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.
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 International University-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 Dominic Byarugaba) at Kampala International University-Western campus and a voucher sample was prepared as herbarium.

**Preparation of the Extract (aqueous extraction)**
Leaves of *Vernonia amygdalina* was collected around Ishaka. The leaves were then air dried for 2 weeks, crushed, and then blended. 12g of the leaves were weighed and used in extraction. Extraction was done by dissolving the ground leaves in 100ml of hot water boiled for thirty minutes in a conical flask and then 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 using aqueous 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* aqueous extract 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 water bath for fifteen minutes then filtered. Three drops of 0.1% ferric chloride were added to the filtrate and observed for the presence of a brownish-green or blue-black coloration indicative of tannins.

iii. **Saponins**
0.2g of the extract was dissolved in 5ml of distilled water and then heated to a boil. The formation of a layer of foam indicated the presence of saponins.
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 the side of the test tube. Turbidity or a white precipitate with this reagent was taken as evidence for the presence of alkaloids.

v. Phlobotannins

0.2g of the extract was dissolved in 5ml of distilled water and filtered. The filtrate was boiled with 2ml of 2% 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 2ml of concentrated sulphuric acid and thereafter 2ml of acetic anhydride was added to the mixture color changed from violet to blue or green indicating the presence of steroids.

vii. Cardiac glycosides

A drop of ferric chloride solution was added to 2ml of glacial acetic acid. This solution was used to treat 0.5g of the extract. The mixture was then underlaid with 1ml of concentrated sulphuric acid. A brown ring at the interphase was indicative of a deoxy-sugar characteristic of cardenolides. A violet ring may also appear below the brown ring while in the acetic acid layer, a greenish ring may form gradually throughout the thin layer indicating the presence of cardiac glycosides.

viii. Reducing sugars

0.2g of the extract was shaken with 5ml of distilled water and filtered. The filtrate 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 cork borer of 6mm diameter and the disc was autoclaved at 121 degrees Celsius for 15 minutes. 0.2g of the extract was then diluted with 10mls of distilled water to give a concentration of 20mg/ml and this was added to the discs. This concentration was chosen as it correlated with the concentration of the standard antibiotic (gentamycin) that was to be used. The plant extract disc was 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 Microbiology Laboratory of Kampala International University-Western campus. The test organisms used were Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli. Staphylococcus aureus and Pseudomonas aeruginosamicroorganisms were cultured on Nutrient Agar plates by dissolving 2.8g of Nutrient Agar in 100mls of water while Escherichia coli was cultured in McConkey 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 microorganisms were later sub-cultured to produce young cultures which were used in the experiment.

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 by diluting 9g of Mueller-Hinton agar in 250ml of distilled water and sterilizing by autoclaving. MHA was allowed to cool to 50 degrees Celsius and 5ml of the molten agar was then added to the petri dishes. Three wells of about 6.0mm diameter were aseptically made on each agar plate using a sterile cork borer. The cultured microorganisms were later inoculated on the Mueller-Hinton Agar plates by spreading the microorganisms uniformly across the Petri dishes. Fixed volumes (20µl) of the plant extract were then introduced into the wells. Control wells containing gentamycin were used as a positive control because it is an antibacterial antibiotic and was set up at the center and distilled water was added to the third well as a negative control. The plates were then incubated at 37 degrees Celsius for 24 hours. The relative susceptibility of the microorganisms to the plant extract was determined by measuring the zones of inhibition. The experiment was done twice for Staphylococcus aureus and Escherichia coli plates and once for Pseudomonas aeruginosa 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 the mean of the zone of inhibition produced by the bacteria to know the effectiveness of each plant extract and the susceptibility of the test organism.
Limitations of the Study
The research results were not as expected due to the failure to isolate and sterilize the 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 were provided by the School of Pharmacy, Kampala International University-Western Campus.

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

Table 1: Phytochemical test

<table>
<thead>
<tr>
<th>TEST</th>
<th>RESULTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>Phlobatannins</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>-</td>
</tr>
<tr>
<td>Steroids</td>
<td>-</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>+</td>
</tr>
<tr>
<td>Reducing sugar</td>
<td>-</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
</tr>
</tbody>
</table>

(+) 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 test microorganism

<table>
<thead>
<tr>
<th>Diameter of inhibition zones (mm)</th>
<th>P. aeruginosa</th>
<th>Staph. aureus</th>
<th>Esch. coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Gentamycin 20mg/ml</td>
<td>27</td>
<td>33.5</td>
<td>23.5</td>
</tr>
<tr>
<td>Aqueous extract 20mg/ml</td>
<td>8</td>
<td>10</td>
<td>-</td>
</tr>
</tbody>
</table>

Aqueous extract (-) = no activity, P. aeruginosa = Pseudomonas aeruginosa, 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 the investigation of medicinal plants \[41-50\]. The present research has tested the crude extracts of Vernonia amygdalina on bacterial strains which comprised of Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli. The therapeutic effect of some species of plants is determined by their constituents \[51-45\]. These constituents increase the body's resistance to disease, retard or delay the processes of natural aging or facilitate the...
adaptation of the organism to certain conditions. Phytochemical screening analysis revealed the presence of tannins, phlobatannins, saponins, terpenoids, glycosides, and alkaloids in the plant extract. Elsewhere it is reported that tannins, alkaloids, saponins, flavonoids, and glycosides could be associated with the antimicrobial activities of some plants. 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 the specific bioactive components against specific organisms; and this has been noted to affect the quality and safety of herbal medicines. The aqueous extract also showed no presence of phytochemicals such as flavonoids, steroids, and reducing sugars. Vernonia amygdalina extracts had inhibition on Staphylococcus aureus (gram-positive) and Pseudomonas aeruginosa (gram negative) but had no inhibition on Escherichia coli (gram negative). This is similar to the observation of the plant’s ability to inhibit E. coli [17]. The aqueous extract of Vernonia amygdalina had slight antituberculosis properties at a percentage of 30% activity on Staphylococcus aureus (10 mm zone of inhibition) when compared to the standard antibiotic activity of gentamycin at 100% (33.5 mm) and 29% activity on Pseudomonas aeruginosa (8 mm zone of inhibition) when compared to the standard antibacterial activity of gentamycin at 100% (27 mm). 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 to the age of the plant used, freshness of plant materials, physical factors (temperature, light water), contamination by fields, and reducing sugars. Vernonia amygdalina showed a greater inhibitory effect on the gram-positive (S. aureus) than on the gram-negative strains (P. aeruginosa, and S. aureus). Gentamycin had the greatest effect on Staphylococcus aureus (33.5 mm zone of inhibition), then on Pseudomonas aeruginosa (27 mm zone of inhibition), and lastly on Escherichia coli (23.5 mm zone 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 on either of the bacterial strains.

CONCLUSION

This study revealed that the Vernonia amygdalina aqueous extract at 20 mg/ml had shown slighter antibacterial action when compared with the vehicle-treated group. However, when compared to standard antibiotics, it had only produced one-fourth of activity. Moreover, it is active against only two organisms such as Pseudomonas aeruginosa, and Staphylococcus aureus but not on Escherichia coli. Hence this plant extract may be used only as an adjuvant to the standard treatment, 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.

REFERENCES

