicrobial resistance, Traditional medicine

INTRODUCTION

The search for novel, effective, and affordable antimicrobial agents has become a global priority as many infectious disease-causing bacteria are developing resistance to commonly used synthetic antibiotics [1]. This growing antimicrobial resistance poses a significant public health concern, especially in developing countries where access to modern medicine is limited. In response to this challenge, researchers are increasingly turning their attention to exploring natural plant-based sources for alternative antimicrobial therapies. Traditional herbal medicine has a long history of use in many Asian, Latin American, and African countries, where up to 80% of the population relies on plant-derived remedies as their primary healthcare [2, 3, 4]. These traditional practices are founded on empirical observations passed down through generations, suggesting that certain medicinal plants possess pharmacological properties beneficial for treating various ailments, including infectious diseases. One such plant that has garnered attention for its potential antimicrobial applications is Vernonia amygdalina, commonly known as bitter leaf. V. amygdalina is a tropical shrub indigenous to sub-Saharan Africa that has been used extensively in traditional medicine [5]. Its leaves, roots, and other plant parts are reported to possess a variety of therapeutic effects, such as antihelminthic, antimalarial, laxative, and fertility-enhancing properties [6, 7, 8]. Furthermore, the leaves are commonly consumed as a vegetable in many African cuisines after undergoing processing to reduce their characteristically bitter taste [9]. Interestingly, observations of wild chimpanzees in Tanzania chewing V.

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amygdalina leaves to extract the bitter juice and subsequently returning to normal activity have lent support to the plant's medicinal value [10]. Traditional healers in various parts of Africa also commonly use V. amygdalina leaf extracts to treat bacterial infections and chronic skin ulcers, even in cases where antibiotic treatments have failed [11 - 15].

Previous studies have investigated the antibacterial properties of V. amygdalina extracts against various bacterial strains. Akinpelu [16] found the plant extract to be effective against *Bacillus subtilis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Shigella dysenteriae*, and *Staphylococcus aureus*, but inactive against Escherichia coli and Serratia marcescens. Similarly, Ashebir and Ashenafi [17] reported that a 7% V. amygdalina extract inhibited the growth of B. cereus, S. aureus, and Shigella flexneri, but had no effect on E. coli. Vernonia amygdalina has also been shown to contain various secondary metabolites, such as tannins, saponins, alkaloids, and terpenoids, which have been associated with antimicrobial properties in other medicinal plants [6, 16, 17]. However, the exact mechanisms by which V. amygdalina exerts its antimicrobial effects, as well as the specific bioactive compounds responsible, remain to be fully elucidated. Further investigation is needed to provide a more comprehensive understanding of the antibacterial potential of this plant and its potential applications in traditional and modern medicine. The present study aims to build upon the existing evidence by evaluating the antibacterial activity of aqueous extracts of V. amygdalina leaves against a panel of clinically relevant bacterial pathogens, including S. aureus, P. aeruginosa, and E. coli. The findings of this research could contribute to the development of plant-derived antimicrobial agents and provide a scientific basis for the traditional medicinal use of *V. amygdalina*.

METHODOLOGY

Study Site

This was an experimental study carried out at Kampala InternationalUniversity-Western Campus Pharmacy laboratory.

Equipment and Materials

Petri dishes, aqueous leaf extract, electronic weighing balance, Distilled water, Beakers, Measuring cylinder, Pen, Water bath, Refrigerator, Nutrient agar, McConkey agar, Spatula, Sterile cork borer, Incubator, Hot air oven.

Plant Material Identification

Vernonia amygdalina plant was identified by a botanist (Professor DominicByarugaba) at Kampala International University-Western campus and avoucher sample was prepared as herbarium.

Preparation of the Extract (aqueous extraction)

Leaves of *Vernonia amygdalina* was collected around Ishaka. The leaves werethen air dried for 2 weeks, crushed, and then blended. 12g of the leaves wereweighed and used in extraction. Extraction was done by dissolving the groundleaves in 100ml of hot water boiled for thirty minutes in a conical flask andthen was soaked for 24 hours. It was then filtered using filter paper and dried in a water bath to obtain the extract. The extract was weighed to obtain the weight/weight.

Phytochemical Screening

Tests for the detection of different secondary metabolites were carried out usingaqueous extracts of *Vernonia amygdalina*according to standard procedures as described by [18, 19, 20].

i. Flavonoids

To 5mls of the dilute ammonia solution, 0.2g of *Vernonia amygdalina* aqueousextract was added followed by the addition of 2ml concentrated sulphuric acid. Observation of a yellow solution that turns colourless was indicative of flavonoids.

ii. Tannins

0.2g of the extract was dissolved in 5mls of distilled water and heated in a waterbath for fifteen minutes then filtered. Three drops of 0.1 %ferric chloride wereadded to the filtrate and observed for the presence of a brownish-green or blue-blackcoloration indicative of tannins.

iii. Saponins

0.2g of the extract was dissolved in 5 ml of distilled water and then heated toa boil. The formation of a layer of foam indicated the presence of saponins.

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iv. Alkaloids (Mayer's test)

0. 5g of the extract was stirred in 1 % aqueous hydrochloric acid in a steambath for five minutes. Two to three drops of Mayer's reagent were added to theside of the test tube. Turbidity or a white precipitate with this reagent wastaken as evidence for the presence of alkaloids.

v. Phlobotannins

0.2g of the extract was dissolved in 5 ml of distilled water and filtered. Thefiltrate was boiled with 2 ml of 2% of aqueous hydrochloric acid solution. Observation of a red precipitate was considered a positive test for Phlobotannins.

vi. Steroids

0.2g of the extract was mixed with 2 ml of concentrated sulphuric acid andthereafter2 ml of acetic anhydride was added to the mixture color changed fromviolet to blue or green indicatingthe presence of steroids.

vii. Cardiac glycosides

A drop of ferric chloride solution was added to 2ml of glacial acetic acid. Thissolution was used to treat 0.5g of the extract. The mixture was then underlaidwith 1 ml of concentrated sulphuric acid. A brown ring at the interphase wasindicative of a deoxy-sugar characteristic of cardenolidesA. A violet ring mayalso appear below the brown ring while in the acetic acid layer, a greenish ringmay form gradually throughout the thin layer indicating the presence ofcardiac glycosides.

viii. **Reducing sugars**

0.2g of the extract was shaken with 5ml of distilled water and filtered. Thefiltrate was then boiled with 3 drops of Fehling's solution A&B for two minutes. An orange solution indicated the presence of a reducing sugar.

Plant Extract Disc Preparation

The plant extract disc was prepared from a labline filter by punching with a corkborer of 6mm diameter and the disc was autoclaved at 121 degrees Celsius for 15minutes. 0.2g of the extract was then diluted with l0mls of distilled water togive a concentration of 20mg/ ml and this was added to the discs. Thisconcentration was chosen as it correlated with the concentration of thestandard antibiotic (gentamycin) that was to be used. The plant extract discwas then dried in an oven and stored in a refrigerator until required for use.

Culturing of the Test Microorganism

The isolates of the microorganism were obtained from the MicrobiologyLaboratory of Kampala International University-Western campus. The testorganisms used were *Staphylococcus aureus*, *Pseudomonas aeruginosa*and*Escherichia coli*. *Staphylococcus aureus and Pseudomonas aeruginosa*microorganisms were cultured on Nutrient Agar plates by dissolving 2.8g ofNutrient Agar in l00mls of water while *Escherichia coli* was cultured inMcConkey agar. The media was autoclaved at 121 degrees Celsius for 15 mins.9mls of this media was poured in plates and left to gel. The microorganismswere later sub-cultured to produce young cultures which were used in theexperiment.

Determination of Antibacterial Activity

i. Agar well diffusion method

This was carried out according to the method described by Opara and Anasa (1993). The growth media Mueller-Hinton Agar (MHA) was prepared bydiluting 9g of Mueller-Hinton agar in 250 ml of distilled water and sterilizing byautoclaving. MHA was allowed to cool to 50 degrees Celsius and 5 ml of themolten agar was then added to the petri dishes. Three wells of about 6.0mmdiameter were aseptically made on each agar plate using a sterilecork borer. The cultured microorganisms were later inoculated on the Mueller-Hinton Agarby spreading the microorganisms uniformly across the Petri dishes. Fixedvolumes (20µl) of the plant extract were then introduced into the wells. Controlwells containing gentamycin were used as a positive control because it is abroad-spectrum antibiotic and was set up at the center and distilled water wasthen added to the third well as a negative control. The plates were incubated at5 degrees for 1 hour to ensure the extract and the controls diffused evenly inthe agar and then were incubated at 37 degrees Celsius for 24 hrs. The relativesusceptibility of the microorganisms to the plant extract was determined bymeasuring the zones of inhibition and the experiment was done two times for*staphylococcus aureus* and *Escherichia coli* plates and once for *Pseudomonas aureginosa* plates and the mean values were calculated and recorded.

Statistical Analysis of Data

Test for significance in the zone of inhibition was done by determining themean of the zone of inhibition produced by the bacteria to know theeffectiveness of each plant extract and the susceptibility of the test organism.

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Limitations of the Study

The research results were not as expected due to the failure to isolate and sterilizethe active principles due to a lack of equipment.

Time Limit

The research study took six months beginning from March 2011 to August 2011.

Sources of Chemicals and Reagents

Chemicals and reagents for phytochemical screening and other reagents wereprovided by the School of Pharmacy, Kampala International University-WesternCampus.

RESULTS

Extraction

After extraction of 12g of leaf extract in l00mls of distilled water, the yield was3.3g of extract. The percentage yield obtained was 27.5% w /w i.e. 3.3/ 12 x 100.

Table 1: Phytochemical test

TEST	RESULTS
Tannins	+
Phlobatannins	+
Saponins	+
Flavonoids	–
Steroids	–
Terpenoids	+
Cardiac glycosides	+
Reducing sugar	–
Alkaloids	+

(+) refers to the presence of the phytochemical and (-) refers to the absence of the phytochemical.

Table 2: Antimicrobial activity of extracts of *Vernonia amygdalina* determined by agar well diffusion method on specific media for each testmicroorganism

	Diameter of inhibition zones(mm)		
	P.aureginosa	*Staph.aureus*	*Esch.coli*
Distilled water	–	–	–
Gentamycin (Dose: 20mg/ml)	27	33.5	23.5
Aqueous extract *Vernonia amygdalina* (20mg/ml)	8	10	–

Aqueous extract:(-) = no activity, *P. aeruginosa= Pseudomonas aureginosa*, *Staph. aureus= Staphylococcus aureus*, *Esch. coli = Escherichia coli*

DISCUSSION

Medicinal plants are used by a large proportion of developing nations [20-30]. The reason for this may be a true improvement in disease conditions after herbal treatment [31-40]. In these countries, the search for new drugs is centered upon theinvestigation of medicinal plants [41-50]. The present research has tested the crude extracts of *V. amygdalina* onbacterial strains which comprised of *Staphylococcus aureus*, *Pseudomonasaeruginosa*, and *Escherichia coli*. The therapeutic effect of some species of plants is determined by their constituents [31-45]. These constituents increase the body's resistance to disease, retardor delay the processes of natural aging or facilitate the

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adaptation of theorganism to certain conditions [22]. Phytochemical screening analysis revealed the presence of tannins, phlobatannins, saponins, terpenoids, glycosides, and alkaloids in the plantextract. Elsewhere it is reported that tannins, alkaloids, saponins, flavonoids, and glycosides could be associated with the antimicrobial activities of someplants [23, 24]. The antibacterial activities of these plants may reside in these active principles as noted by [25]. What has not been resolved is the separation of thespecific bioactive components against specific organisms; and this has been noted to affect the quality and safety of herbal medicines [26]. The aqueous extract also showed no presence of phytochemicals such asflavonoids, steroids, and reducing sugars. *Vernonia amygdalina* extracts had inhibition on Staphylococcus aureus (grampositive) and Pseudomonas aeruginosa (gram negative) but had no inhibition onEscherichia coli (gram negative). This is similar to the observation of the plants'ability to inhibit *E. coli* [17]. The aqueous extract of *Vernonia amygdalina*had slightantibacterial properties at a percentage of 30% activity on Staphylococcusaureus (10mm zone of inhibition) when compared to the standard antibioticactivity of gentamycin at 100% (33.5mm) and 29% activity on *Pseudomonasaeruginosa* (8mm zone of inhibition) when compared to the standard antibioticactivity of gentamycin at 100% (27mm). The extract showed no activity on*Escherichia coli* and could not be compared with the standard antibiotic. The extract may be used as an adjuvant to the standard treatment. However, such an addition of therapy needs to be explored whether associated with any drug interactions. The difference in antimicrobial properties of a plant extract is attributable tothe age of the plant used, freshness of plant materials, physical factors (temperature, light water), contamination by field microbes, adulteration andsubstitution of plants, incorrect preparation and dosage [28, 29]. *Vernonia amygdalina* showed a greater inhibitory effect on the gram-positive (*S. aureus*) than on the gram-negative strains (*P. aureus*) [17, 30, 31]. The standard antibiotic used which was gentamycin showed zones of inhibition on *E. coli*, *P. aeruginosa*, and *S. aureus*. Gentamycin had the greatest effect on *Staphylococcus aureus* (33.5mm zone of inhibition), then on *Pseudomonasaeruginosa* (27mm zone of inhibition), and lastly on *Escherichia coli* (23.5mmzone of inhibition). Gentamycin showed a stronger retardation effect on the gram-positive bacterial strains than on the gram-negative ones. Distilled water which was used as a negative control showed no effects oneither of the bacterial strains.

CONCLUSION

This study revealed that the *Vernonia amygdalina* aqueous extract at 20 mg/ mlhad shown slighter antibacterial action when compared with the vehicle-treatedgroup. However, when compared to standard antibiotics, it had only producedone-fourth of activity. Moreover, it is active against only two organisms such as*Pseudomonas aeruginosa*, and *Staphylococcus aureus* but not on *Escherichiacoli*. Hence this plant extract may be used only as an adjuvant to the standardtreatment, provided it does not produce any drug interactions.

RECOMMENDATIONS

Ethanol and other extractions of the plant extract should be carried out to ascertain the presence of other phytochemicals and determine their antibacterial activity. It is essential to isolate the specific component responsible for the antibacterial activity of the extract. Improving the quality of results will require upgrading the equipment used in isolating the active principles. Furthermore, to comprehensively assess the antibacterial efficacy of the extract, conducting minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) tests is recommended. Fractionation of the extract should also be pursued to identify which phytochemical component exhibits the strongest antibacterial activity.

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