



ANTIBACTERIAL ACTIVITY OF *Vernonia amygdalina* (BITTER LEAF) STEM BARK AGAINST *Escherichia coli* ASSOCIATED WITH DYSENTERY

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DOI: <https://doi.org/10.70382/caijbhr.v8i3.008>

Abstract

Dysentery remains a significant global health concern, particularly in regions with limited access to healthcare and sanitation. This study investigates the potential antibacterial properties of *Vernonia amygdalina*, commonly known as bitter leaf, specifically focusing on the stem bark in aqueous and ethanolic extracts against *Escherichia coli*, one of the major causative agents of dysentery. Additionally, the phytochemical composition of the extracts was analysed to gain insights into the bioactive components of the plant. This research employed agar well diffusion method and also a range of concentrations (20-40mg/ml) in both the aqueous and ethanolic extracts. This study revealed promising results, lowest zones of inhibition measuring 8.2mm and 12.1mm in the aqueous and ethanolic extracts, respectively with minimum inhibitory concentration of 10mg/ml and 15mg/ml respectively. These results suggest the potential of bitter leaf stem bark in combating *E. coli*; a key pathogen associated with dysentery. This study therefore underscores the medicinal potential of *Vernonia amygdalina* stem bark in the context of dysentery. The observed inhibitory effects against *E. coli* provide a strong foundation for further research in the pursuit of safe and efficacious treatments for dysentery. This research contributes to the broader field of natural medicine, drug discovery and have a significant impact on global health, especially in regions where dysentery remains a major public health concern.

Keywords: Antibacterial; *Vernonia amygdalina*; Bitter leaf; Dysentery; Stem bark; Plant Extract.

Introduction

Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs. In many parts of the world, medicinal plants have been used as traditional treatments for various human ailments for thousands of years. The use of plant and its products has a long history in maintaining human health that began

with folk medicine and through the years has been incorporated into traditional medicine (Belay *et al.*, 2019). *Vernonia amygdalina* is a tropical plant belonging to the family Compositae and is widely known as vegetable and medicinal plant. It has the common name bitter leaf due to its bitter taste (Ibrahim *et al.*, 2000; Adetunji *et al.* 2013). The Yoruba name is 'Ewuro' 'Olugbu' in Igbo and 'Shiwaka' in Hausa. The shrub is usually about 5m high. The leaves are simple and entire (5×15cm), finely glandular below and displaying few lateral nerves. The flowers occur in a panicle, white and fragments (Iwu, 1999). It does not produce seeds and has to be distributed or propagated through cutting. It grows under a range of ecological Zones in Africa and produces a larger mass of forage and it is drought tolerant; it is majorly used for human consumption and has to be washed to remove the bitter taste. Its bitter taste is due to anti nutritional factors such as alkaloids, saponins, tannins and glucosides (Adetunji *et al.*, 2013).

Vernonia amygdalina have been used in many homes in the eastern parts of Nigeria as food especially in the preparation of soups. The characteristic bitter taste is believed to have after taste of sweetness. The peeled stem is often used as chewing stick for cleaning the teeth and is very effective as anticaries (Adekunle, 2002; Arekemase *et al.*, 2013). This plant contains complex active components that are pharmacologically useful. The roots and the leaves are used in ethno-medicine to treat fever, hiccups, kidney problems and stomach discomfort. The stem and root divested of the bark are used as chewing sticks in many West African countries like Cameroon, Ghana and Nigeria (Adetunji *et al.* 2013). It can be propagated either by cutting or seedling.

According to Kaper *et al.*, 2004, Dysentery refers to an acute inflammatory reaction of the intestinal tract characterized by watery stool, blood and pus. It is caused by any one of several members of Enterobacteriaceae. *E. coli*, a member of the bacterial family of Enterobacteriaceae, is the most prevalent commensal inhabitant of the gastrointestinal tracts of humans and warm-blooded animals, as well as one of the one of the major causative agents of dysentery. The Enterobacteriaceae or enteric bacteria are a large heterogeneous group of gram-negative rods whose natural habitat is the intestinal tracts of human and some other animals (Jawatz *et al.*, 2004; Arekemase *et al.*, 2013).

In some African countries including Nigeria, this plant species is traditionally used to treat many ailments including diabetes, malaria, helminth infections, fever, promote wound healing and to treat microbial infections. Also, the Hausa tribe of the northern part of Nigeria used the root and twig of *V. amygdalina* to treat stomach-ache and gastrointestinal troubles (Audu *et al.*, 2018). It is also prescribed to nursing mother as it improves lactation (Anibijuwon *et al.*, 2012; Audu *et al.*, 2018). According to Akujobi *et al.*, 2004 and Adetunji *et al.*, 2013, Botany and medicine have been closely linked throughout history. Prior to this century, medical practitioners whether allopath (medical doctors), homeopaths, naturopaths, herbalist or shamans had to know the plants in the area and how to use them since many of their drugs were derived from plants. Around 1900, 80% of the drugs were derived from plants. However, in the decades that followed, the development of synthetic drugs from petroleum products caused a sharp decline in the pre-eminence of drugs from live plant sources.

V. amygdalina is been used traditionally in a lot of homes in Nigeria but it is still considered as under exploited crop of medicinal significance and Medicinal plants have been recognized as potential sources of new compounds for therapeutic use.

Literature Review

Medicinal plants have long served as sources of bioactive compounds with antimicrobial properties, especially in regions with limited access to synthetic pharmaceuticals. Among such plants, *Vernonia amygdalina*, commonly known as bitter leaf, has received considerable scientific attention due to its wide ethnomedicinal applications, including antimicrobial use (Ijeh et al., 2011). Various parts of the plant—including the leaves, roots, and stem bark—have been traditionally utilized for treating gastrointestinal infections, fevers, and microbial diseases (Adetunji et al., 2013). Recent research has increasingly focused on validating these traditional claims through laboratory-based investigations.

Several studies have demonstrated the antibacterial potential of *V. amygdalina* leaf extracts against *E. coli* strains. Ijeh and Ejike (2011) confirmed the efficacy of bitter leaf extracts in controlling *E. coli* growth, attributing the antimicrobial activity to phytochemical constituents such as flavonoids, alkaloids, and tannins. However, most of these studies have primarily centered on the leaves of *V. amygdalina*, with relatively limited attention given to the stem bark.

Research specifically examining the stem bark of *V. amygdalina* is comparatively sparse but promising. A study by Audu et al. (2018) investigated the antibacterial properties of the stem bark extracts against various bacterial species, including *E. coli*. The findings indicated that both aqueous and ethanolic extracts of the stem bark demonstrated measurable zones of inhibition against *E. coli*, with the ethanolic extracts showing superior antimicrobial potency. This suggests that ethanol is more efficient at extracting the active antibacterial compounds from the plant matrix, aligning with observations from related studies involving other plant species (Ezekwesili et al., 2014).

Further supporting evidence was provided by Eze et al. (2017), who examined the antimicrobial activity of *V. amygdalina* stem bark in combination with other medicinal plants against *E. coli*. Their results confirmed that *V. amygdalina* stem bark contributed significantly to the observed antibacterial activity in multi-plant preparations, emphasizing its potential as a standalone antimicrobial agent.

Phytochemical analyses of *V. amygdalina* stem bark have revealed the presence of bioactive secondary metabolites such as saponins, alkaloids, tannins, and sesquiterpene lactones (Okolie et al., 2020). Given that *E. coli* possesses a relatively robust outer membrane, the effectiveness of these phytochemicals underscores their therapeutic potential.

Despite these positive findings, there remains a notable gap in systematic investigations specifically targeting the antibacterial activity of *V. amygdalina* stem bark against *E. coli*. Most available studies focus on generalized antibacterial properties or utilize mixed parts of the plant, leaving a need for targeted research on the stem bark alone. Moreover, inconsistencies in extraction methods, bacterial strains used for testing, and variations in phytochemical composition depending on geographical

origin highlight the necessity for standardization in future studies (Ijeh & Ejike, 2011; Eze et al., 2017).

Aim and Objectives

This study aimed to determine the anti-dysenteric activity of *V. amygdalina* stem bark on *E. coli* through the following Objectives:

- a) To determine its antibacterial activities on *Escherichia coli*
- b) To determine the Zone of inhibition on the test organism.
- c) To determine the minimum inhibitory concentration.

Significance of the study

Antibiotics are undeniably one of the most important therapeutic discoveries of the 20th century that had effectiveness against serious bacterial infections. However, only one third of the infectious diseases known to have been treated from these synthetic products (Sharma, 2011; Belay *et al.*, 2019). According to Njoroge and Bussmann, 2006, Medicinal plants have been recognized as potential sources of new compounds for therapeutic use. Findings from researchers and pharmaceutical entrepreneurs have pointed out that ethno botanically derived compounds have greater activity than compounds derived from random screening and thus a greater potential for novel products developed.

Also, there has been the development of multiple drug resistance, thus there is need to develop alternative antimicrobial drugs from medicinal plants for the treatment of infectious diseases; because antimicrobials of plant origin have been found to have enormous therapeutic potential (Abdulhamid *et al.*, 2016).

Scope of the study

This study investigates the antibacterial effect of *Vernonia amygdalina* stem bark extract on *Escherichia coli* using the zone of inhibition method and also determine the Minimum inhibitory concentration.

Limitation of the study

This study focuses on *E. coli* and does not account for the effect of *Vernonia amygdalina* on other pathogens that may cause dysentery, it also uses crude extracts rather than isolated active compounds, which may lead to variability in results due to the presence of multiple constituents in the extract.

Material and Methods

Collection of plant materials.

Stem barks of *V. amygdalina* plant were collected in a sterile polythene bag from a garden at Sabo layi area of Bali, Taraba State, Nigeria. They were taken to the Department of Biological Sciences,

School of Science and Technology, Federal Polytechnic Bali, Taraba state, Nigeria for proper identification and authentication. The samples were air-dried for three weeks after which they were first grounded with mortar and pestle. It was further ground to fine powder using an electric blender. It was kept in air-tight containers till further use.

Preparation of extracts: Aqueous Extract

50grams of the finely ground powder was introduced into a conical flask and 200 ml of distilled water was added, covered with a cork, mixed together properly. The extraction was done for 48 hours as described by Adetunji *et. al.*, 2013. After 48 hours, the extract was filtered through a muslin cloth and the obtained filtrate was further sterilised by filtration through membrane filter HC type. The filtrate obtained was evaporated to dryness at 45°C, and the obtained residue was kept in a foil paper.

Ethanollic Extract

50grams of the finely ground powder was introduced into a conical flask and 200 ml of 95% ethanol was added, covered with a cork, mixed together properly. After 48 hours, the extract was filtered through a muslin cloth and the obtained filtrate was further sterilised by filtration through membrane filter HC type. The filtrate obtained was evaporated to dryness at 45°C, and the obtained residue was kept in a foil paper.

Sterility Test of the Extracts

Both extracts (Aqueous and ethanolic) were tested for sterility by inoculating 1ml of each of them on nutrient agar and incubated at 37°C for 24 hours. Then the plates were observed for growth. There was no growth on the plates after incubation which indicates that the extracts were sterile.

Test Organism and Standardization

The test Organism used in this study is *Escherichia coli*. It was obtained from the General Hospital Laboratory Bali, Taraba state, Nigeria and was standardized by picking few colonies into sterile test-tube containing sterile nutrient broth and incubated at 37°C for 24 h. The turbidity of the suspensions was adjusted to 0.5 turbidity McFarland standard as described by Audu *et. al.*, 2018.

Antibacterial Activity Assay

This was done using agar-well diffusion method by picking 0.2 mL of the adjusted suspensions using a sterile wire loop, were inoculated on the solidified Mueller Hinton agar Plates. The agar medium was punched out four times using a sterile cork-borer of 8 mm diameter, then a sterile Pasteur pipette was used to introduce sterile distilled water and subsequently 20mg/ml, 30mg/ml and 40mg/ml of the extracts into the wells and incubated at 37°C for 24 hours as described by Adetunji *et. al.*, 2013.

Minimum Inhibitory Concentration was determined by putting 5mg/ml, 10mg/ml and 15mg/ml Each of Aqueous and Ethanolic Extracts into separate tubes of already prepared Mueller Hinton broth. The tubes were then inoculated with standardized *E. coli* suspension and were incubated. Sterile distilled water served as the control.

Results

The results in Table 1 show the zones of inhibition with different concentrations of both aqueous and ethanolic extract.

Table 1: Zones of Inhibition for Aqueous and Ethanolic extract

Concentration(mg/ml)	Zone of Inhibition(mm)		
	Aqueous extract	Ethanolic extract	Control
20	8.2	12.1	-
30	10.3	15.5	-
40	11.5	20.2	-

Key: - no visible zone of inhibition

Table 2: Minimum Inhibitory Concentration for Aqueous and Ethanolic extract

Concentration(mg/ml)	Extracts		
	Aqueous extract	Ethanolic extract	Control
5	Growth	Growth	-
10	No Growth	Growth	-
15	No Growth	No Growth	-

Discussion

Zone of Inhibition was used to determine the antibacterial effect of *Vernonia amygdalina*. The result of aqueous and ethanolic extracts are presented in Table 1.

The lowest zone of Inhibition recorded for aqueous extract against *E. coli* was 8.2mm at 20mg/ml while ethanolic extract have 12.1mm zone of inhibition also at 20mg/ml. The Minimum Inhibitory Concentration (MIC) observed for both extracts as shown in Table 2 indicates that the aqueous extract exhibited inhibitory activity at a concentration of 10 mg/mL, while the ethanolic extract showed inhibitory activity at 15 mg/ml. This suggests that the aqueous extract was more effective against *E. coli* at a lower concentration compared to the ethanolic extract. The clarity of the broth served as an indicator of bacterial growth: clear tubes denoted inhibition of growth (no turbidity), whereas cloudy tubes indicated visible bacterial growth. These findings demonstrate that increasing the concentration of the extracts reduced bacterial proliferation, with complete inhibition observed at the recorded MIC values.

The highest zone of inhibition recorded was with ethanolic extract and this may be due to the higher volatility of the ethanol which tends to extract more active compounds from the samples than water (Adetunji *et al.*, 2013). Doughari *et al.*, 2007 stated that another reason for organic extract to be more active than water extract is due to the better solubility of the active components in organic solvents.

Audu *et al.*, 2018 shows the presence of alkaloids, steroids, Glycosides, flavonoids, tannins, saponins, phlebotomine, and phenolics in the extracts of *V. amygdalina* stem bark which explains the effectiveness of these extracts.

Conclusion

The findings of the study have provided compelling evidence supporting the traditional use of *V. amygdalina* in ethnomedicine, particularly in the treatment of Infection caused by *E. coli*. The results demonstrated that both aqueous and ethanolic extracts of *V. amygdalina* stem bark exhibited inhibitory effects against *E. coli*. However, the ethanolic extract produced a higher zone of inhibition compared to the aqueous extract. This observation suggests that ethanol is more efficient at extracting the bioactive antimicrobial constituents from the plant material. The superior efficacy of the ethanolic extract may be attributed to its ability to solubilize a broader range of phytochemicals, including phenolic compounds, flavonoids, alkaloids, and tannins, which have been associated with antimicrobial properties.

The antibacterial action observed aligns with previous studies that have reported the therapeutic potential of *V. amygdalina* extracts against various bacterial strains. Traditional healers across many African communities have long used bitter leaf extracts in the treatment of gastrointestinal infections, wounds, and respiratory tract infections. The confirmation of antibacterial activity in this study further validates these traditional claims and underscores the potential of this plant as a source of novel antimicrobial agents.

Moreover, the global increase in antibiotic-resistant strains of *E. coli* presents a serious public health challenge, necessitating the urgent search for alternative therapies and new antibacterial agents. The findings from this research contribute to the growing body of evidence that medicinal plants can serve as potential reservoirs for drug discovery. By targeting plants with proven ethnobotanical uses, researchers can identify novel bioactive compounds that may overcome antibiotic resistance mechanisms. It is important to recognize that the antimicrobial efficacy of plant extracts can be influenced by various factors, including the part of the plant used, the solvent employed in extraction, the concentration of the extract, and the bacterial strain tested. In this study, the use of the stem bark of *V. amygdalina* proved to be an effective choice, although other parts of the plant, such as leaves and roots, may also harbor antibacterial compounds.

Beyond the demonstrated antibacterial activity, the use of natural products like *V. amygdalina* carries significant advantages, including potential biocompatibility, reduced risk of toxicity, and accessibility for populations with limited access to synthetic antibiotics. Medicinal plants are an

integral part of primary healthcare in many developing regions, providing affordable and culturally acceptable remedies for a wide range of ailments.

Recommendations

1. Further research is recommended to isolate, identify, and characterize the specific bioactive compounds responsible for the antibacterial activity of *Vernonia amygdalina* stem bark. Use advanced analytical techniques such as chromatography and spectroscopy for compound identification.
2. Toxicity studies are also to be prioritize in order to scientifically assess the safety of both the crude extracts and the purified compounds. This is necessary despite the plant's traditional use, especially before any clinical application.
3. There is also need to perform in vivo studies using appropriate animal models to determine whether the antibacterial activity observed in vitro is replicable in living organisms.
4. Well-structured clinical trials are also advised, particularly in areas with a high incidence of *E. coli* infections and increasing antibiotic resistance, to evaluate therapeutic effectiveness in humans.

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