PHYTOCHEMICAL, PROXIMATE COMPOSITION AND ANTBACTERIAL ACTIVITY OF *Vernonia amygdalina* LEAVES AGAINST CLINICAL ISOLATES OF *Staphylococcus aureus* AND *Escherichia coli* IN ENUGU STATE

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APPROVAL

This research work titled "Phytochemical, Proximate Composition And Antbacterial Activity Of *Vernonia amygdalina* Leaves Against Clinical Iso`lates of *Staphylococcus aureus* And *Escherichia coli* In Enugu State **"** has been assessed and approved by the committees of the Department of Biological Sciences and the Faculty of Natural Sciences and Environmental Studies, Godfrey Okoye University, Enugu, Nigeria.

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CERTIFICATION

This is to certify that this project report titled: Phytochemical, Proximate Composition And Antbacterial Activity Of *Vernonia amygdalina* Leaves Against Clinical Isolates of *Staphylococcus aureus* And *Escherichia coli* In Enugu State was carried out by Obi Vivian Chidimma with Registration Number GOU/U22/MCB/532 under the supervision in the department of Biological Sciences, Godfrey Okoye University Enugu Nigeria.

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DEDICATION

This project work is dedicated to God Almighty who has always been faithful to me.

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ABSTRACT

Vernonia amygdalina (bitter leaf) is a plant usually cultivated in East and West Africa. It is known to contain several antimicrobial substances such as flavonoids, steroid glycoside, tannins etc against various microorganisms. This study aimed to assess the antibacterial properties, qualitative phytochemical profile, and quantitative proximate composition of *Vernonia amygdalina.* Ethanol and aqueous extracts of *Vernonia amygdalina* were tested against clinical isolates of *Staphylococcus aureus* and *Escherichia coli* to validate its medicinal and nutritional relevance. Fresh *V. amygdalina* leaves from Abakpa market, Enugu, were air dried, pulverized, and extracted via a 72hour cold maceration process using 95% ethanol and distilled water. Phytochemicals were screened using standard chemical tests, proximate composition was evaluated and antibacterial susceptibility metrics including Minimum Inhibitory Concentration and Minimum Bactericidal Concentration were determined through agar well diffusion and macro broth tube dilution assays and analyzed by mean values and standard deviations. Phytochemical screening revealed the presence of flavonoids, alkaloids, tannins, saponins, steroids, and cardiac glycosides in the ethanolic matrix. Proximate analysis yielded total carbohydrates, 40.50%, crude protein 16.50%, crude fiber 12.30%, ash content 8.50%, crude fat 4.20%, and an air-dried moisture content of 18.00%. Agar well diffusion showed concentration dependent activity, with the ethanolic extract outperforming the aqueous extract, yielding maximum inhibition zones of 30 ± 1.15 mm against *S. aureus* at 200mg/ml and 100mg/ml concentrations, and 29.33 ± 1.15 mm against *E. coli* at 200 mg/mL. The aqueous extract yielded a much lower zone of inhibition, 20.33 ± 0.58mm at 200mg/ml for *E. coli* and 24.67 ± 0.58mm for *S. aureus.* Conversely, the aqueous extract failed to exhibit bactericidal capacity, acting strictly as a bacteriostatic agent against *E.coli*. These findings demonstrate that while both bitter leaf extracts possess definitive antibacterial activity, the ethanolic extract exhibits superior efficacy.

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CHAPTER ONE

INTRODUCTION

1.0 Background of the Study

Herbal medicine is the oldest healthcare system known to mankind, and it is estimated that more than 50% of all drugs used in modern medicine are derived from natural sources. They are also essential in the field of pharmaceutical development (Preethi *et al*., 2010). According to the World Health Organization (WHO, 2002), 80% of the population in the world depends on traditional or herbal medicine for primary health care, in which plants are the basis of traditional medicine systems. They are considered useful as potential lead compounds for new drug discovery (Sofowora, 1993; Akinjogunla *et al*., 2011). The revival of the plant/health connection has also given rise to new concepts of multi-component botanical medicines, dietary supplements and plant-based recombinant proteins (Akinjogunla *et al*., 2011). However, multidrug-resistant (MDR) bacteria are a serious problem in the medical field and in the pharmaceutical industry. In such a situation, the need to discover new highly effective, affordable, acceptable, and accessible treatments is heightened (Martino *et al*., 2002).

The bitter leaf, *Vernonia amygdalina*, is a member of the Asteraceae family and is known for its bitter taste. In traditional medicine, it has been either applied as a leafy vegetable, having the leaves softened in soups, or as a tonic of water to cure various diseases (Igile *et al*., 1994). It is a well-known medicinal plant in folk medicine, used in the treatment of many diseases (Allen, 1987). The leaves are definitely bitter, which can be minimised by boiling or by soaking in fresh water several times (Burkill, 1985). The presence of saponins, alkaloids, tannins and glycosides is responsible for this bitter flavor. The compounds are useful as a bittering agent and as a biological control agent for microbial growth in beer brewing without reducing the quality of the malt (Farombi and Owoeye, 2011). In traditional medicine, the root and leaf are used for treating fever, hiccups, kidney disorders, stomach discomfort and various other diseases (Burkill,1985).

The stems, roots and leaves are extensively used to make aqueous and alcoholic extracts, which are used as purgatives, anti-malarial agents and are used to treat eczema (Kupchan, *et al* 1971). The *Vernonia amygdalina* has come to be of great importance in recent years, as studies in human medicine have revealed that it contains potent anti-tumorigenic properties (Izevbigie *et al*., 2004),

while in nutrition it is mainly used for soup preparation in the tropical region and as an appetite stimulant and a fever reducer (Iwu *et al*., 1996). It has also been included in foods for children during weaning with success (Eleyinmi *et al*., 2005).

The leaf extract of the plant has been pharmacologically investigated and found to be hypoglycemic and hypolipidemic in experimental animals, indicating its possible use in diabetes mellitus control (Ebong *et al*., 2008). According to a few studies, the plant contains a wide variety of bioactive components such as flavonoids, saponins, alkaloids, tannins, phenolics, terpenes, steroidal glycosides, triterpenoids, and multiple forms of sesquiterpene lactones (Luo *et al*., 2017). It possesses a wide range of pharmacological activities such as antimicrobial, antimalarial, anti-diabetic, laxative, hypoglycemic, anti-inflammatory, cathartic, anticancer, antifungal, and antibacterial activities among others, owing to these compounds (Alara *et al*., 2017).

The aqueous extract of *V. amygdalina* leaves exhibited antimicrobial activity against pathogenic microorganisms such as *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa, Klebsiella spp*. and *Candida albicans*, while ethanolic extract displayed activity against *Staphylococcus aureus* and *Candida albicans*. In another study, ethanol-based leaf extract and water-based leaf extract showed that both extracts had an effective effect on *Streptococcus mutans*, with a minimum inhibitory concentration (MIC) of 12.5 mg/ml and 55 mg/ml, respectively (Akinpelu,1999; Anibijunwon *et al*., 2012).

1.2 Statement of the Problem

Due to the rising occurrence of antimicrobial resistance, alternative antibacterial agents from medicinal plants are needed. *Vernonia amygdalina* extracts have been shown to exhibit antibacterial properties against pathogenic bacteria like *Staphylococcus aureus* and *Salmonella spp*., with the strength varying depending on the method of extraction and solvent used (Ahmad *et al*., 2020; Arekemase *et al*., 2021). Although this activity has been observed, the relationship between these antibacterial activities and the phytochemical and nutritional constituents of the plant is not completely understood.

There is a lack of scientific data that can be used to validate, standardise, and utilise the use of *Vernonia amygdalina* as a natural antimicrobial agent and functional food. Therefore, it is

important to conduct an integrated research study to assess the antibacterial activity of bitter leaf, its phytochemical constituents and its proximate composition so that there is credible evidence that will support the medicinal and nutritional importance of bitter leaf.

Vernonia amygdalina is a bitter leaf plant and popular vegetable food and medicine in many African countries, including Nigeria due to its nutritional and therapeutic properties. Previous studies have identified some plant bioactive phytochemicals such as flavonoids, tannins, saponins, alkaloids, steroids, and phenolics that are responsible for the antimicrobial and health-promoting properties of the plant (Arekemase *et al*., 2021; Ali *et al*., 2019). Moreover, the nutritional composition of bitter leaf has been substantiated through nutritional analysis, which has established its value in the human diet (Mgbemena and Amako, 2017).

Although these benefits have been reported, extensive research is still lacking that has the ability to assess bitter leaf's antibacterial activity, phytochemical profile, and nutritional composition all at once, particularly from specific local sources. Most current literature emphasizes only one of these factors, such as antimicrobial activity, phytochemical composition, or proximate composition. This dismembered approach restricts the overall knowledge of the plant's potential (Mgbemena and Amako, 2017).

1.2 Aim and Objectives

1.2.1 Aim

The aim of the study is to evaluate *Vernonia amygdalina* antibacterial activity , identifying *Vernonia amygdalina* phytochemical constituents and proximate analysis.

1.2.2 Objectives

- To determine the proximate composition of *Vernonia amygdalina* leaves.
- To determine the phytochemical constituents of the ethanol extract of leaves.
- To evaluate the antibacterial activity of aqueous and ethanol extract of against *V. amygdalina* against clinical isolates of *staphylococcus aureus and Escherichia coli.*
- To determine the minimal inhibitory concentration of *Vernonia amygdalina* extracts against the test isolates

- To determine the minimum bactericidal concentration of *Vernonia amygdalina* extract against the test isolates.

CHAPTER TWO

LITERATURE REVIEW

2.0 Botanical Profile and Ethnomedicinal Relevance of *Vernonia amygdalina*

Most people would simply recognize *Vernonia amygdalina* as bitter leaf (Alara *et al*. 2017). This resilient and adaptable flora is widely available in the tropics of sub-Saharan Africa. Due to its use in folk medicine and as an indigenous food source, in West Africa it is considered a staple (Kadiri and Olawoye, 2016).

In Nigeria, it goes by a number of vernacular names depending on the community: the Yoruba refer to it as Ewuro, the Igbo as Onugbu and the Hausa as Shuwaka. The shrub has an aggressive way of growing, putting on height to anywhere from 2 to 5 metres. Its green, lanceolate leaves are puberulent and have a very pronounced bitterness to them.

2.1. Traditional Medicinal Value and Ethnobotanical Applications

There is a sound basis for the laboratory work now being done on crude extracts of *Vernonia amygdalina* (Degu *et al.*, 2024) in that it is rooted in how the plant has been used, both in the past and today, in traditional African medicine. One need only look at indigenous communities to see this; well before we had our modern disk diffusion or MIC assays, they made extensive use of bitter leaf infusions to put an end to all manner of infectious, metabolic and systemic troubles (Adewuyi *et al.,* 2025; Degu *et al*., 2024).

2.2. Management of Gastrointestinal Disorders and Gastroenteritis

Researchers have observed indigenous communities and have come to appreciate the role of the *V*. *amygdalina* in traditional medicine long before Mic and disk diffusion assay, bitter leaf was used for a number of infections (Adewuyi *et al*., 2025; Degu *et al*., 2024)

2.3. Ethnobotanical Treatment of Skin Infections and Wound Healing

People have been using the crude leaf paste and juices of *V. amygdalina* on open wounds and skin infections for a long time, a practice documented by Degu *et al.*, 2024. In traditional communities, the plant is used as an antiseptic bandage of sorts to stop rotting and speed up the healing of tissue (Mgwenya *et al*., 2025).

2.4. Antimalarial and Antipyretic Properties

The bitter leaf in Sub-Saharan Africa is used as a traditional cure for curing malaria and related high fevers (Degu *et al.*, 2024). Boiling the roots or leaves in water produces a very bitter decoction that the patient drinks regularly to alleviate severe chills and body aches (Hussen and Endalew, 2023). The phytochemical profile has shown that the bitter sesquiterpene lactones and alkaloids responsible for the bitterness help in eliminating blood-borne parasites and reducing high body temperatures, thus making it an effective and cheap treatment in the rural areas (Degu *et al.*, 2024).

2.5. Effect of different processing methods on nutritional composition of bitter leaf

Calcium, potassium, iron, and vitamin C vanish from the leaves when people perform common ways of preparation because boiling, squeeze washing, and salting happen together (Tsado *et al*., 2015). It has been observed that nutrients leave the leaves when the bitterness is removed so that the flavor profile of the bitter leaves becomes more pleasant for the individual, but when the bitter leaves remain in boiling water without squeezing to help the beta carotene concentration grow, the vitamins that dissolve in water are lost instead (Nkechi, 2023). Agomuo *et al*., 2016 in their study, discovered that the action of squeezing bitter leaves together with palm oil helps with keeping the healthful components inside, and experts claim that this technique provides a remedial answer to stop the loss. According to the findings of (Yakubu *et al*., 2012), using water to soak the leaves overnight, blanching, or even the act of squeezing the bitter leaves with or without salt, are some of the procedures that A lot decrease the antioxidant power, the amount of protein, and the level of water in the cell. The fat quantity was made less when blanching and rubbing without salt occurred, even though the fat quantity increased when the bitter leaves stayed in water or were rubbed with salt. Crude fiber amounts became smaller because of the soaking, while squeezing with salt caused the fiber to increase in the bitter leaves. Ash components became more plentiful

when squeezing was done, while blanching and keeping the leaves in water made the ash levels much lower. Mineral, tannin, and phytate parts of the bitter leaves were removed away when the procedural ways of soaking for the nocturnal duration, blanching, and squeezing were used (Yakubu *et al*., 2012).

Regarding the research of the fresh leaves and the leaves that were left for 5 days in a room to dry by itself, experts claim that a great amount of minerals was found inside them. These minerals did not decrease after drying. (Ifesan *et al.,* 2014).

2.6 Phytochemical Solubility Profiles of Ethanolic vs. Aqueous Solvents

The isolation of bioactive components from *Vernonia amygdalina* leaves depends fundamentally on the chemical principle that a solvent will selectively dissolve molecules with a similar polarity index (Alara *et al.*, 2021). The plant matrix contains a diverse array of secondary metabolites that range from highly hydrophilic (water loving) molecules to moderately lipophilic (fat loving) chemical chains (Nowak *et al.,* 2022). Consequently, choosing between an organic alcohol like ethanol and a completely polar solvent like distilled water changes the final chemical yield of the crude extract (Adewuyi *et al.*, 2025).

As the dielectric constant of ethanol (Usman *et al*., 2025) remains balanced, it is believed that ethanol functions as a common polar organic liquid for dissolving substances. The small ethyl chain and the end hydroxyl group allow ethanol to break down the walls of plant cells with strength, so that very polar and medium nonpolar chemical structures like flavonoids, tannins, and alkaloids (Nowak *et al.,* 2022; Usman *et al.,* 2025) are pulled out from the material. Distilled water works as a very polar substance which melts away loose glycosides, botanical sugars, and saponins with great speed because distilled water carries high polarity (Hussen and Endalew, 2023).

2.7 Comparative Phytochemical Screening of Bitter Leaf Extracts

Both qualitative and quantitative laboratory screenings reveal that the chemical makeup of both water based and ethanol based preparations varies significantly (Chijindu *et al*., 2024). These differences result in the performance of each extract during benchtop antimicrobial assays (Afolabi *et al.,* 2025).

The aqueous extract is a solution that is prepared by soaking leaf powder in water to dissolve in water (Degu *et al.,* 2024). Water extracts a big amount of simple tannins and free plant carbohydrates (Ali, 2020).

In contrast, the ethanolic extract has a much higher density of complex flavonoids, alkaloids, and terpenoids (Usman *et al*., 2025). These compound families do not dissolve well in pure water but move easily into organic alcohols, which is why the ethanolic extract is a more concentrated storage of the plant's specific defenses (Nowak *et al*., 2022). Water allows the extraction of highly soluble saponins, while ethanol allows the extraction of a much broader and more chemically active spectrum of antimicrobial components (Alara *et al*., 2021; Usman *et al*., 2025).

2.8 Phytochemical Architecture and Mechanisms of Action

Vernonia amygdalina medicinal efficacy is derived from its secondary metabolites, which constitite small molecular weight organic structures synthesized by the plant in order to survive environmental challenges and defend against microbial attack (Nowak *et al*., 2022). Qualitative chemical screenings of ethanolic leaf extract of the plant consistently show a diverse chemical profile rich in highly bioactive agents.

2.8.1. Saponins

Scientists claim that saponins represent a large group of amphiphilic glycosides which are abundant in the sharp tasting foliage and directly contribute to its bitter profie (Degu *et al*., 2024). A fat soluble hydrophobic core is joined to a water soluble hydrophilic sugar chain so that saponins function as organic cleaning agents. Because saponins interact with a microscopic organism, saponins connect immediately to the structural lipids that exist within the outer biological boundary.

2.8.2. Tannins

Polyphenolic polymers which dissolve in water and alcohol are recognized as tannins, and these substances exist in large amounts inside the plant body (Tura *et al.,* 2024). Experts claim that tannins show their ability to fight bacteria through their ability to collect proteins and join with important metal ions.

2.8.3. Flavonoids

Flavonoids contain diverse polyphenolic arrangements, including luteolin and apigenin derivatives can be effectively recovered through polar organic solvents like ethanol (Nowak *et al*., 2022). These compounds exhibit potent radical-scavenging capabilities, robust antioxidant protection, and a high level of antibacterial efficacy. Because flavonoids easily permeate bacterial lipid bilayers, they disrupt the cellular framework of the cytoplasmic membrane and suppress the production of intracellular nucleic acids, thereby halting active replication and deactivating internal enzymes.

2.8.4. Alkaloids

Basic chemical entities containing nitrogen which are extracted from the foliage of *V*. *amygdalina* are represented by alkaloids (Alara *et al*., 2017). Scientists have stated that alkaloids provide a strong defense against harmful microscopic organisms because they possess specific bactericidal effects. When alkaloids encounter the protective barriers of a bacterium, alkaloids move through these structures with ease to reach the internal fluid known as cytoplasm. It has been observed that alkaloids then attach themselves directly into the genetic code of the bacterial organism due to them bonding with the bacterial DNA in this specific manner, the biological function of transcription is halted immediately. Natural processes like the production of new cells are prevented by the chemical presence of alkaloids. It is believed that, alkaloids disrupt the cycle of life by stopping cell division, thereby making it highly effective against these pathogens.

2.9. Influence of Extraction Solvents

The success of the medicinal dosage and the final bactericidal ability of a botanical extract rests fully on the polarity and effectiveness of the solvent used (Alara *et al*., 2017). The pharmacological results are influenced by the solvent because the solvent dictates what parts of the plant are pulled out. Experts claim that traditional community medicine mostly uses water boiling to get results. Systematic laboratory work shows that organic solvents obtain a much better collection of bioactive elements because organic solvents have better strength than water.

Scientists have observed that ethanol serves as a very powerful polar organic liquid for the removal of bioactive secondary metabolites from dried plant matter (Nowak *et al*., 2022). Ethanol contains a structural hydroxyl group that links easily with water loving plant elements, including saponins and polyphenols because ethanol possesses a small ethyl chain, it has the capacity to attract molecules which are moderately lipophilic. Studies have shown that *V. amygdalina* ethanol extract exhibit enhanced antimicrobial zones alongside reduced MIC values when tested against common microorganisms than extracts made from water (Tura *et al*., 2024). Water is considered to be less effective because water cannot dissolve complex organic rings attached to cell walls entirely.

2.10. Nutritional and Proximate Matrix

Researchers perform proximate analysis because this process separates the biological matter of a plant into six basic chemical segments which include ash, raw protein content, moisture, structural fiber, total fat alongside total carbohydrates (Kadiri and Olawoye, 2016). researchers believe that recording these specific measurements for *Vernonia amygdalina* (bitter leaf) provides evidence regarding the character of the plant. *Vernonia amygdalina* is categorized as a functional nutritional source by medical professionals because *Vernonia amygdalina* also serves as a very reliable pharmaceutical raw material. This information is documented so that the dual nature of *Vernonia amygdalina* remains clear for individuals with high educational backgrounds.

2.10.1 Moisture Content

Moisture percentage shows the amount of free water that exists inside the structural parts of the leafy vegetable. Due to these fresh leaves possessing a high level of retained water, this elevated dampness promotes the accelerated multiplication of microorganisms and the degradation of enzymes. Free water is extracted from the leafy vegetable through air drying the raw materials at room temperature in the shade until the moisture content reaches a steady level of 8% to 12%. Drying the raw materials protects fragile chemical parts that are sensitive to heat from being destroyed by high temperatures, while the drying also prevents the spreading of fungus or any microbe while the leafy vegetable is kept in storage.

2.10.2 Ash Content

Total organic substance is calculated through ash content when heat reaches elevated thermal levels like 550°C. Pure inorganic minerals are produced by ash content because organic matter is removed through heat. Experts claim that *V. amygdalina* possesses remarkably high ash content proportions which range from 10% to 17% of dry leaf mass (Kadiri and Olawoye, 2016). This elevated ash content yield demonstrates an abundant accumulation of fundamental nutritional macro minerals and micro minerals because *V. amygdalina* provides magnesium, calcium, potassium, iron, and zinc. These specific minerals serve as vital helper components for biological chemical activities while the body functions.

2.10.3 Crude Protein, Lipids, and Fiber

Dry bitter leaf contains very high levels of crude protein for a leaf for human consumption. A study carried out by Kadiri and Olawoye, 2016 identified the nutritional contents of bitter leaf. The findings revealed that the crude protein content in dry bitter leaf was very high containing very high vegetable content of dietary nitrogen either in the form of amino acids or as pure nitrogen. It ranged between 21% and 28%. The crude lipid in dry bitter leaf were between 3.5% and 5.5% which were essentially waxes and essential fatty acids. The crude fiber of dry bitter leaf were between 9% and 18% which were indigestible and were made up of cellulose that occurred in plant structures and lignin that offered health benefits to human digestive system.

2.11 Pathogenic Profiles of Target Clinical Isolates

Staphylococcus aureus and *Escherichia coli* may differ in their susceptibility to plant-derived antimicrobial agents, first and foremost because of their cell wall structure differences (Adamczak, Oarowski, and Karpiski, 2019). *Staphylococcus aureus* is a Gram-positive bacterium with a thick peptidoglycan cell wall. However, *Escherichia coli* is a Gram-negative bacterium that has an outer membrane complex, mainly consisting of lipopolysaccharides (Shamsudin *et al*., 2022).

Plant extracts containing bioactive flavonoids, such as luteolin and apigenin derivatives, can kill clinical isolates through multiple mechanisms of action (Adamczak *et al*., 2019; Shamsudin *et al*., 2022). The major fraction of plant extracts, i.e. flavonoids, such as luteolin and apigenin derivatives, are efficiently extracted from plant materials with the aid of polar organic solvents, for example ethanol (Navarro-Hoyos, 2023). These compounds easily interact with the lipid bilayer forming the outer membrane of target bacteria cells.

The flavonoids are then able to permeate across the lipid layer of the outer membrane. This is able to cause severe damage to both *S. aureus* and *E. coli* by disrupting the cell's cytoplasmic membrane. The cell's cytoplasmic membrane is responsible for preserving the cell's internal environment, and maintaining both the structure and the permeability of the cell. It enables the bacteria to synthesize new intracellular nucleic acids, and any disruption to the membrane, causes severe problems to the bacteria. The study on the clinical isolates found that the flavonoids were able to stop active replication of the bacteria and also inactivate important enzymes produced by the bacteria (Gallegos, 2022; Shamsudin *et al*., 2022). These characteristics enable the flavonoids to express effective and broad-spectrum antibacterial activity (Gallegos, 2022; Shamsudin *et al*., 2022).

2.12 Determination of Minimum Inhibitory Concentration (MIC)

Minimum Inhibitory Concentration (MIC), is identified as the lowest concentration amount of a bacteriostatic substance required to completely suppress detectable growth of a target microorganism after a standardized time of incubation (David and Musyoki, 2024). When modern laboratory tests of plant medicines are performed, calculating the lowest concentration required for bitter leaf ethanol extracts to inhibit growth is highly necessary because this calculation helps

the transition from looking at basic zones of inhibition to getting exact mathematical numbers (Afolabi *et al*., 2026). By utilizing the lowest concentration, the true power of the bitter leaf component against pathogenic organisms that resist multiple drugs is successfully measured by investigators (Upla *et al*., 2025).

2.13 Determination of Minimum Bactericidal Concentration (MBC)

The minimum bactericidal concentration of *Vernonia amygdalina* extracts is observed to determine its bacteriostatic or bactericidal effect on the isolates. The MBC values for *Vernonia amygdalina* extracts are different consequent to the choice of extraction solvent.

The ethanolic extract of *Vernonia amygdalina* has a lot of compounds like alkaloids and flavonoids that can disrupt the membranes of bacteria. This means it can inhibit at low concentrations close to the Minimum Inhibitory Concentration point as found by Usman *et al*., 2025. This shows that the ethanolic extract obtained from bitter leaf demonstrate potent inhibitory action against bacteria even at low doses (Afolabi *et al*., 2025).

Conversely, water based extract of *V. amygdalina* typically exhibit a pronounced disparity between their MIC and MBC values. At first the saponins, in the extract of *Vernonia amygdalina* stress the bacteria and stop them from growing which is a good sign as noted by Degu *et al*., 2024. However if these organisms are transferred to a growth medium without the extract it may grow. This means the aqueous extract of bitter leaf needs to be used at high concentrations to really inhibit the bacteria.

2.14 Susceptibility Variations Between Gram Positive and Gram Negative Bacteria

In the course of research work, the determination of the antibacterial potency of *Vernonia amygdalina* ethanol extract revealed that the structural variances in the cell walls of the target pathogens led to quite different results (Afolabi *et al*. 2025). It has been noted that researchers in the lab have noted that it is because of these physical barriers that bacteria are divided into Gram-positive and Gram-negative categories based on their cell wall properties (Tura *et al*. 2024). Experimental tests demonstrate that because of structural difference in their cell walls, *S. aureus* which is Gram positive regularly demonstrate higher sensitivity marked by larger clearer zones

and reduced MICs than the more shielded E.coli which is Gram negative (Afolabi *et al*., 2025; Tura *et al*., 2024).

It has also been observed that the cause of this distinct variation is recognized easily when a microscope is used. According to the study of (Degu *et al*., 2024), the cell wall of *Staphylococcus aureus* is constructed from a thick and porous peptidoglycan substance, even though *Staphylococcus aureus* completely lacks an external protective envelope of lipids. Because of this open and porous structure, the active saponins, flavonoids, and tannins which exist in the bitter leaf extract penetrate inside without encountering any blocking obstacle (Tura *et al*., 2024). Consequently, these organic compounds reach their target locations rapidly, which causes the bacteria to become highly vulnerable to low concentrations of the bitter leaf extract (Degu *et al*., 2024).

On the other hand, *E. coli* bacteria have a highly specialized layout of their cell walls to act as a protective shield for the bacteria (Itah and Akinjogunla, 2024). While the thickness of the peptidoglycan layer that makes up the bacteria's cell wall is quite thin, there is a tough outer membrane that surrounds the bacteria that contains lipopolysaccharides (Alozie *et al*., 2024). This outer membrane acts as a barrier that prevents polar and other heavy plant molecules from entering the bacterium's cells (Itah and Akinjogunla, 2024), because of this extra defensive layer, researchers must apply much higher concentrations of the crude extract to break through the outer barrier of *E. coli* and stop its growth (Afolabi *et al*., 2025; Alozie *et al*., 2024).

2.15 Mechanisms of Plant Extracts on Bacterial Cell Membranes

Scientists claim that the primary method by which the ethanol extract of *Vernonia amygdalina* destroys or stops the growth microorganisms involves the direct breaking of the bacterial cytoplasmic membrane (Masyita *et al*., 2022). This bacterial cytoplasmic membrane is vital for the life of the microorganism because the bacterial cytoplasmic membrane acts as a wall that keeps necessary food components for the cell inside (Di Matteo *et al*., 2024). Many experts believe that this process is the most effective way to stop infections. Saponins, which are found in great quantity inside the bitter leaf, have a special molecular build. The molecular build of saponins is very similar to a cleaning liquid or a strong soap (Degu *et al*., 2024). Because saponins possess

this soap like quality, the protective skin of the bacteria is dissolved. It has been observed that the ethanol extract of *Vernonia amygdalina* causes the contents of the organism to leak out. Such leakage happens because the ethanol extract of *Vernonia amygdalina* creates holes in the bacterial cytoplasmic membrane. Since the bacterial cytoplasmic membrane can no longer hold the necessary materials, the microbe eventually dies.

When the extract comes into contact with a bacterial cell, the saponins and oil loving terpenoids dissolve directly into the lipid layers of the bacterial membrane (Alozie *et al*., 2024; Masyita *et al*., 2022). This infiltration causes the neat arrangement of the bacterial membrane to tear and loosen, forming physical cracks and pores (Di Matteo *et al*., 2024). Through these open pores, vital internal contents such as potassium ions, core metabolic enzymes, and essential cell proteins rapidly leak out into the surrounding environment (Alozie *et al*., 2024). Without its internal contents and steady membrane pressure, the bacterial cell loses its structural balance, stops producing energy, and physically collapses in a process known as cellular lysis (Di Matteo *et al*., 2024; Masyita *et al*., 2022).

CHAPTER THREE

MATERIALS AND METHODS

3.0 Materials

Sterile laboratory coat, hand gloves, beaker, safety goggles, Agars, test tubes, conical flasks, cotton wool, sterile swab sticks, syringe, detergent, spatula, filter paper, test tube racks, petri dishes, micropipettes, cork borer, inoculating loops, masking tape, ziplock bag and meter rule

3.0.1 Equipment

Weighing balance, petri dish, gas, incubator, refrigerator, Bunsen burner, hot air oven, dissicator, muffle furnace, crucibles, measuring cylinder, sterile blender, and water bath

3.0.2 Reagents

Analytical grade ethanol (99.9%, sigma – Aldrich), distilled water, analytical reagent hydrochloric acid (37.0%w/w conc., loba chemie) wagner reagent (Iodine 1.27g + potassium iodide 2.0g in 100ml of H_2O) ferric chloride (0.1% to 1.0% w/v aqueous solution, loba chemie), sulfuric acid (95.0% v/v conc., loba chemie).

3.1 Sample Collection and Preparation

Fresh leaves of *Vernonia amygdalina* were got from Abakpa market Enugu. The leaves were washed with distilled water to get rid of any dirt or pests, They were then air dried at room temperature for 7 days. Direct sunlight was avoided to prevent the breakdown of plant chemicals, A clean sterile blender was used to grind the dried leaves into a powdered form. The powder was then kept in a sealed ziplock bag at room temperature until it was used for extraction.

Vernonia amygdalina was identified by Alire Raffeneau Delile in 1826.

3.2 Extraction Procedure (Cold Maceration Method)

To obtain the bioactive compounds from the powdered leaves of *Vernonia amygdalina*, the cold extraction (maceration) method was employed in this study, as it preserves thermolabile secondary

metabolites. (i.e., heat-sensitive). Two separate solvent extraction systems were established: one with a 95% ethanol organic solvent system and the other with a sterile distilled water aqueous solvent system.

3.2.1 Ethanolic Extraction Phase

Using a digital weighing balance, 100 g of the milled powder of *Vernonia amygdalina* leaves was measured and put into a clean 1000 mL glass conical flask. A volume of 1L of 95% ethanol solvent was then poured into the flask, which creates a sampling optimum that allows for an accurate sample: solvent ratio target up to 1:10. The flask was tightly covered with aluminum foil and masking tape to minimize solvent loss.

The sample was kept at normal room temperature for 72 h. The flask was shaken vigorously three times a day to maintain the concentration gradient at the maximum level so that mass transfer from plant cells could occur.

After 72-hour maceration, the plant residue was filtered out by passing through clean muslin cloth, followed by a second filtration step using Whatman No. 1 filter paper. The clear filtrate was later concentrated at reduced pressure on a rotary evaporator with a 40^0C water bath to remove the alcohol solvent. This dark green ethanolic crude extract was concentrated, put in sterile airtight specimen bottles, labelled, and stored in a refrigerator at 4^0C until needed for further assays (Edeoga *et al*. 2005).

3.2.2 Aqueous Extraction Phase

To prepare two extracts simultaneously, a 100g sample of powdered *Vernonia amygdalina* leaves (the different plants having an equal weight) was poured into a sterile 1000mL glass conical flask, and one liter of sterile distilled water was added to it. The flask was closed, and the mixture was left to macerate at room temperature for 72 hours; it had manual shaking intervals of the same duration as ethanolic extraction. The aqueous crude extract was filtered first through a muslin cloth and then through a Whatman No. 1 filter paper to obtain a clear aqueous extract filtrate free from any suspension. The filtrate was dried by evaporation in a water bath; the temperature of the laboratory water bath was constantly held at 70^0C to eliminate the water without degrading the

extract. The aqueous crude extract, which had turned into a semi-solid, was scraped, put into a sterile container, properly labeled, and stored at 4^0C (Hussain *et al*. 2011).

3.3 Standardization and Preparation of Inoculum

Pure clinical isolates of *Staphylococcus aureus* and *Escherichia coli* were sourced from Park Lane Hospital, Enugu, and validated. To regulate the bacterial cell density for the research, the organisms were initially subcultured on nutrient agar slants and incubated at 37^0C for 24 hours (Kate and Lucky, 2012).

Bacterial colonies from fresh cultures were picked and suspended in agar slant nutrient tubes. Tubes were tightly capped and labeled for identification. Each bacterial suspension's turbidity was adjusted visually by adding sterile saline or organisms until it matched the optical density of a freshly prepared 0.5 McFarland turbidity standard. This adjustment ensures a bacterial density of 1.5 x 10^8 CFu/mL approximately standardizing the in vitro workload across all test plates mathematically (Chessbrough, 2005).

3.4 Antimicrobial Sensitivity testing (Agar Well Diffusion)

Standardized bacterial inocula were seeded on Müller-Hinton Agar (MHA) freshly prepared plates well labeled with the names of each microorganism (*Staphylococcus aureus* and *Eshericia coil*) and type of extract (Ethanolic or Aqueous). The sterile cotton swab was dipped into the diluted microbial suspension, and the extra liquid was removed by pressing the swab really hard against the inside surface of the tube. Then the swab was applied to inoculate the MHA plate in three spreading directions so that a uniform and confluent bacterial growth was achieved.
When the plates were dry (15 min), wells equidistant from each other was made cut in the agar matrix using a sterile 6 mm cork borer. A sterile needle was used to remove the agar plugs. To prevent the extracts from seeping from the agar layer, each well was sealed at the bottom by adding a drop of molten, uninoculated (MHA) to the plates.

Adjustable micropipette tips were used for exactly taking 0.1ml of the various concentrations of diluted extracts (i.e. crude ethanolic extract at 200mg/mL, 100mg/mL, 50mg/mL and 25mg/mL,

crude aqueous extract at 200mg/mL, 100mg/mL, 50mg/mL and 25mg/mL, negative control: distilled water) into separate designated wells (Manandhar *et al*. 2019).

The plates were left alone for 1 hour at room temperature for the active secondary metabolites to diffuse from the agar grid before the growth occurred. Afterwards, the plates were incubated in a laboratory incubator at 37C for 24 h (Gobezie *et al*. 2020).

The diameter of zones of inhibition was determined using a meter ruler (mm) after the incubation period. This test was performed in triplicate.

3.5 Determination of Minimum Inhibitory Concentration (MIC)

Both ethanolic and aqueous extracts of *Vernonia amygdalina* were tested for the Minimum Inhibitory Concentration (MIC), which is the minimum amount of a chemical required to inhibit bacterial growth. The macro broth tube dilution method was used in this study. The extracts, after being melted, were mixed with the designated solvents. Afterward, a serial two -fold dilution was performed with sterile Nutrient Broth tubes to yield 200mg/mL, 100mg/mL, 50mg/mL and 25mg/mL concentrations.

After that, all dilutions were inoculated with a standardized 0.5 McFarland bacterial suspension before incubation, two controls were used during the procedure: a first control with nutrient broth only (Positive Control) and the second one with nutrient Broth, the bacterial inoculum, and without the plant extract (Negative Control), This way confirming the viability of the organism. After shaking and mixing the tubes, they were placed in a 37 degree C incubator for 24 hrs. Clouding or turbidity in the tubes are usually seen as visual indicator of bacterial growth and was checked after 24hrs of incubation. The MIC is the minimum concentration of extract that completely inhibits the rise in turbidity when viewed as a comparison with the clear sterility control tube (Evbuomwan *et al*. 2018).

3.6 Determination of Minimum Bactericidal Concentration (MBC)

To find out if the *Vernonia amygdalina* extracts kill bacterial cells (bactericidal) or just stop their growth (bacteriostatic), the Minimum Bactericidal Concentration (MBC) test was done. Samples

were taken from all the tubes in the MIC test set that showed no visible growth or no turbidity of bacteria.

With a sterile inoculating loop, a small amount of fluid from the clear test tube was spread on a new Mueller-Hinton Agar plate.

The plates were turned over and then placed at 37^0C for 24 to 48 hours so that any surviving bacteria could reproduce. After incubation, the plates were scrutinized for colony growth. The Minimum Bactericidal Concentration (MBC) was identified as the minimum concentration of the extract leading to no bacterial colony growth on the agar, which means that 99.9% of the initial bacterial population was killed (Evbuomwan *et al*. 2018).

3.7 Phytochemical Screening (Qualitative)

3.7.1 Test for Alkaloids (Wagner's Test)

Alkaloids are nitrogen-containing chemicals that are often the active agents in medicinal plants.

A small amount of (approx. 0.5g) crude extract was dissolved in 5ml of 1% Hydrochloric acid (HCl) in a test tube. The mixture was filtered to remove any undissolved particles. 23 drops of Wagner's reagent were added to the filtrate by the side of the test tube. The generation of a reddish-brown precipitate at the bottom or along the side of the test tube is indicative of the presence of alkaloids (Trease and Evans, 1983).

3.7.2 Test for Saponins (Frothing Test)

Saponins are natural cleaning agents; *Vernonia amygdalina* has been known as a plant with a very high level of saponins, and this is the reason it has that "foamy" quality when washed.

We made use of 0.5g of the extract which was diluted with 20ml of distilled water in a graduated cylinder. The mixture was shaken vigorously for 15 minutes. The presence of saponins is indicated

by the formation of a stable, persistent froth or foam layer at the surface of the liquid that does not immediately dissolve (Trease and Evans, 1983).

3.7.3 Test for Tannins (Ferric Chloride Test)

Tannins are a group of polyphenol compounds capable of interacting with and precipitating proteins.

To experiment, 0.5g of the extract was mixed with 10ml of distilled water and then filtered. The resulting solution was treated with a few drops of a 0.1% Ferric Chloride (FeCl) solution. A significant change in color to brownish green, blue black, or deep blue is a clear indication of the tannins' presence (Trease and Evans, 1983).

3.7.4 Test for Flavonoids (Shinoda Test)

Flavonoids are very potent antioxidants.

0.5g of the extract was dissolved in 2ml of Ethanol. Several pieces of Magnesium ribbon or turnings were added to it, and concentrated Hydrochloric acid (HCl) was added dropwise. This was followed by a color change, which made the liquid pink, crimson-red, or sometimes magenta. This result confirms the occurrence of flavonoids (Trease and Evans, 1983).

3.7.5 Test for Cardiac Glycosides (Keller-Killiani Test)

These compounds are capable of directly affecting the muscles of the heart. 0.5g of the extract was dissolved in 2ml of Glacial Acetic Acid containing one drop of Ferric Chloride solution.

This mixture was underplayed with 1ml of concentrated Sulfuric Acid (H_2SO_4) by pouring it carefully down the side of the test tube. A brown ring appeared at the dividing layer of the two liquids, while the upper acetic acid layer slowly turned a blue-green or clear blue color, which indicates the presence of cardiac glycosides (Trease and Evans, 1983).

3.7.6 Test for Steroids (Libermann-Burchard Test)

These are often found in the n-Hexane extract of *Vernonia amygdalina*.

We made use of 0.5g of the extract which was dissolved in 2ml of Chloroform, 10 drops of Acetic Anhydride was added, 2 drops of concentrated Sulfuric Acid were added carefully along the side of the tube. A color progression moving from red/pink to a final deep green or blue-green appearance indicates the presence of steroids (Trease and Evans, 1983).

3.8 Proximate Analysis

In Proximate Analysis, you are essentially breaking down the *Vernonia amygdalina* into its fundamental nutritional components.

3.8.1 Determination of Moisture Content (Air-Oven Method)

This determines the water content, which is something important in the shelf-life of your plant material. Firstly, a clean silica dish was thoroughly washed and then dried in an oven running at 105^0C for 1 hour. It was then taken out, cooled in a desiccator, and finally weighed (W1). 2.0g of fresh or air-dried powdered sample was put into the dish, and it was again weighed (W2). The dish was put back in the oven at 105^0C for the drying process, which took approximately 24 hours. Finally, the dishes were taken out, cooled in the desiccator, and weighed (W3) (AOAC, 2005).

Formula:

$$\%\text{Moisture} = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

3.8.2 Determination of Ash Content (Muffle Furnace Method)

Ash is the residue of inorganic mineral matter (like calcium, magnesium, etc.) left after all organic substances have been burnt off. A clean, dry porcelain crucible was weighed (W1); 2.0 g of the sample was put in the crucible and weighed (W2). The crucible was put into a Muffle Furnace, and the temperature was increased to 550^0C for approximately 4-6 hours until the sample turned into white/gery ash. The furnace was left to cool, the crucible was put in a desiccator, and weighed (W3) (AOAC, 2005).

Formula:

$$\%\text{Ash} = \frac{W_3 - W_1}{W_2 - W_1} \times 100$$

3.8.3 Determination of Crude Lipid/Fat (Soxhlet Extraction)

Firstly, a clean and dry round-bottom flask was weighed (W1). Then, 5.0 g of the powdered sample was wrapped in a filter thimble and placed in the Soxhlet apparatus. 150ml of n-Hexane was poured into the flask. Extraction proceeded for 8 hours. At the end, the solvent was removed by a Rotary Evaporator. The flask (now with the extracted oil) was heat-dried in an oven at 60C for 30 minutes to ensure complete evaporation of the solvent, then cooled down and weighed (W2) (AOAC, 2005)

Formula:

$$\%\text{Crude Fat} = \frac{W_2 - W_1}{\text{Weight of Sample}} \times 100$$

3.8.4 Determination of Crude Fiber (Acid-Base Digestion)

The method determines the indigestible cellulose and lignin fractions. Fat-free samples (residue from the fat extraction) weighing 2.0g were refluxed with 200ml of 1.25% H_2SO_4 for 30 minutes. Then it was filtered through a muslin cloth and washed with boiling water until the wash water was no longer acidic. The residue was subjected to a second acid digestion with 200ml of 1.25% NaOH for 30 min, then filtered and washed with boiling water, and finally with a little ethanol. The residue was transferred into a crucible, dried at 105C to constant weight (W1), and then incinerated in a muffle furnace at 550C for 2 hours to obtain the weight of the ash (W2) (AOAC, 2005).

- **Formula:**

$$\%\text{Crude Fiber} = \frac{W_1 - W_2}{\text{Weight of Sample}} \times 100$$

3.8.5 Determination of Crude Protein (Kjeldahl Method)

This involves three stages: Digestion, Distillation, and Titration.

- **Digestion:** we made use of 0.5g of sample which was mixed with 10ml of conc. H2SO4 and a Kjeldahl catalyst tablet in a digestion flask, it was heated until the solution becomes clear (light green).
- **Distillation:** The digest was diluted with distilled water and 40% NaOH was added to make it alkaline. the ammonia was distilled into a flask containing Boric Acid indicator.
- **Titration:** The trapped ammonia was titrated with 0.1M HCL**,** (AOAC, 2005).

Formula: Calculate %Nitrogen (N) based on the titration volume.

% Crude protein = %N x 6.25

(6.25 is the standard conversion factor for plant proteins).

3.8.6 Determination of Total Carbohydrate

This is usually determined by "difference," meaning you subtract all other percentages from 100, (AOAC, 2005).

Formula: %CHO =100 − (%Moisture+%Ash+%Crude Fat+%Crude Fiber+%Crude Protein)

CHAPTER FOUR

RESULTS

Table 4.1 shows the zone of inhibition of the *Vernonia amygdalina* ethanolic extract against the target clinical isolate was measured in millimeters (mm),

Table 4 1. Antibacterial susceptibility test of *Vernonia amygdalina* ethanolic extract against *Escherichia coli* and *Staphylococcus aureus*

	Concentration (mg/mL)/ Zone of inhibition (milimeters)			
Isolates	**200mg/Ml**	**100mg/mL**	**50mg/mL**	**25mg/mL**
Escherichia Coli	29.33 ± 1.15	27.67 ± 0.58	17.67± 0.58	15.33± 0.58
Staphylococcus aureus	31.33 ± 1.15	30	25	20

Table 4.2 shows the zone of inhibition of the *V. amygdalina* aqueous extract were significantly smaller across all concentrations from 200mg/mL to 25mg/mL compared to the *V. amygdalina* ethanolic extract.

Table 4.2. Antibacterial susceptibility test *Vernonia amygdalina* Aqueous extracts against *Escherichia coli* and *Staphylococcus aureus*

	Concentration (mg/mL)/ Zone of inhibition (milimeters)			
Isolates	**200mg/Ml**	**100mg/mL**	**50mg/mL**	**25mg/mL**
Escherichia coli	20.33 ± 0.58	18	16	12
Staphylococcus aureus	24.67± 0.58	20	17	14

Table 4.3 indicates the lowest established inhibitory concentration of the ethanol extract that could be able to pause or stop the growth of the isolates which was seen at 50mg/mL for *Staphylococcus aureus* and *Escherichia coli.*

Table 4.3. Minimum inhibitory concentration (MIC) of *Vernonia amygdalina* ethanol extract

Isolates	**200mg/mL**	**100mg/mL**	**50mg/mL**	**25mg/mL**
E.coli	-	-	+	-
S.aureus	-	-	+	-

Key: (+) = MIC

(-) = Nil

In Table 4.4 the parameters showed the bactericidal threshold where the extract achieved complete cell death, resulting in a 99.9% reduction of the initial bacterial inoculum, the MBC for *E.coli* was seen at 100mg/mL while *S. aureus* was at 50mg/mL indicating a highly efficient bactericidal mechanism.

Table 4.4 Minimum bactericidal concentration (MBC) of *Vernonia amygdalina* ethanol extract

Isolates	**200mg/mL**	**100mg/mL**	**50mg/mL**	**25mg/mL**
E.coli	-	+	-	-
S. aureus	-	-	+	-

Key: (+) = MBC

(-) = Nil

In table 4.5 *E.coli* exhibited a higher MIC threshold of 100mg/mL whereas *S. aureus* remains inhibited at 50mg/mL which also shows that the Gram negative cell wall structure of *E.coli* requires a higher concentration of water soluble compounds to halt active replication.

Table 4.5 Minimum inhibitory concentration of *Vernonia amygdalina* aqueous extract

Isolates	**200mg/mL**	**100mg/mL**	**50mg/mL**	**25mg/mL**
E.coli	-	+	-	-
S. aureus	-	-	+	-

Key: (+) = MIC

(-) = Nil

Table 4.6 shows a complete lack of bactericidal endpoint within the tested range for *E. coli* while *S. aureus* required a higher concentration of 200mg/mL for complete lethality.

Table 4.6 Minimum bactericidal concentration of *Vernonia amygdalina* aqueous extract

Isolates	**25 mg/mL**	**50 mg/mL**	**100 mg/mL**	**200 mg/mL**
E.coli	-	-	-	-
S. aureus	-	-	-	+

Key: (+) = MBC

(-) = Nil

The qualitative phytochemical screening of V. *amygdalina* ethanol extract presented in table 4.7 confirmed the presence of diverse secondary metabolites within the ethanolic extract, which includes alkaloids, saponins, tannins, flavonoids, cardiac glycoside and steroids, these bioactive compounds serve as the active agents responsible for the observed zones of inhibition and cell death.

4.7 Phytochemical component of *V. amygdalina* ethanolic extract

Key: (-) absent, (+) present

Bioactive Constituents	**Ethanolic extract**
Alkaloids	+
Saponins	+
Tannins	+
Flavonoids	+
Cardiac glycoside	+
Steriods	+

Table 4.8 breaks down the quantitative nutritional constituents of the dried *Vernonia amygdalina* leaves, total carbohydrates represented the largest fraction at 40.50% followed by moisture at 18.00%, crude protein at 16.50%, crude fiber at 12.30%, ash content at 8.50%and lipid formed the smallest component at 4.20%. These nutritional percentage outlined the mineral and organic substrate available to support microbial growth.

Table 4.8 Proximate composition of *Vernonia amygdalina* leaves

Proximate constituent	***Vernonia amygdalina*** (%)
Moisture content	18.00%
Crude protein	16.50%
Ash content	8.50%
Crude fiber	12.30%
Crude fat/lipid	4.20%
Total carbohydrate	40.50%

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.0 DISCUSSION

The antibacterial activity evaluation of the raw extracts of *Vernonia amygdalina* revealed that the extracts have quite a potential in growth inhibition of both *Escherichia coli* and *Staphylococcus aureus*. The data in Tables 4.1 and 4.2 reveal that the ethanolic extract produced bigger zones of inhibition than the aqueous extract at all concentration levels. At the highest concentration of 200 mg/mL, the ethanolic extract showed the largest zones of inhibition of 31.33±1.15 mm for *S. aureus* and 29.33±1.15 mm for *E. coli*, whereas the aqueous extract at the same concentration resulted in lower inhibition zones of 24.67± 0.58 mm for *S. aureus* and 20.33±0.58 mm for *E. coli*.

This clear superiority of the ethanolic solvent extraction is in line with previous findings by (Udochukwu *et al*., 2015; Ahmad *et al*., 2024) where ethanolic preparations of bitter leaf possessed more pronounced antibacterial properties than water based extractions. The lower performance of the aqueous extract can be linked to its inability to adequately dissolve non-polar bioactive plant components. Furthermore, the observation that *Staphylococcus aureus* was generally more sensitive to both extracts than *Escherichia coli* agrees with established microbiological terms regarding Gram positive and Gram negative cell envelope structures (Inusa *et al*., 2018). This difference in sensitivity may be explained by the structural difference in the cell wall composition of these organisms. Gram-positive bacteria do not have an outer lipopolysaccharide membrane, unlike Gram-negative bacteria, which makes *S. aureus* very susceptible to the entry of active plant metabolites.

MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bactericidal Concentration) were able to confirm the great healing potential of the ethanolic extract. For the ethanolic extract, the MICs were set at 50mg/mL for both *E. coli* and *S. aureus*, with these bactericidal effects being reached at the higher concentration (Evbuomwan *et al*. 2018).

The qualitative phytochemical screening depicted in Table 4.7 was consistent with (Raji *et al.,* 2025), who also found that the examined plant contained a great amount of bioactive secondary metabolites, which were, among others, alkaloids, tannins, flavonoids, saponins, cardiac

glycosides, and steroids. These findings affirmed chemical data indicating that the combined action of flavonoids and alkaloids is an important protective measure that controls microbial growth (Akinjogunla *et al*. 2011), whereas tannins and saponins together effectively precipitate bacterial proteins and render bacterial membranes permeable, which results in cell death due to the loss of essential nutrients.

Simultaneously, the proximate composition analysis presented in Table 4.8 demonstrates that *Vernonia amygdalina* functions as an exceptional nutritional resource alongside its proven medicinal properties. The leaves yielded a high carbohydrate content (40.50%), serving as the primary caloric and energy supplier, complemented by a substantial crude protein level (16.50%). This significant protein value confirms the leaf meal as a potent source of essential amino acids capable of supporting tissue repair and cellular growth, further validating its status as a nutrient-dense botanical resource.

The ash content recorded at 8.50% reflects a high total inorganic mineral concentration. This elevated mineral reserve suggests a rich composition of essential macro and micro elements (such as calcium, potassium, magnesium, and iron) necessary for key physiological functions, metabolic enzymatic cofactors, and osmotic balance in human and animal nutrition. Furthermore, the crude fiber content (12.30%) and crude lipid (fat) profile (4.20%) align with established baseline nutritional standards for healthy green leafy vegetables. The moderate crude fiber percentage plays a key role in promoting gastrointestinal motility and aiding digestion without impairing overall nutrient absorption, while the lipid content represents a healthy, low-fat yield likely composed of essential fatty acids and fat-soluble vitamins.

The moisture content of the processed plant material was recorded at a stable 18.00%. Maintaining a moisture level below the critical 20.00% threshold is biologically vital in post-harvest processing. This controlled water activity effectively restricts the physiological water available for the growth of opportunistic spoilage microorganisms (including molds, yeasts, and bacteria) and minimizes endogenous enzymatic autolysis during storage. Consequently, this moisture stabilization preserved the structural integrity of the active, cell-disrupting phytochemical constituents documented in Table 4.7, ensuring they remained concentrated and biologically potent

enough to elicit the pronounced zones of inhibition observed across all susceptibility assays in this study.

5.1 CONCLUSION

These results show that *Vernonia amygdalina* leaf extracts possess a very strong and wide antibacterial activity against the two bacteria used in this study, i.e., *Staphylococcus aureus* and *Escherichia coli*. It is known that these plant extracts are very rich in plant secondary metabolites like flavonoids, tannins, and alkaloids, which are believed to be responsible for their antimicrobial activities through mechanisms like the disturbance of bacterial membrane lipid bilayers, the impairment of cytoplasmic membrane integrity, and the inhibition of key enzymes. In addition, the studies show that the ethanolic extract of *Vernonia amygdalina* has quite a bit higher bacteriostatic and bactericidal effects compared to the aqueous extract, as it caused larger zones of inhibition and had a lower minimum inhibitory concentration (MIC). The proximate analysis of *Vernonia amygdalina* also revealed its significant structural constituents. Overall, these studies lend scientific support to the traditional knowledge of *Vernonia amygdalina* as a beneficial plant source for the development of therapeutic agents.

5.2 RECOMMENDATION

Due to the focus on crude fractions in the study, it is essential for future investigations to move toward advanced isolation and characterization techniques like High Performance Liquid Chromatography (HPLC) or Gas Chromatography Mass Spectrometry (GC-MS). Using these tools allows researchers to identify, purify, and seperate the exact active flavonoid derivatives such as luteolin and apigenin responsible for the membrane damage. Immediately these specific molecules are isolated, the research can naturally progress into exploring synergy and testing how these pure plant compounds interact when combined with conventional antibiotics to see if they can break down or reverse existing drug resistance mechanisms in stubborn clinical isolates.

Beyond the petri dish, the actual medical or industrial potential of *Vernonia amygdalina* cannot be fully realized without moving into animal models for detailed in vivo toxicity profiling and pharmacokinetic testing. This step is crucial to determine how the body metabolizes the extract, what a safe therapeutic dosage looks like, and if the compounds can cause any cellular damage

before anyone can think about human clinical trials. At the same time, because pathogens like *Staphylococcus aureus* and *Escherichia coli* often form tough, defensive communities in real world infections, future studies should evaluate the extract's ability to disrupt cell to cell communication and dismantle mature microbial biofilms. Finally, to turn these findings into a practical product, further research must evaluate the chemical stability of these secondary metabolites under different temperatures and pH levels to establish clear rules for shelf life and formulation

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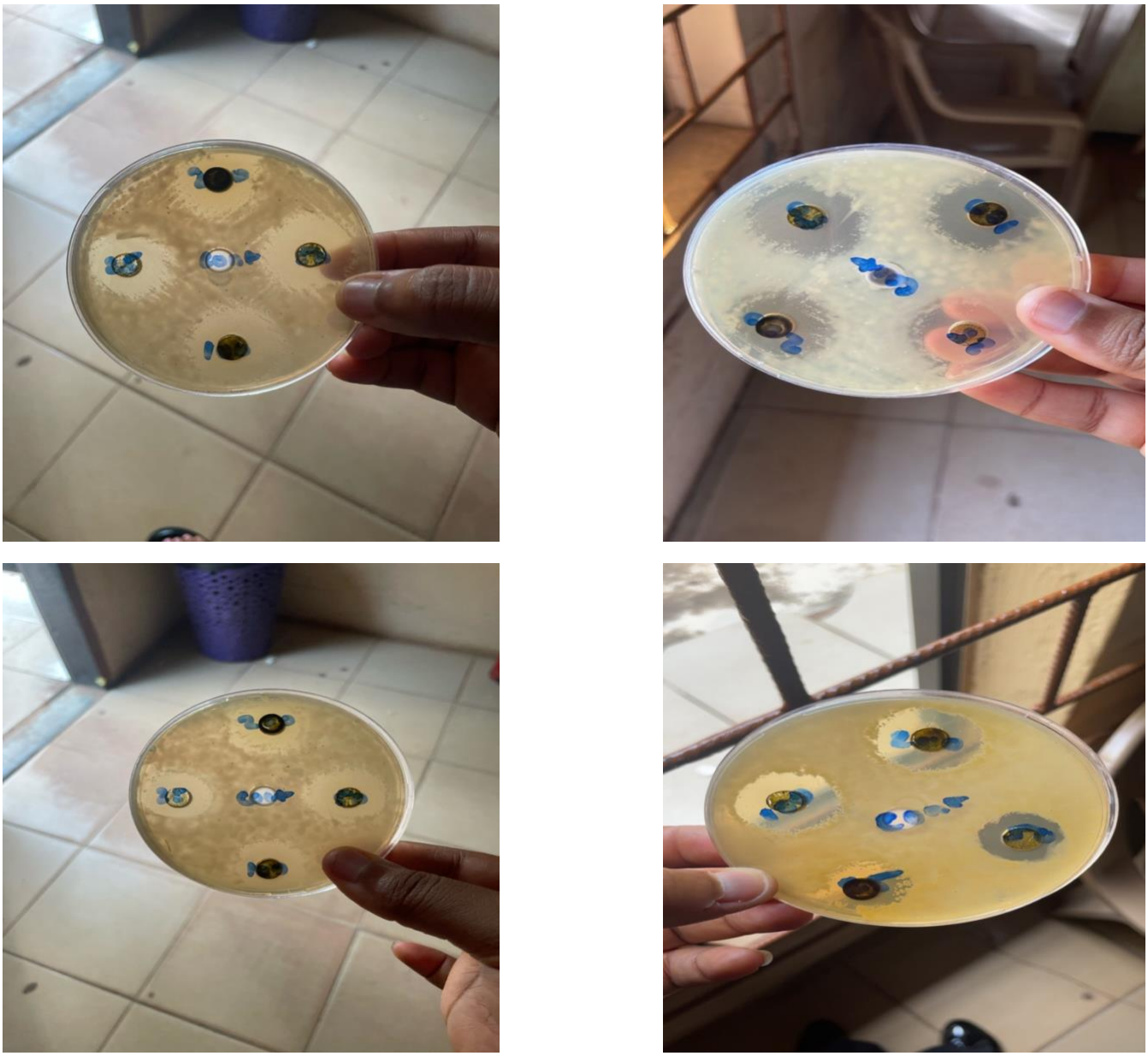
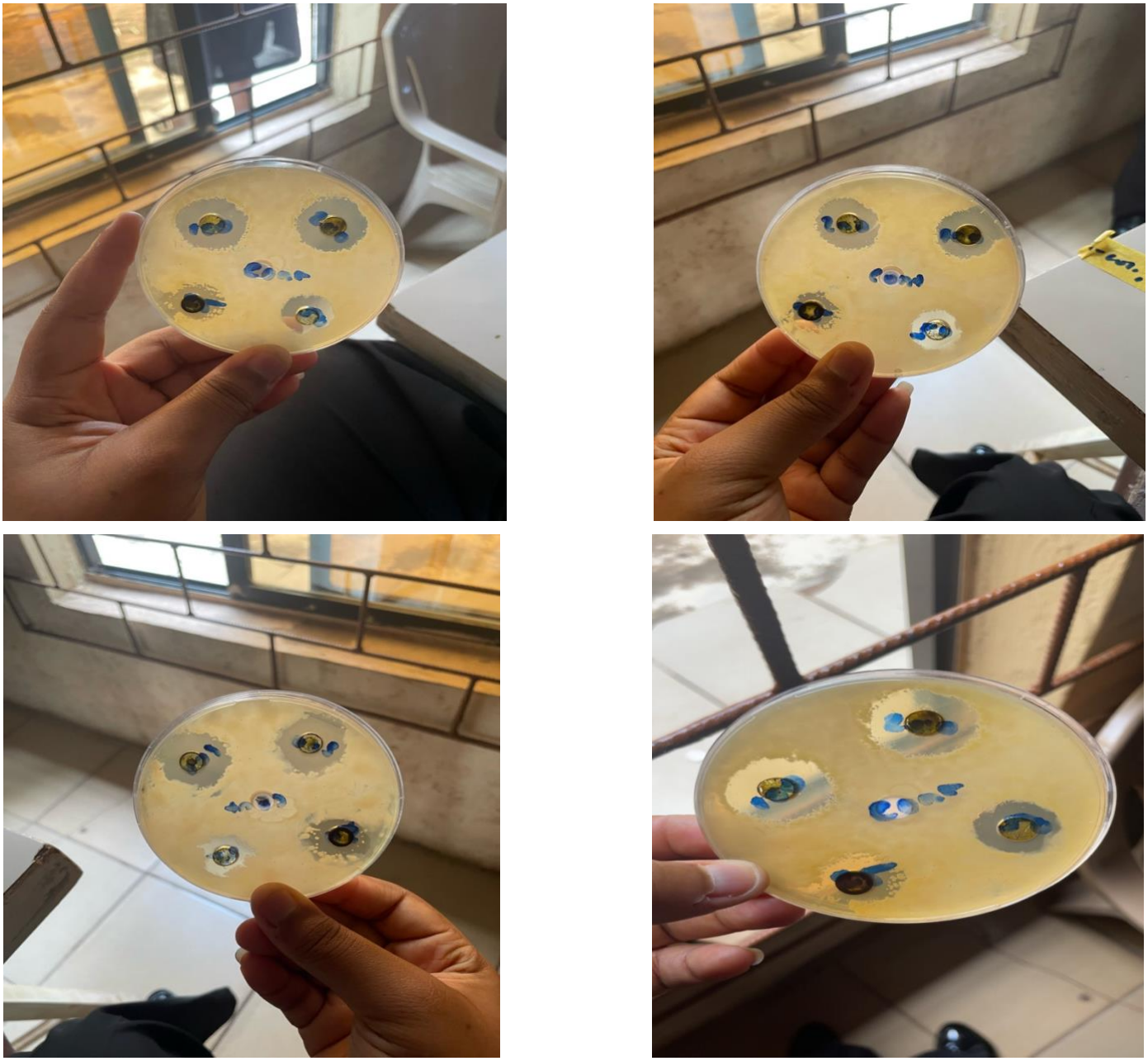
APPENDICES

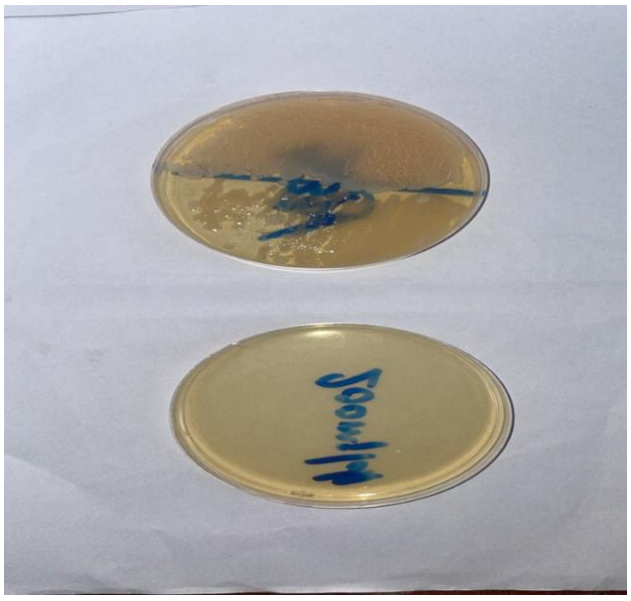


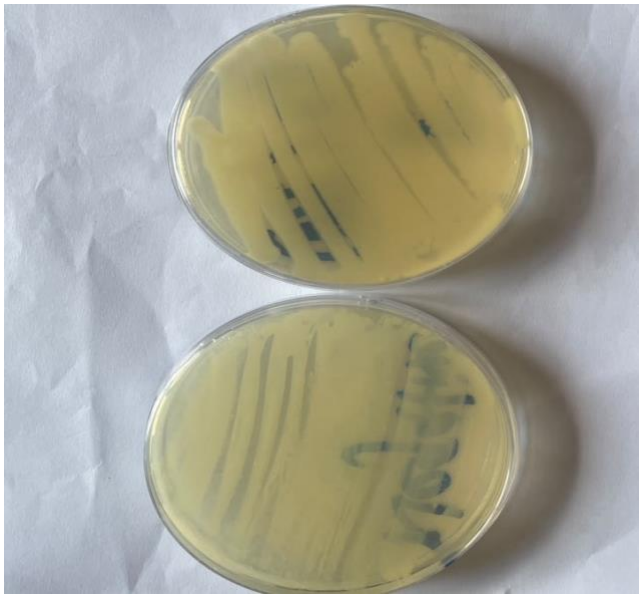
APPENDIX 1 Antisuceptibility test indicating the zones of inhibition of *S. aureus* and *E.coli* using *V. amygladina* ethanol extract.



APPENDIX 2 Antisuceptibility test indicating the zones of inhibition of *S. aureus* and *E.coli* using *V. amygladina* aqueous extract.

APPENDIX 3 Minimum inhibitory concentration and Minimum bactericidal concentration of *V. amygdalina* ethanol extract against the target isolates.





APPENDIX 4 Minimum inhibitory concentration and Minimum bactericidal concentration of *V. amygdalina* aqueous extract against the target isolates.