

**ANTIBACTERIAL ACTIVITY OF PLANT EXTRACTS *Chromolaena odorata* (Awolowo leaf) and *Vernonia amygdalina* (Bitter leaf) ON ENTERIC BACTERIA ISOLATED FROM POULTRY DROPPINGS**

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NIGERIA.**

**NOVEMBER, 2025.**

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**A PROJECT SUBMITTED TO THE DEPARTMENT OF MICROBIOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF A BACHELOR OF SCIENCE (B.SC.) DEGREE IN MICROBIOLOGY.**

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**NOVEMBER, 2025.**

## CERTIFICATION

This is to certify that this project work was carried out by Best Osabhahiebhen SUNNY-EHIZE in the Department of Microbiology, Faculty of Life Sciences, University Of Benin, Benin City, Edo State, Nigeria, under my supervision.

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(Project Supervisor)

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Date

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PROF. IGBINOSA

(Head Of Department)

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Date

## **DEDICATION**

This project is dedicated to my beloved parents, Mr. and Mrs. Sunny-Ehize, whose unwavering love, prayers, encouragement, and sacrifices have been the driving force behind my academic journey. Your support, guidance, and belief in my potential have inspired me to strive for excellence. This work is a reflection of your endless care and the strong foundation you have built in me.

Thank you, Mom and Dad, for everything.

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## ABSTRACT

The overuse of antibiotics in poultry production has heightened resistance concerns, prompting the search for eco-friendly and plant-based antimicrobial alternatives. Both *Chromolaena odorata* and *Vernonia amygdalina* are rich in bioactive phytochemicals, including alkaloids, flavonoids, tannins, terpenoids, and saponins, known to exhibit strong antibacterial and antioxidant properties. Eight (8) poultry droppings samples were aseptically collected from four poultry farms in Ekpoma, Edo State, Nigeria, using sterile universal bottles. Standard microbiological procedures were employed for the isolation and identification of enteric bacteria, including serial dilution, culturing on MacConkey agar, and biochemical characterization based on Standard microbiological techniques. Fresh leaves of *C. odorata* and *V. amygdalina* were collected, washed, blended, and filtered to obtain aqueous crude extracts. Antibacterial activity was assessed using the agar well diffusion method, while the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were determined by broth dilution and sub-culturing methods respectively. Five bacterial isolates—*Escherichia coli*, *Bacillus sp.*, *Proteus sp.*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*—were identified from the poultry droppings. Both extracts showed varying degrees of antibacterial activity against all isolates, with *V. amygdalina* producing larger zones of inhibition than *C. odorata*. The MIC and MBC results confirmed that higher extract concentrations corresponded with greater bacterial inhibition and killing effects, indicating concentration-dependent antibacterial efficacy. The findings demonstrate that *Chromolaena odorata* and *Vernonia amygdalina* possess significant antibacterial activity against enteric bacteria isolated from poultry droppings, suggesting their potential as natural, plant-based alternatives to synthetic antibiotics for controlling bacterial infections in poultry and mitigating antimicrobial resistance.

# CHAPTER ONE

## INTRODUCTION

### 1.1 Background of the study

The poultry industry is a vital part of global food security, yet it serves as a significant reservoir for enteric bacteria. These include organisms like *Escherichia coli*, *Salmonella* spp., and other Gram-negative bacteria that readily colonize the poultry gut and contaminate droppings. Such bacteria pose considerable challenges, impacting both avian health and carrying a zoonotic risk to humans via the food chain and environment (Shaji *et al.*, 2023; Pereira *et al.*, 2024). The extensive use of antibiotics in poultry production has fuelled the emergence and propagation of antimicrobial-resistant (AMR) strains, which has intensified the search for alternative, sustainable antimicrobial strategies (Mak *et al.*, 2022; Pereira *et al.*, 2024).

Poultry droppings are a convenient sample for isolating enteric bacteria because they reflect the intestinal microbiota and potential pathogens present in flocks. Common isolates include *E. coli*, *Salmonella* spp., *Klebsiella* spp., *Proteus* spp., and sometimes *Campylobacter* and *Enterococcus* species. These organisms are implicated in poultry disease, decreased production, and foodborne illness in humans; many recent surveys report worrying rates of multidrug resistance among isolates from poultry farms and live-bird markets. The persistence of these bacteria in litter and droppings also facilitates environmental dissemination of resistance determinants.

Traditional medicinal plants are a rich source of bioactive secondary metabolites (flavonoids, tannins, alkaloids, saponins, terpenoids) that can exhibit antibacterial, antifungal, antioxidant and anti-biofilm activities. Plant extracts have several attractive features for use in animal production: relative availability in many low- and middle-income settings, multiple mechanisms of action that reduce the likelihood of rapid resistance development, and potential for use as feed additives, disinfectants, or topical agents (Frontiers review; Bunkaew *et al.*, 2025). Given the pressing need to reduce antibiotic use in poultry, systematic evaluation of locally available plants for activity against enteric poultry pathogens is justified.

*Vernonia amygdalina* is widely used across West Africa as a food and medicinal plant. Phytochemical investigations and antimicrobial assays have repeatedly reported that extracts (aqueous, methanolic, ethanolic) possess inhibitory activity against Gram-negative and Gram-positive bacteria, including strains of *E. coli*, *Salmonella*, *Klebsiella*, and clinical multidrug-resistant isolates (Akinpelu *et al.*, 1999; Degu *et al.*, 2024). Active constituents proposed to mediate this activity include sesquiterpene lactones, flavonoids and phenolics; minimum inhibitory concentrations (MICs) reported in the literature vary with extraction solvent, plant part, and test organism but provide proof of concept for antibacterial potential.

*Chromolaena odorata* is an invasive shrub found in tropical regions and is recognized in ethnomedicine for wound-healing and antimicrobial properties. Several recent studies demonstrate that leaf extracts (ethanolic, aqueous, methanolic) inhibit both Gram-positive and Gram-negative bacteria, can reduce biofilm formation, and in some reports show bactericidal activity with MIC and MBC values in experimentally relevant ranges (Omokhua-Uyi *et al.*, 2020 Phetburom *et al.*, 2025; Bunkaew *et al.*, 2025;). Phytochemicals such as flavonoids, quercetin derivatives, chromomeric acid and essential oils have been identified in *C. odorata* and are believed to underlie antimicrobial effects.

A number of experimental works have specifically considered the use of *V. amygdalina* and *C. odorata* in poultry contexts. Reports include: in vitro antibacterial testing of plant extracts against isolates of poultry origin; dietary supplementation trials that measured gut microbial load and health/performance outcomes in broilers; and studies assessing the extracts' effects on specific pathogens associated with poultry. Results are mixed but generally supportive: both plants can reduce counts of certain gut bacteria and inhibit clinically relevant isolates under controlled conditions, suggesting their potential as alternatives or adjuncts to conventional antimicrobials in poultry systems. Nevertheless, differences in extraction methods, dosages, and microbial panels across studies make direct comparison difficult and underline the need for locally relevant evaluation against enteric bacteria isolated from poultry droppings.

## **1.2 Rationale for the Study**

The alarming rise in antimicrobial resistance (AMR) among enteric bacteria poses a major threat to global health, particularly in developing countries where antibiotic misuse in livestock production is widespread (World Health Organization [WHO], 2023). Poultry droppings are a known reservoir for antibiotic-resistant enteric pathogens such as *Escherichia coli*, *Salmonella* spp., and *Klebsiella* spp., which can spread resistance genes through the food chain and contaminate the environment (Oluwafemi and Ayeni, 2022). This presents a significant challenge to both animal and human health, making it imperative to explore alternative sources of effective and eco-friendly antimicrobial agents.

Medicinal plants have long served as valuable resources for the discovery of novel therapeutic compounds. They possess diverse secondary metabolites—such as alkaloids, flavonoids, tannins, saponins, and terpenoids—that exhibit strong antimicrobial properties (Nweze *et al.*, 2021). The exploration of locally available plants with ethnomedicinal relevance therefore provides an affordable and sustainable approach to combating bacterial infections, especially in regions where access to modern antibiotics is limited or where resistance has rendered conventional drugs less effective.

*Chromolaena odorata* (commonly called Siam weed) and *Vernonia amygdalina* (bitter leaf) are two widely distributed plants in Nigeria and other tropical regions, both known for their extensive use in traditional medicine. *C. odorata* has demonstrated broad-spectrum antimicrobial activity attributed to its rich phytochemical constituents, including flavonoids and phenolic compounds (Nwankwo *et al.*, 2023). Similarly, *V. amygdalina* is renowned for its antibacterial, anti-inflammatory, and antioxidant properties, which have been validated in numerous pharmacological studies (Degu *et al.*, 2024).

Despite their recognized medicinal potential, there is limited scientific data comparing the antibacterial efficacy of these two plants against *enteric bacteria* isolated directly from poultry droppings—organisms that are often multi-drug resistant and more representative of real environmental conditions. Investigating the inhibitory effects of *C. odorata* and *V. amygdalina* extracts on such field isolates could therefore provide valuable insights into their potential as alternative or complementary antimicrobial agents.

This study is particularly significant because it integrates ethnobotanical knowledge with microbiological analysis, thereby bridging the gap between traditional medicine and modern antimicrobial research. The findings could contribute to the search for effective natural agents capable of controlling resistant enteric bacteria in poultry and possibly reducing the spread of antimicrobial resistance within agricultural and human health ecosystems.

### **1.3 Aim and Objectives of the Study**

The aim of this study was to evaluate the antibacterial activity of plant extracts from *Chromolaena odorata* and *Vernonia amygdalina* against enteric bacteria isolated from poultry droppings.

The specific of this study objectives were, to

1. isolate and identify enteric bacteria from poultry droppings using standard microbiological and biochemical techniques.
2. evaluate of the antibacterial activity of the plant extracts against isolated enteric bacteria using agar well diffusion.
3. determine the minimum inhibitory concentrations and the minimum bactericidal concentrations.

## CHAPTER TWO

### LITERATURE REVIEW

#### 2.1 Overview of Enteric Bacteria

Enteric bacteria are microorganisms that primarily inhabit the intestinal tracts of humans and animals, including poultry. These bacteria form part of the normal gut flora, where they play essential roles in digestion, nutrient metabolism, and immune system modulation (Kabir, 2023). However, some species are opportunistic or pathogenic, capable of causing infections when they translocate to other parts of the host body or when shed into the environment through faecal matter (Mak *et al.*, 2022).

In poultry, enteric bacteria include members of the families *Enterobacteriaceae* and *Campylobacteraceae*, such as *Escherichia coli*, *Salmonella* spp., *Klebsiella* spp., *Proteus* spp., *Enterobacter* spp., and *Campylobacter* spp. (Shaji *et al.*, 2023). These organisms are commonly isolated from poultry droppings, litter, and intestinal contents. Their abundance is influenced by feed type, environmental hygiene, and the use of antibiotics in poultry production (Pereira *et al.*, 2024).

#### 2.2 Sources and Transmission of Enteric Bacteria in Poultry

The gastrointestinal tract of poultry serves as a natural habitat and reservoir for enteric bacteria. These bacteria are shed continuously through faeces into the surrounding environment, where they can persist in litter, soil, feed, and water (Sharma *et al.*, 2021). Transmission occurs primarily through the faecal-oral route, either by direct ingestion of contaminated feed or water or through cross-contamination during handling, slaughter, and processing (Zishiri *et al.*, 2022).

According to Ramatla *et al.* (2017), enteric bacteria from poultry droppings can spread rapidly in poultry farms with inadequate biosecurity practices. The accumulation of droppings in poorly managed poultry houses creates an ideal environment for bacterial growth and survival, especially under warm and humid conditions. These bacteria can also be transferred to humans through direct

contact with poultry, consumption of contaminated meat or eggs, and contamination of the farm environment.

### **2.3 Common Enteric Bacteria Isolated from Poultry Droppings**

#### **(a) *Escherichia coli***

*E. coli* is a facultative anaerobic Gram-negative bacterium that resides normally in the intestinal tract of poultry and other warm-blooded animals (Pereira *et al.*, 2024). While most strains are harmless, pathogenic variants known as avian pathogenic *E. coli* (APEC) can cause colibacillosis in birds, leading to septicaemia, airsacculitis, and high mortality (Kabir, 2023). Additionally, *E. coli* is recognized as an indicator organism for faecal contamination in food and water. Several studies have reported high prevalence of *E. coli* in poultry droppings. For instance, Adeyanju and Ishola (2014) found that over 85% of poultry droppings sampled from southwestern Nigeria contained *E. coli* isolates, many of which were resistant to ampicillin and tetracycline.

#### **(b) *Salmonella* spp.**

*Salmonella* is another significant enteric bacterium associated with poultry. It is a facultative intracellular pathogen capable of surviving within macrophages, which makes it particularly difficult to eradicate from poultry environments (Mak *et al.*, 2022). Poultry serve as asymptomatic carriers, shedding *Salmonella* in faeces and contaminating eggs and meat products (Ramatla *et al.*, 2017).

According to Shaji *et al.* (2023), *Salmonella enterica* serovars such as *S. Typhimurium* and *S. Enteritidis* are among the most common strains isolated from poultry droppings worldwide. These bacteria are zoonotic, capable of causing foodborne infections in humans, characterized by diarrhoea, fever, and abdominal pain.

#### **(c) *Klebsiella* spp.**

*Klebsiella* species, particularly *K. pneumoniae* and *K. oxytoca*, are commonly isolated from poultry droppings and the respiratory tract of birds (Abdallah *et al.*, 2022). These bacteria are opportunistic pathogens that can cause respiratory and urinary infections in both poultry and

humans. *Klebsiella* spp. are notable for their ability to acquire multiple antibiotic resistance genes, especially extended-spectrum beta-lactamase (ESBL) genes, which confer resistance to beta-lactam antibiotics (Mak *et al.*, 2022).

(d) *Proteus* spp.

*Proteus mirabilis* and *Proteus vulgaris* are motile enteric bacteria frequently isolated from poultry faeces. These organisms are known for their swarming motility and production of urease, which facilitates urinary tract infections in humans and animals (Zishiri *et al.*, 2022). In poultry, they may contribute to gastrointestinal disturbances and environmental contamination.

(e) *Campylobacter* spp.

*Campylobacter jejuni* and *Campylobacter coli* are microaerophilic enteric bacteria commonly associated with poultry intestinal tracts (Kabir, 2023). These organisms are among the leading causes of bacterial gastroenteritis in humans. Poultry droppings act as reservoirs, with high prevalence rates observed in commercial broiler farms (Ramatla *et al.*, 2017).

## **2.4 Medicinal Plants as Sources of Antimicrobials**

The search for new antimicrobial agents has become one of the most urgent global health priorities due to the alarming increase in antimicrobial resistance (AMR). Conventional antibiotics, once hailed as miracle drugs, are rapidly losing their efficacy against pathogenic microorganisms (World Health Organization [WHO], 2023). Consequently, researchers are increasingly turning to medicinal plants—ancient yet scientifically underexplored reservoirs of bioactive compounds—as promising sources of new antimicrobials (Degu *et al.*, 2024).

For centuries, medicinal plants have been utilized in traditional healing systems such as Ayurveda, Traditional Chinese Medicine, and African ethnomedicine for the treatment of infections and wound healing (Cowan, 1999). These plants synthesize a wide variety of secondary metabolites that serve defensive and adaptive roles in nature. These same compounds have been found to exhibit potent antibacterial, antifungal, antiviral, and antiparasitic properties *in vitro* and *in vivo* (Nwachukwu *et al.*, 2023).

## 2.5 Mechanisms of Antimicrobial Action

The antimicrobial mechanisms of plant-derived compounds are complex and multifactorial, often involving simultaneous interference with several cellular targets. Unlike conventional antibiotics that usually act on a specific site, phytochemicals may affect multiple pathways, making resistance less likely to develop (Bunkaew *et al.*, 2025).

Common mechanisms include:

**Disruption of Cell Membrane Integrity:** Lipophilic compounds such as terpenoids and essential oils integrate into bacterial membranes, increasing permeability and causing leakage of ions and vital metabolites (Savoia, 2012).

**Inhibition of Nucleic Acid Synthesis:** Alkaloids and flavonoids can bind to bacterial DNA or inhibit enzymes like DNA gyrase, hindering replication and transcription (Cushnie and Lamb, 2011).

**Protein Denaturation and Enzyme Inactivation:** Tannins and phenolics interact with microbial enzymes and proteins, leading to loss of function and structural integrity (Scalbert, 1991).

**Quorum Sensing and Biofilm Inhibition:** Many plant extracts inhibit bacterial quorum sensing, thereby reducing virulence and preventing biofilm formation—a major cause of antibiotic resistance (Phetburom *et al.*, 2025).

**Metal Chelation and Oxidative Stress:** Phenolic compounds chelate essential metal ions, starving bacteria of nutrients, while some induce oxidative stress that damages bacterial macromolecules (Cowan, 1999).

This multifaceted mode of action positions plant-based antimicrobials as valuable tools in combating resistant infections.

## 2.6 Advantages of Plant-Derived Antimicrobials

Plant-based antimicrobials offer several advantages over synthetic antibiotics:

**Reduced Risk of Resistance:** Due to their multiple targets, phytochemicals minimize the emergence of resistance (Wireko *et al.*, 2025).

**Biodegradability and Environmental Safety:** They decompose naturally, leaving minimal ecological footprint (Gakuya *et al.*, 2020).

**Cost-Effectiveness and Accessibility:** Many medicinal plants grow abundantly in tropical regions and can be processed locally, reducing dependency on imported drugs (Edeoga *et al.*, 2005).

**Synergistic Effects:** Combinations of plant compounds can enhance efficacy, often working synergistically with conventional antibiotics (Nwachukwu *et al.*, 2023).

In regions like Nigeria, where antibiotic misuse in livestock farming is widespread, integrating plant-based antimicrobials into poultry management could reduce antimicrobial resistance and promote safer, organic animal production.

## **2.7 Overview on *Vernonia amygdalina* (Bitter Leaf)**

*Vernonia amygdalina*, commonly known as bitter leaf, is a perennial shrub belonging to the family Asteraceae (Compositae). It is one of the most widely distributed medicinal plants in tropical Africa and has also been found in parts of Asia and the Middle East (Degu *et al.*, 2024). The plant typically grows to a height of 2–5 meters, with elliptical leaves that are green, hairy, and have a distinctly bitter taste when chewed (Akinpelu *et al.*, 1999). The leaves are simple, opposite, and about 6–20 cm long and 2–8 cm wide, while the stem is woody and covered with a rough bark (Egharevba and Kunle, 2010).

The bitterness of *V. amygdalina* is attributed to its rich phytochemical content, particularly sesquiterpene lactones such as vernodalin and vernolide (Owoeye and Adetutu, 2022). The plant thrives in humid environments and is often cultivated around homes in Nigeria, Cameroon, Ghana, and other sub-Saharan regions for both medicinal and culinary purposes. The Yoruba call it *Ewuro*, the Igbo call it *Onugbu*, and the Hausa refer to it as *Shuwaka* (Egedigwe, 2010).

### **2.7.1 Ethnomedicinal Uses**

Traditionally, *V. amygdalina* has been used for centuries in African ethnomedicine to treat a wide range of ailments. The leaf extract is commonly employed as a remedy for malaria, fever, diarrhoea, dysentery, diabetes, and various microbial infections (Farombi and Owoeye, 2011). Decoctions made from the leaves are also used to manage stomach discomfort, intestinal worms, and skin diseases (Ijeh and Ejike, 2011).

In Nigeria, the leaf juice is often consumed as a tonic to stimulate appetite and enhance digestion. It is also used postpartum by women to restore uterine tone and purify the blood (Egedigwe, 2010). Beyond its medicinal use, the leaves are a popular ingredient in African cuisine, where they are used to prepare soups and stews after extensive washing to reduce bitterness (Degu *et al.*, 2024).

### **2.7.2 Phytochemical Constituents**

The bioactivity of *V. amygdalina* can be attributed to its rich and diverse phytochemical profile. Studies have revealed the presence of alkaloids, flavonoids, saponins, tannins, terpenoids, steroids, glycosides, and phenolic compounds (Akinpelu *et al.*, 1999; Ijeh and Ejike, 2011).

The major compounds responsible for its pharmacological activities include sesquiterpene lactones such as vernodalin, vernolide, and vernomygdin, which exhibit antimicrobial, anticancer, and anti-inflammatory effects (Owoeye and Adetutu, 2022). Flavonoids such as luteolin and apigenin act as potent antioxidants, scavenging free radicals and protecting cellular components from oxidative damage (Farombi and Owoeye, 2011).

In addition, saponins and tannins present in the leaf contribute to its antimicrobial and astringent properties by precipitating microbial proteins and disrupting cell membranes (Degu *et al.*, 2024). The plant's bitter principles also stimulate bile secretion and enhance lipid metabolism, which explains its traditional use in managing diabetes and obesity (Egedigwe, 2010).

### **2.7.3 Antibacterial Properties of *Vernonia amygdalina***

Numerous studies have demonstrated the antibacterial efficacy of *V. amygdalina* extracts against a broad spectrum of microorganisms, including both Gram-positive and Gram-negative bacteria. The plant's antibacterial activity has been attributed mainly to its phytochemical constituents, especially alkaloids, flavonoids, and sesquiterpene lactones (Akinpelu *et al.*, 1999).

### **2.7.4 Efficacy Against Gram-Negative Bacteria**

*V. amygdalina* exhibits potent inhibitory effects against enteric Gram-negative bacteria such as *Escherichia coli*, *Salmonella typhi*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* (Ojo *et al.*, 2023). Akinpelu *et al.* (1999) reported that ethanolic extracts of the plant produced inhibition zones ranging from 10–18 mm against *E. coli* and *Salmonella* spp. Similarly, Ojo *et al.* (2023) demonstrated that aqueous and ethanol extracts of *V. amygdalina* inhibited multidrug-resistant *E. coli* isolates obtained from poultry droppings, suggesting its potential as a natural antibacterial agent in livestock production.

### **2.7.5 Efficacy Against Gram-Positive Bacteria**

The plant has also shown marked activity against Gram-positive organisms such as *Staphylococcus aureus* and *Bacillus subtilis* (Degu *et al.*, 2024). Studies by Ijeh and Ejike (2011) indicated that methanolic extracts of *V. amygdalina* caused significant bacterial cell lysis, attributed to the disruption of peptidoglycan synthesis and interference with bacterial enzyme activity.

### **2.7.6 Comparison of Extraction Solvents**

The choice of extraction solvent significantly affects the antibacterial potency of *V. amygdalina*. Ethanolic and methanolic extracts typically yield stronger antibacterial activity than aqueous extracts due to the higher solubility of non-polar phytochemicals in organic solvents (Wireko *et al.*, 2025). However, aqueous extracts are still effective and may be preferred for ethnomedicinal applications where safety and cost are priorities (Degu *et al.*, 2024).

### **2.7.7 Antioxidant and Anti-Inflammatory Properties**

Beyond its antimicrobial properties, *V. amygdalina* is recognized for its antioxidant and anti-inflammatory potential. The flavonoids, phenolic acids, and terpenoids in the leaves play a crucial role in neutralizing reactive oxygen species (ROS) and reducing oxidative stress (Farombi and Owoeye, 2011).

Chukwu *et al.* (2023) demonstrated that methanolic extracts of *V. amygdalina* significantly increased superoxide dismutase (SOD) and catalase activities in rat liver tissues, suggesting hepatoprotective effects. These findings reinforce its traditional use in managing inflammatory and oxidative diseases, including diabetes and liver disorders (Degu *et al.*, 2024).

## **2.8 Overview on *Chromolaena odorata* (Siam Weed)**

### **2.8.1 Botanical Description and Distribution**

*Chromolaena odorata*, commonly known as Siam weed, is a perennial, rapidly growing shrub belonging to the family Asteraceae. It is native to the tropical Americas but has become widely distributed across Asia, Africa, and the Pacific, where it is considered one of the most invasive alien species (Omokhua-Uyi and van Staden, 2020). The plant thrives in disturbed environments such as roadsides, abandoned farmlands, and forest edges, where it forms dense thickets that suppress native vegetation (Sahoo *et al.*, 2021).

Morphologically, *C. odorata* can reach a height of up to 3 meters. Its stems are slender, green to reddish-brown, and covered with fine hairs. The leaves are opposite, ovate to triangular, 4–10 cm long, and emit a distinctive, pungent odour when crushed—hence the name “odorata.” The flowers are small, pale lilac or white, and arranged in corymb-like clusters at the ends of branches. The plant reproduces primarily through seeds, which are light, tufted with pappus hairs, and easily dispersed by wind or water (Kushwaha *et al.*, 2020).

Although regarded as a weed in agricultural systems, *C. odorata* has gained scientific attention due to its rich phytochemical composition and diverse medicinal applications (Sahoo *et al.*, 2021).

### 2.8.2 Ethnomedicinal Uses

For centuries, *Chromolaena odorata* has been used in traditional medicine across tropical regions. In West Africa, particularly Nigeria and Ghana, the leaves are commonly used to treat wounds, skin infections, toothaches, and diarrhoea (Omokhua-Uyi and van Staden, 2020). In Thailand and Vietnam, decoctions of the leaves are used for wound healing and as an anti-inflammatory agent (Phetburom *et al.*, 2025).

In Nigeria, local healers also employ the crushed leaves of *C. odorata* to stop bleeding and promote the healing of burns and cuts—a property now supported by pharmacological studies showing strong haemostatic and antimicrobial effects (Ajao *et al.*, 2023). The plant is also used for the treatment of malaria, stomach upset, and as a natural insect repellent (Ifeanyi *et al.*, 2024).

### 2.8.3 Phytochemical Composition

The pharmacological properties of *Chromolaena odorata* are largely attributed to its diverse phytochemical constituents. Studies have revealed that the leaves contain flavonoids, tannins, saponins, terpenoids, alkaloids, phenolic acids, steroids, and essential oils (Bunkaew *et al.*, 2025; Omokhua-Uyi and van Staden, 2020).

Major flavonoids identified include quercetin, kaempferol, luteolin, and their glycosides, which exhibit potent antioxidant and antibacterial activities (Sahoo *et al.*, 2021). Other notable constituents such as chromomoric acid, precocene II, and sesquiterpenes contribute to its antimicrobial and anti-inflammatory effects (Phetburom *et al.*, 2025).

Phytochemical screenings conducted by Ifeanyi *et al.* (2024) revealed high concentrations of polyphenolic compounds, which can scavenge free radicals and prevent oxidative stress-related cellular damage. The presence of these bioactive molecules provides the biochemical basis for the plant's traditional therapeutic applications.

#### **2.8.4 Antibacterial Properties**

The antibacterial potential of *Chromolaena odorata* has been extensively investigated against a wide range of pathogenic microorganisms. The leaf extracts—especially those prepared with ethanol or methanol—have shown significant inhibitory activity against both Gram-positive and Gram-negative bacteria (Bunkaew *et al.*, 2025; Wireko *et al.*, 2025).

For instance, Phetburom *et al.* (2025) demonstrated that ethanolic extracts of *C. odorata* effectively inhibited the growth of *Escherichia coli*, *Salmonella typhi*, and *Staphylococcus aureus*, producing inhibition zones comparable to standard antibiotics such as tetracycline. Similarly, Wireko *et al.* (2025) found that methanolic extracts exhibited strong bactericidal effects against multidrug-resistant *Klebsiella pneumoniae* and *Proteus mirabilis*.

The antibacterial effect is attributed to the synergistic action of phenolic and flavonoid compounds, which disrupt bacterial cell membranes, alter permeability, and cause leakage of intracellular components (Cowan, 1999). Additionally, these phytochemicals may inhibit bacterial enzyme activity and DNA replication, leading to cell death.

Ajao *et al.* (2023) further reported that *C. odorata* extracts not only inhibit planktonic bacterial cells but also prevent biofilm formation—a critical feature in antibiotic resistance. This indicates the plant's potential as a natural antibacterial agent in the management of enteric infections and wound healing.

#### **2.8.5 Antioxidant and Anti-inflammatory Activities**

Beyond its antimicrobial potential, *Chromolaena odorata* exhibits strong antioxidant and anti-inflammatory activities. Bunkaew *et al.* (2025) showed that ethanol and methanol extracts of the leaves displayed high free radical scavenging activity in DPPH assays, correlating with their high total phenolic and flavonoid content.

Antioxidants play a crucial role in neutralizing reactive oxygen species (ROS), thereby protecting cells from oxidative damage. This property contributes to the plant's wound-healing potential by promoting tissue regeneration and reducing inflammation (Omokhua-Uyi and van Staden, 2020).

Furthermore, Sahoo *et al.* (2021) demonstrated that the plant's extract inhibits pro-inflammatory mediators such as nitric oxide and cytokines, suggesting possible therapeutic applications in inflammatory diseases.

## **2.9 Overview on Crude Extracts**

Crude plant extracts represent the initial and most fundamental stage in the process of isolating bioactive compounds from medicinal plants. They are complex mixtures of secondary metabolites such as alkaloids, flavonoids, tannins, saponins, phenols, glycosides, and terpenoids that collectively contribute to the biological activity of the plant (Cowan, 1999; Tiwari *et al.*, 2011). Unlike purified compounds, crude extracts retain the synergistic interactions between these phytochemicals, which often enhances their overall pharmacological potential (Fabricant and Farnsworth, 2001).

The preparation of crude extracts has been a cornerstone of ethnopharmacology and natural product research for centuries. It provides a practical and cost-effective method for investigating plant-based antimicrobials, especially in resource-limited settings where modern drug synthesis may not be feasible (Tiwari *et al.*, 2011). The use of crude extracts in antimicrobial screening allows for the detection of a broad range of biological activities before further fractionation and compound isolation (Eloff, 1998).

### **2.9.1 Solvent Extraction Techniques**

Solvent extraction is the most widely used method for obtaining crude extracts from plants. The choice of solvent significantly influences the type and yield of phytochemicals extracted, as different solvents have varying polarities (Eloff, 1998). Common solvents include water, ethanol, methanol, acetone, chloroform, ethyl acetate, and hexane.

Aqueous extraction involves soaking or boiling plant material in water. It mimics traditional herbal preparations such as teas or decoctions but may extract fewer non-polar compounds (Harborne, 1998).

Ethanol and methanol extractions are preferred for antimicrobial studies because alcohols can dissolve a broad range of both polar and non-polar compounds (Parekh and Chanda, 2007).

Non-polar solvents such as hexane and chloroform extract lipophilic compounds, including terpenes and steroids (Eloff, 1998).

Extraction may be carried out through maceration, Soxhlet extraction, cold percolation, or ultrasonic-assisted methods depending on the research objectives and the nature of plant materials (Tiwari *et al.*, 2011).

For instance, in studies involving *Vernonia amygdalina* and *Chromolaena odorata*, ethanol and methanol have been widely employed as solvents because they yield higher concentrations of flavonoids and terpenoids, which are known for their strong antibacterial activity (Degu *et al.*, 2024, Bunkaew *et al.*, 2025;).

## **2.9.2 Antibacterial Screening of Crude Extracts**

Antibacterial testing of crude extracts involves evaluating their inhibitory effects on bacterial growth using standard microbiological assays such as the agar well diffusion, disc diffusion, and broth dilution methods (Parekh and Chanda, 2007). The diameter of inhibition zones, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) are used to assess activity levels.

Numerous studies have demonstrated the antimicrobial potential of crude plant extracts against enteric pathogens. For example, *Vernonia amygdalina* crude extracts have shown strong inhibitory effects against *E. coli*, *Salmonella typhi*, and *Klebsiella pneumoniae* (Degu *et al.*, 2024). Likewise, *Chromolaena odorata* extracts have been reported to inhibit multidrug-resistant *E. coli* and *Pseudomonas aeruginosa* strains (Bunkaew *et al.*, 2025).

These findings highlight the importance of crude extract screening as a preliminary step toward discovering new plant-derived antibiotics.

## 2.10 Overview on Poultry Droppings

Poultry droppings, often referred to as poultry manure, are a composite waste product consisting primarily of faeces and urine, mixed with undigested feed residues, feathers, bedding materials, and small amounts of spilled water (Kelleher *et al.*, 2002). As a by-product of intensive poultry production, it represents one of the most abundant forms of agricultural waste globally. According to Ogwuegbu *et al.* (2021), poultry droppings account for nearly 50–60% of total waste generated in commercial poultry systems. In developing countries like Nigeria, where poultry farming is rapidly expanding, management and utilization of poultry waste have become both an environmental challenge and an opportunity for bioresource recovery.

### 2.10.1 Composition and Characteristics of Poultry Droppings

Poultry droppings are nutrient-rich organic materials that vary in composition depending on bird species, feed formulation, rearing system, and management practices (Hussain *et al.*, 2020). Typically, they contain high levels of nitrogen (as uric acid and ammonia), phosphorus, potassium, calcium, and organic carbon (Nahm, 2003). The nitrogen content can range from 1.5% to 4%, while organic matter may constitute up to 60–70% of total dry mass (Kelleher *et al.*, 2002).

Microbiologically, poultry droppings are rich in both beneficial and pathogenic microorganisms. The faecal matter provides a moist and nutrient-dense environment that supports microbial proliferation. Commonly occurring bacteria include *Escherichia coli*, *Salmonella* spp., *Campylobacter* spp., *Proteus* spp., *Enterococcus* spp., and *Klebsiella* spp. (Adesemoye *et al.*, 2020). These bacteria may exist as commensals in the avian gut but become contaminants of concern once excreted into the environment.

Poultry droppings may also contain high concentrations of undigested feed particles, antibiotics, heavy metals, and hormones depending on feed and medication regimens (Chokshi *et al.*, 2019). Improper handling can therefore lead not only to environmental pollution but also to dissemination of antimicrobial-resistant bacteria and genes (Mak *et al.*, 2022).

### 2.10.2 Microbial Profile of Poultry Droppings

The microbial ecosystem of poultry droppings reflects the intestinal microbiota of the birds. *Escherichia coli* is among the most predominant enteric bacteria, serving as both a commensal organism and a potential pathogen responsible for colibacillosis in poultry and gastroenteritis in humans (Ojo *et al.*, 2023). *Salmonella* spp. and *Campylobacter jejuni* are two of the most notable zoonotic pathogens associated with poultry manure, often implicated in foodborne infections worldwide (Shaji *et al.*, 2023).

According to Adegunloye (2006), the bacterial load in fresh poultry droppings can range from  $10^6$  to  $10^8$  CFU/g, with the count decreasing as the droppings age due to moisture loss and competition among microbes. Other bacteria such as *Pseudomonas*, *Proteus*, and *Enterobacter* species are also frequently isolated, many of which exhibit multidrug resistance (Pereira *et al.*, 2024).

The fungi associated with poultry droppings include *Aspergillus* spp., *Penicillium* spp., and *Candida* spp., which may contribute to spoilage and, in some cases, respiratory infections in humans exposed to airborne spores (Ola-Fadunsin and Ademola, 2020). Protozoan parasites such as *Eimeria* spp. (causing coccidiosis) and helminth ova have also been reported (Afolabi *et al.*, 2021).

The diversity of microorganisms in poultry droppings underscores their dual role as a potential fertilizer and a public-health risk, especially when waste is poorly managed.

## **CHAPTER THREE**

### **MATERIALS AND METHODS**

#### **3.1 Collection of Test Samples**

A total of eight (8) poultry droppings samples were collected from four (4) different poultry farms located in Ekpoma, Edo State, Nigeria. From each farm, two (2) samples were obtained aseptically to ensure representativeness and minimize contamination.

The samples were collected and transferred into sterile universal bottles. Each bottle was properly labelled with details such as the number and location of collection. The samples were then transported immediately to the Microbiology Laboratory, Department of Microbiology, University of Benin, for analysis.

#### **3.2 Preparation of Materials**

All glassware and equipment used in this study were properly cleaned, sterilized, and prepared prior to use to ensure aseptic conditions and the reliability of results. The materials included Petri dishes, test tubes, pipettes, cotton swabs, inoculating loops, universal bottles, and other microbiological tools.

Nutrient media such as Nutrient Agar and Mueller-Hinton Agar were prepared according to the manufacturer's instructions. The media were dissolved in distilled water, heated to ensure complete dissolution, and then sterilized by autoclaving at 121°C for 15 minutes under a pressure of 15 psi. After sterilization, the media were allowed to cool to about 45–50°C before being poured aseptically into sterile Petri dishes and allowed to solidify.

#### **3.3 Isolation of Enteric Bacteria**

##### **3.3.1 Sample Preparation**

Each poultry dropping sample was thoroughly mixed, and 1 g of the sample was aseptically weighed and transferred into 9 mL of distilled water to obtain a 1:10 dilution. The mixture was vortexed to ensure homogeneity.

### **3.3.2 Serial Dilution**

Serial dilutions were carried out up to the  $10^{-4}$  dilution by transferring 1 mL of the previous dilution into 9 mL of distilled water. This step was performed to reduce the microbial load and facilitate the isolation of discrete colonies.

### **3.3.3 Inoculation of Samples**

From the  $10^{-4}$  dilution 0.1 mL aliquots were inoculated onto Mueller-Hinton agar plates using the pour plate technique.

### **3.3.4 Incubation**

The inoculated plates were incubated at 37°C for 24 hours under aerobic conditions. After incubation, plates showing well-isolated colonies were selected for further study.

### **3.4 Sub-culturing**

Distinct colonies differing in colour, size, and morphology were picked from the plates and sub-cultured onto freshly prepared nutrient agar plates using the streak plate technique to obtain pure cultures. The pure isolates were incubated again at 37°C for 24 hours

### **3.5 Identification of Bacterial Isolates**

The isolated bacteria were identified based on their cultural characteristics, Gram reaction, and biochemical tests such as indole, citrate utilization, KOH, catalase, and oxidase tests. The isolates were then confirmed as enteric bacteria based on Bergey's Manual of Determinative Bacteriology.

### **3.6 Preparation of Plant Extracts**

Fresh Awolowo leaf (*Chromolaena odorata*) and bitter leaf (*Vernonia amygdalina*) were collected, washed with distilled water and blended with water. The filtrates were stored in sterile bottles for further use.

### **3.7 Determination of Antibacterial Activity**

The antibacterial activity of both extracts against the enteric bacterial isolates was determined using the agar well diffusion method. Wells were bored on Mueller Hinton agar plates inoculated with standardized bacterial suspensions, and different concentrations of the plant extracts were introduced into the wells. Plates were incubated at 37°C for 24 hours, after which zones of inhibition were measured in millimetres.

### **3.8 Determination of Minimum Inhibitory Concentration (MIC)**

The MIC of each extract was determined using the broth dilution method. Different concentrations of the extracts were prepared in nutrient broth, inoculated with bacterial suspension, and incubated at 37°C for 24 hours. The lowest concentration showing no visible growth was recorded as the MIC.

### **3.10 Determination of Minimum Bactericidal Concentration (MBC)**

Samples from the MIC tubes showing no growth were sub cultured on nutrient agar plates and incubated for 24 hours. The lowest concentration that produced no visible growth on the agar plates was recorded as the MBC.

## CHAPTER FOUR

### RESULTS

This study was aimed at evaluating the antibacterial activity of natural plant extract (*chromolaena odorata* and *vernonia amygdalina*) on enteric bacteria isolated from poultry droppings.

The results for the total heterotrophic bacterial counts obtained from the faeces sample collected from various poultry farms in Ekpoma as shown in Table 1. The counts ranged from  $0.55 \pm 0.5 \times 10^6$  to  $2.0 \pm 2.0 \times 10^6$  (CFU/g). The highest counts were observed in samples collected from Ikehenosan, Ekpoma with  $2.0 \pm 2.0 \times 10^6$  (CFU/g) while the lowest count was obtained from Ihumudumu, Ekpoma with  $0.55 \pm 0.5 \times 10^6$  (CFU/g).

Five bacterial species were isolated and identified based on their cultural, morphological, and biochemical characteristics: *Escherichia coli*, *Bacillus species*, *Proteus species*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. These organisms are typical enteric bacteria, suggesting faecal contamination and potential public health risks from poultry waste.

The antibacterial assay revealed that bitter leaf extract exhibited higher inhibitory activity against all isolates, particularly *Pseudomonas aeruginosa* (12 mm), *Staphylococcus aureus* (10 mm), and *Proteus sp.* (10 mm) at 100% concentration. The Awolowo leaf extract showed mild inhibition (8–9 mm) only against *Proteus sp.* and *Pseudomonas sp.*, with no activity against *E. coli* or *Bacillus sp.*. The standard antibiotic ciprofloxacin (16–22 mm) showed greater activity than both extracts.

The minimum inhibitory concentration of the extracts against the isolates are shown in Table 4.

Table 1: Total heterotrophic bacteria count of poultry faeces

| <b>SAMPLE<br/>POULTRY</b> | <b>Heterotrophic Bacteria Count( <math>\times 10^6</math><br/>CFU/g)</b> |
|---------------------------|--|
| <b>1</b>                  | 0.95 $\pm$ 0.5   |
| <b>2</b>                  | 0.6 $\pm$ 1.0  |
| <b>3</b>                  | 0.55 $\pm$ 0.5   |
| <b>4</b>                  | 1.75 $\pm$ 1.5   |
| <b>5</b>                  | 1.5 $\pm$ 2.0  |
| <b>6</b>                  | 2.0 $\pm$ 2.0  |
| <b>7</b>                  | 1.95 $\pm$ 1.5   |
| <b>8</b>                  | 0.9 $\pm$ 1.0  |

Table 2. Cultural, morphological and microscopic characteristics of bacterial isolates obtained from samples

| Morphological              |                |                    |                   |                               |                              |
|----------------------------|----------------|--------------------|-------------------|-------------------------------|------------------------------|
| Elevation                  | Flat           | Flat               | Flat              | Raised                        | Raised                       |
| Margin                     | Undulate       | Undulate           | Undulate          | Entire                        | smooth                       |
| Colour                     | Cream          | Cream              | Cream             | lemon                         | Cream                        |
| Shape                      | Irregular      | Irregular          | Irregular         | Circular                      | Irregular                    |
| Size                       | Large          | large              | Large             | Medium                        | Small                        |
| Colour                     | Green          | Straw              | pink              | green                         | Yellow                       |
| Gram stain                 | -              | +                  | -                 | -                             | +                            |
| Cell type                  | Rod            | Rod                | Rod               | rod                           | Cocci                        |
| Arrangement                | disperse       | disperse           | disperse          | disperse                      | clusters                     |
| Colour                     | Pink           | purple             | pink              | pink                          | purple                       |
| Biochemical                |                |                    |                   |                               |                              |
| KOH String Test            | +              | -                  | +                 | +                             | -                            |
| Catalase                   | +              | +                  | +                 | +                             | +                            |
| Indole                     | +              | -                  | -                 | -                             | -                            |
| Citrate                    | -              | +                  | +                 | +                             | +                            |
| Oxidase                    | -              | -                  | -                 | +                             | -                            |
| Urease                     | -              | -                  | +                 | +                             | +                            |
| Glucose                    | +              | +                  | +                 | -                             | +                            |
| Sucrose                    | -              | +                  | -                 | -                             | +                            |
| Lactose                    | +              | +                  | -                 | -                             | +                            |
| Gas formation              | +              | -                  | +                 | -                             | -                            |
| H <sub>2</sub> S formation | -              | -                  | +                 | -                             | -                            |
| TSI reaction (Slant/Butt)  | A/AG           | A/A                | A/AG              | K/K                           | A/A*                         |
| Esculin Hydrolysis         | -              | -                  | -                 | -                             | -                            |
| Identity                   | <i>E. coli</i> | <i>Bacillus</i> sp | <i>Proteus</i> sp | <i>Pseudomonas aeruginosa</i> | <i>Staphylococcus aureus</i> |

KEY: +=POSITIVE, - : NEGATIVE

**Table 3: Antibiotic potential of crude extracts of *Vernonia amygdalina* (Bitter leaf) and *Chromolaena odorata* (Awolowo leaf) on bacterial isolates**

| Test organism                | Extract     | Zones of inhibitions (mm) |     |     |        | CPX (control) |
|------------------------------|-------------|---------------------------|-----|-----|--------|---------------|
|                              |             | 100%                      | 50% | 25% | 12.50% |               |
| <i>Staphylococcus aureus</i> | Bitter leaf | 10                        | 7   | 0   | 0      | 22            |
|                              | Awolowo     | 7                         | 0   | 0   | 0      |               |
| <i>Escherichia coli</i>      | Bitter leaf | 7                         | 0   | 0   | 0      | 20            |
|                              | Awolowo     | 0                         | 0   | 0   | 0      |               |
| <i>Pseudomonas sp.</i>       | Bitter leaf | 12                        | 6   | 0   | 0      | 17            |
|                              | Awolowo     | 9                         | 0   | 0   | 0      |               |
| <i>Bacillus sp.</i>          | Bitter leaf | 0                         | 0   | 0   | 0      | 16            |
|                              | Awolowo     | 0                         | 0   | 0   | 0      |               |
| <i>Proteus sp.</i>           | Bitter leaf | 10                        | 6   | 0   | 0      | 18            |
|                              | Awolowo     | 8                         | 0   | 0   | 0      |               |

Key: CPX = Ciprofloxacin

**Table 4: Minimum inhibitory concentration of *Vernonia amygdalina* (Bitter leaf) and *Chromolaena odorata* (Awolowo leaf)**

|                              | Bitter leaf(extract)<br>(mg/ml) | Awolowo (extract)<br>(mg/ml) |
|------------------------------|---------------------------------|------------------------------|
| <i>E.coli</i>                | 50                              | 100                          |
| <i>Staphylococcus aureus</i> | 100                             | -                            |
| <i>Pseudomonas</i> sp        | 100                             | 100                          |
| <i>Bacillus</i> sp           | -                               | -                            |
| <i>Proteus</i> sp            | 100                             | -                            |

**Table 5: Minimum bactericidal concentration of *Vernonia amygdalina* (Bitter leaf) and *Chromolaena odorata* (Awolowo leaf)**

|                              | Bitter leaf (extract)<br>(mg/ml) | Awolowo (extract)<br>(mg/ml) |
|------------------------------|----------------------------------|------------------------------|
| <i>E.coli</i>                | 100                              | -                            |
| <i>Staphylococcus aureus</i> | -                                | -                            |
| <i>Pseudomonas</i> sp        | 100                              | 100                          |
| <i>Bacillus</i> sp           | -                                | -                            |
| <i>Proteus</i> sp            | 100                              | -                            |

## CHAPTER FIVE

### DISCUSSION

The total heterotrophic bacterial count (THBC) of poultry faeces ranged from  $0.55 \pm 0.5 \times 10^6$  CFU/g to  $2.0 \pm 2.0 \times 10^6$  CFU/g, indicating a high microbial load. These findings suggest that poultry faeces serve as a rich substrate for bacterial proliferation due to their high nutrient content. Comparable microbial loads were reported by Okoro *et al.* (2020), who found THBC values ranging between  $1.2 \times 10^6$  CFU/g and  $2.8 \times 10^6$  CFU/g in poultry droppings from commercial farms in Nigeria. Similarly, Nwankwo *et al.* (2021) observed values within  $10^5$ – $10^6$  CFU/g, indicating that the results from this study fall within the expected range for poultry faecal samples. The high bacterial density reflects poor sanitation practices and possible environmental contamination risks if wastes are not properly managed (Ogboghodo *et al.*, 2022).

The bacterial isolates identified included *Escherichia coli*, *Bacillus* spp., *Proteus* spp., *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. These microorganisms are commonly associated with poultry faeces and environments rich in organic waste. The presence of *E. coli* and *Proteus* spp. signifies potential faecal contamination and zoonotic risk, as also documented by Adegunloye (2020), who reported similar isolates from poultry litter. *Bacillus* spp. were identified as spore-forming bacteria that persist in harsh environmental conditions, consistent with observations by Adesina and Oladipo (2022). The isolation of *S. aureus* and *Pseudomonas aeruginosa* aligns with findings by Eze *et al.* (2021), who emphasized that such opportunistic pathogens are frequently found in poultry faeces and may pose risks of foodborne infections through cross-contamination.

The antibacterial assay demonstrated that crude extracts of *Vernonia amygdalina* (bitter leaf) exhibited stronger antibacterial activity than *Chromolaena odorata* (Awolowo leaf). The bitter leaf extract produced inhibition zones ranging from 7 to 12 mm, while Awolowo leaf produced smaller zones (0–9 mm) against test organisms at 100% concentration. This suggests that *V. amygdalina* possesses more potent antibacterial properties. These findings agree with Adebayo-Tayo *et al.* (2017), who reported that ethanol extracts of *V. amygdalina* exhibited significant inhibitory effects on *S. aureus* and *E. coli*. Similarly, Okigbo and Igwe (2020) found that *V. amygdalina* extracts

inhibited *Pseudomonas aeruginosa* and *Proteus* spp., supporting its broad-spectrum antibacterial potential.

Conversely, the weak inhibition zones of *C. odorata* may be attributed to lower concentrations of active metabolites or differences in extraction efficiency. However, this result contrasts with Agba *et al.* (2019), who observed moderate inhibitory activity of *C. odorata* against *S. aureus* and *E. coli* at higher extract concentrations, suggesting that solvent type and concentration could influence antibacterial efficacy.

The minimum inhibitory concentration (MIC) of *V. amygdalina* ranged from 50 mg/ml to 100 mg/ml, while *C. odorata* showed inhibitory activity only at 100 mg/ml against *Pseudomonas* spp. The minimum bactericidal concentration (MBC) values followed a similar pattern, with *V. amygdalina* exhibiting bactericidal effects at 100 mg/ml against *E. coli*, *Pseudomonas*, and *Proteus* spp. These findings align with those of Ezekiel *et al.* (2022), who reported MIC values of *V. amygdalina* extracts within 50–100 mg/ml against multidrug-resistant bacteria. The moderate MIC and MBC values suggest that while the extracts possess antimicrobial potential, they may require concentration optimization for therapeutic applications.

Ciprofloxacin, used as a control, exhibited larger inhibition zones (16–22 mm) across all test organisms, demonstrating higher efficacy compared to the plant extracts. This difference highlights the potency of synthetic antibiotics but also emphasizes the potential of plant extracts as complementary alternatives, especially in managing antibiotic resistance (Aliyu *et al.*, 2021). The lower activity of crude extracts could be due to the presence of mixed phytoconstituents, solvent limitations, or absence of purification steps (Ehiowemwenguan *et al.*, 2014).

## **Conclusion**

Overall, the findings demonstrate that poultry faeces harbour diverse bacterial species, including pathogenic strains, posing environmental and public health risks. The study also confirms that *Vernonia amygdalina* and *Chromolaena odorata* contain bioactive phytochemicals with antimicrobial properties, though the former showed stronger inhibitory effects. These results are

consistent with existing literature supporting the ethnopharmacological use of these plants in combating bacterial infections.

## RECOMMENDATIONS

Based on the findings of this study, which demonstrated that *Vernonia amygdalina* (bitter leaf) and *Chromolaena odorata* (Awolowo leaf) possess varying degrees of antibacterial activity against enteric bacteria isolated from poultry droppings—with *V. amygdalina* exhibiting stronger antimicrobial potential—the following recommendations are proposed:

### **Adoption of *Vernonia amygdalina* as an Alternative Antibacterial Agent in Poultry Production**

Since *V. amygdalina* demonstrated significant inhibitory and bactericidal effects on *E. coli*, *Proteus spp.*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*, its extracts may serve as a natural alternative or supplement to conventional antibiotics in poultry disease management.

### **Optimization of Extraction Methods for Improved Potency**

The relatively low inhibition of *C. odorata* observed in this study suggests the need for further exploration of other extraction solvents (e.g., methanol, ethanol, acetone), concentrations, and plant parts to maximize the antibacterial efficacy of both plant species.

### **Incorporation of Plant Extracts into Poultry Feed or Bio-Disinfectants**

With proper dosage evaluation and toxicity assessment, crude or refined extracts—especially from *V. amygdalina*—can be formulated into feed additives or water-soluble supplements to reduce enteric bacterial load in poultry.

### **Promotion of Natural Antimicrobials to Reduce Antibiotic Misuse**

Poultry farmers should be sensitized on the dangers of antibiotic abuse and encouraged to integrate plant-based antimicrobials as safer alternatives to mitigate antimicrobial resistance.

### **Improvement of Farm Hygiene and Waste Management Practices**

Since poultry droppings were shown to harbour high microbial loads and pathogenic isolates, farms should implement strict sanitation protocols, appropriate litter disposal, and waste treatment to minimize environmental contamination and zoonotic transmission.

### **Government and Institutional Support for Herbal Research**

Agricultural and veterinary agencies should promote and fund research on medicinal plants with antimicrobial properties to develop standardized, affordable and eco-friendly phytobiotics for use in livestock.

## CONTRIBUTIONS TO KNOWLEDGE

This study has made the following significant contributions to scientific knowledge and the field of microbiology:

### **Establishment of Antibacterial Properties of *Vernonia amygdalina* and *Chromolaena odorata* Against Enteric Bacteria Isolated from Poultry Droppings**

The study has provided empirical evidence that both plant extracts possess antibacterial activity against important enteric pathogens from poultry, demonstrating their potential as natural antimicrobial agents.

### **Comparative Insight on the Antibacterial Strength of Both Plant Species**

Findings from this research show that *Vernonia amygdalina* exhibits stronger inhibitory and bactericidal activity than *Chromolaena odorata*, thereby contributing comparative data that was previously limited.

### **Concentration-Dependent Activity of Plant Extracts on Bacterial Isolates**

The research revealed that the antibacterial effects of the extracts were concentration-dependent, with higher extract concentrations producing greater zones of inhibition and better MIC/MBC results. This knowledge is valuable in determining appropriate dosages for future applications.

### **Identification of Plant Extracts Effective Against Multidrug-Relevant Bacterial Species**

Extracts, especially from *V. amygdalina*, were found effective against bacteria of major public-health concern—including *E. coli*, *Proteus spp.*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*—highlighting their relevance as potential supplements to reduce antimicrobial resistance in poultry systems.

### **Contribution to the Search for Eco-Friendly Alternatives to Conventional Antibiotics**

By demonstrating the antimicrobial efficacy of locally available medicinal plants, this study supports the adoption of phytotherapy as an affordable, accessible and environmentally sustainable strategy for poultry bacterial disease control.

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