

The Synergistic Antimicrobial Effect of *Vernonia Amygdalina* (Bitter Leaf) Extract and Ciprofloxacin on *Escherichia Coli*

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Abstract

Since many microorganisms are developing resistance to synthetic medications, finding newer sources of antibiotics is a global challenge that occupies research organizations, pharmaceutical firms, and academia. This study is aimed to evaluate and determine the synergistic antimicrobial effect of *vernonia amygdalina* (bitter leaf) extract and antibiotics on *Escherichia coli*. Plant leaves were air dried, crushed and immersed in ethanol. Wastewater samples were collected from the NDDC Hostel wastewater dump site using sterile, screw-capped sample containers. The samples were aseptically collected to prevent external contamination and were properly labelled before being transported immediately to the Microbiology Laboratory for analysis. The result revealed that Total Heterotrophic bacteria ranged from $1.1 \pm 0.12 \times 10^7$ CFU/ml to $3.2 \pm 0.26 \times 10^7$ CFU/ml with Sample W2 having the higher counts, while Total Coliform Count ranged from $1.5 \pm 0.14 \times 10^5$ CFU/ml to $2.9 \pm 0.26 \times 10^5$ CFU/ml with Sample W2 having the highest count; Total Fecal Counts (TFC) ranged from $8.0 \pm 0.10 \times 10^3$ CFU/ml to $11.0 \pm 0.23 \times 10^3$ with Sample W2 having the highest counts. The susceptibility results revealed considerable variation in the antibacterial activity of ciprofloxacin, *Vernonia amygdalina* extract, and their combinations against the bacterial isolates. Ciprofloxacin alone produced little inhibition in some isolates indicating some level of resistance or intermediate susceptibility (12-16mm) against most isolates and (18mm) on some isolates, while the plant extract alone exhibited very weak antibacterial activity (0-30 mm) at varying concentrations. However, the ciprofloxacin-plant extract mixtures produced markedly larger zones of inhibition, reaching 22 mm in isolate E4 and 30 mm in E2. These findings suggest that combining ciprofloxacin with *Vernonia amygdalina* extract enhanced antibacterial activity against some isolates, indicating a possible synergistic interaction between the antibiotic and the phytochemicals present in the plant extract.

Keywords: Bitter leaf, Plant leaves, Wastewater samples, Antibacterial activity and plant extract.

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I. Background of Study

Since many microorganisms are developing resistance to synthetic medications, finding newer sources of antibiotics is a global challenge that occupies research organizations, pharmaceutical firms, and academia (Gull *et al.*, 2022). The problem has become even more challenging as a result of the microorganisms' rapid development of multi-drug resistance to the current antimicrobial drugs. The development of pathogen resistance to antibiotics brought on by the indiscriminate use of contemporary antibiotics such as medicinal plants has drawn more attention in the last 20 years (Eja *et al.*, 2020). In various regions of the world, the use of medicinal plants to treat illnesses has been practiced since ancient times. The main benefit of using plants as a source of medicine is that they are still the most affordable and efficient alternatives (Patel *et al.*, 2021).

Medicinal plants contain certain substances which possess the healing properties known as "phytochemicals". Phytochemicals are biologically active, non-nutritive chemical substances that naturally occur in these plants and give them their distinctive color, flavour, and aroma. The two main categories of phytochemicals are primary components and secondary metabolites (Saad *et al.*, 2021). Proteins, amino acids, simple sugars, and chlorophyll are primary elements, whereas glycosides, alkaloids, phenolic compounds, flavonoids, saponins, essential oils, tannins, and terpenoids are secondary constituents. The medical value of plants is therefore found in their corresponding phytochemical composition, as the majority of phytochemicals have considerable therapeutic capabilities (Shaista *et al.*, 2019).

For instance, among the numerous medicinal plants recognized for their health benefits which include, *Vernonia amygdalina* (bitter leaf), *Azadirachta indica* (neem), *Zingiber officinale* (ginger), and *Ocimum gratissimum* (scent leaf). These plants are valued for their antimicrobial, anti-inflammatory, and therapeutic properties and are commonly used in many parts of the world, especially in tropical regions (Okwu, 2022). *Vernonia amygdalina*, commonly known as bitter leaf, is one of the most versatile medicinal plants. Almost every

part of the plant, including the leaves, bark, seeds, roots, and flowers, possesses medicinal value (Shaista *et al.*, 2019). Bitter leaves contain bioactive compounds such as nimbidin, azadirachtin, and flavonoids, which exhibit strong antibacterial, antifungal, antiviral, and antiparasitic properties. Traditionally, bitter leaf extracts are used to treat skin infections, wounds, eczema, and acne due to their antiseptic effects.

The majority of contemporary drugs used to treat various ailments nowadays are based on plants and plant-based substances (Lopez *et al.*, 2019). This demonstrates the abundant natural resources that the plant kingdom has made available for the treatment of numerous ailments. As secondary metabolites, bioactive substances are normally stored in all plant cells, but the levels differ according to the plant component, season, temperature, and specific growth phase. To treat specific disorders, some of these active substances can be used singly or in combination (Lopez *et al.*, 2019).

More so, the combination of some antibiotics (ciprofloxacin, ampicillin or penicillin) and the leaf extracts from medical plants such as the bitter leaf can potentially have antimicrobial effects against gram-negative bacteria such as *Escherichia coli* which are common bacterial isolated from human (Kotloff *et al.*, 2020). However, the effectiveness of such a combination would depend on several factors, such as the concentration of antibiotics, the specific compounds in the plant extract, and the susceptibility of the bacteria strains to the plant extract (Ishiwu *et al.*, 2021).

Aim of Study

The study is aimed to evaluate and determine the synergistic antimicrobial effect of *vernonia amygdalina* (bitter leaf) extract and antibiotics on *Escherichia coli*.

Statement of Problem

The increasing prevalence of antibiotic-resistant strains of *Escherichia coli* has become a major public concern in both clinical and community settings. *Escherichia coli*, although a normal flora of the intestinal tract, is responsible for a wide range of infections such as urinary tract infections, diarrhoea, wound infections, and septicemia. The situation is worsened by the frequent misuse and overuse of antibiotics, which has contributed to the development of multidrug-resistant strains that are more difficult to treat with conventional antimicrobial therapy. This has led to treatment failures, prolonged illness, and increased healthcare costs.

At the same time, medicinal plants such as *Vernonia amygdalina* are widely used in traditional medicine for the treatment of infections due to their perceived antimicrobial properties.

However, there is still limited scientific evidence on how these plant extracts interact with conventional antibiotics when used together against resistant bacterial strains. Therefore, this study seeks to address these gaps by investigating the synergistic effect of *Vernonia amygdalina* extracts and selected antibiotics on *Escherichia coli*, with the aim of providing scientific evidence that may guide improved antimicrobial therapy and support alternative strategies for managing antibiotic resistance.

II. LITERATURE REVIEW

Antimicrobial Resistance (Drug Resistance)

Drug resistance refers to the ability of microorganisms, such as bacteria, viruses, fungi, parasites, to withstand the effects of medications designed to inhibit them or stop their growth and this phenomenon poses a significant public health challenge as it reduces the effectiveness of drugs, leading to prolonged infections, increased healthcare costs, and higher mortality rates (Ladipo *et al.*, 2022). However, drug resistance can develop due to genetic mutations or the acquisition of resistance genes from other microorganisms, making treatment difficult. Antibiotic resistance occurs when bacteria become resistant to antibiotics, leading to infections that are harder to treat, and examples of such bacteria include *Escherichia coli*, methicillin-resistant *Staphylococcus aureus* (MRSA) and multidrug-resistant *Mycobacterium tuberculosis* (MDR-TB) (AlMasaudi *et al.*, 2024).

Examples of Drug Resistant Bacteria

Escherichia coli

Escherichia coli is a Gram-negative, rod-shaped, facultative anaerobic bacterium that belongs to the Enterobacteriaceae family. It naturally resides in the intestines of humans and warm-blooded animals, where most strains are harmless and play a role in maintaining gut health (Fernández *et al.*, 2020). However, some strains are pathogenic and can cause a range of illnesses, particularly foodborne diseases. Pathogenic *Escherichia coli* strains are classified into groups based on their virulence factors and the type of illness they cause (Gandra *et al.*, 2020). The most notable strain is *Escherichia coli* O157:H7, a Shiga toxin-producing *Escherichia coli* (STEC), which can lead to severe gastroenteritis, bloody diarrhea, and potentially life-threatening complications like hemolytic uremic syndrome (HUS), especially in young children and the elderly (Gopal *et al.*, 2021). However, contamination with *Escherichia coli* can occur through the consumption of

undercooked meat (especially beef), drinking contaminated water or unpasteurized milk, poor hygiene and improper handling of raw foods and most importantly, contact with fecal matter during slaughter or food processing (Heinitz *et al.*, 2022). More so, in food microbiology and public health, the presence of *Escherichia coli* is considered an indicator of fecal contamination and poor sanitation (Heng *et al.*, 2021). Moreover, some strains of *Escherichia coli* have developed antibiotic resistance, which can make treating infections caused by these bacteria more challenging.

Staphylococcus epidermidis

Staphylococcus epidermidis is a species of Gram-positive cocci bacteria that is commonly found on human skin and mucous membranes, particularly in the upper respiratory tract, as part of the normal human microbiota (Zeedan *et al.*, 2024). It is one of the coagulase-negative staphylococci (CoNS). *Staphylococcus epidermidis* is considered a commensal bacterium, meaning it typically coexists harmlessly with the human body and it plays a role in preventing more harmful bacteria from colonizing the skin and mucous membranes (Zeedan *et al.*, 2023). However, while *Staphylococcus epidermidis* is generally harmless, it can become an opportunistic pathogen in certain situations. It is known for its ability to form biofilms on medical devices like catheters, prosthetic joints, and heart valves. These biofilms can lead to infections, especially in individuals with compromised immune systems. More so, some strains of *Staphylococcus epidermidis* have developed antibiotic resistance, which can make treating infections caused by these bacteria more challenging. *Methicillin-resistant Staphylococcus epidermidis* (MRSE) is an example of a strain with antibiotic resistance (Abdullahi, 2017). *Staphylococcus epidermidis*, while typically a commensal bacterium found on the skin and mucous membranes, can cause various pathological conditions, especially in certain vulnerable populations or under specific circumstances (Lai *et al.*, 2022). *Staphylococcus epidermidis* is a common cause of healthcare-associated infections (HAIs) or nosocomial infections. Patients in hospitals and healthcare facilities, especially those with indwelling medical devices, are at an increased risk. Healthcare-associated infections involving *Staphylococcus epidermidis* can include catheter-associated urinary tract infections, bloodstream infections (septicemia), and surgical site infections (Ishiwu *et al.*, 2021). However, in some cases, *Staphylococcus epidermidis* bloodstream infections can lead to sepsis, a potentially life-threatening condition. Sepsis occurs when the body's response to infection becomes dysregulated and can result in widespread inflammation and organ dysfunction. *Staphylococcus epidermidis* can also cause infective endocarditis, an infection of the inner lining of the heart chambers and heart valves (Justina and Solomon, 2021). This condition is more common in individuals with pre-existing heart conditions or artificial heart valves. Some strains of *Staphylococcus epidermidis* have developed antibiotic resistance, including methicillin resistance (MRSE) and resistance to other commonly used antibiotics, this can complicate treatment and increase the risk of persistent or recurrent infections (Kotloff *et al.*, 2020).

Vancomycin Resistant *Enterococcus* (VRE)

Vancomycin-Resistant *Enterococcus* (VRE) is a type of bacteria that has developed resistance to the antibiotic vancomycin, which is often considered one of the last-resort antibiotics for treating serious bacterial infections (Adegbola and Adewoye, 2022). *Enterococcus* is a genus of bacteria that can be found in the human gastrointestinal tract and is also commonly found in the environment. The resistance of *Enterococcus* to vancomycin is primarily due to changes in the structure of the bacterial cell wall (Akinjogunla *et al.*, 2019). Vancomycin normally works by binding to specific cell wall components, preventing the bacteria from building a strong and stable cell wall (Carnwath *et al.*, 2020). Vancomycin-Resistant *Enterococcus* has modified cell wall structures that no longer bind vancomycin effectively (Dash *et al.*, 2020). While *Enterococcus faecalis* and *Enterococcus faecium* are the most common species associated with Vancomycin-Resistant *Enterococcus* infections, resistance can occur in other *Enterococcal* species as well (Gheldof *et al.*, 2021). Vancomycin-Resistant *Enterococcus* can cause a variety of infections, including urinary tract infections, bloodstream infections (bacteremia), surgical site infections, and infections in other body systems (Omoya *et al.*, 2022). These infections can be challenging to treat due to limited treatment options. Treating VRE infections can be challenging due to limited antibiotic options. Alternative antibiotics, such as linezolid or daptomycin, may be used, but their efficacy can vary depending on the specific strain of Vancomycin Resistant *Enterococcus*. In some cases, a combination of antibiotics may be necessary (Orafidiya *et al.*, 2022).

Carbapenem Resistant *Enterobacteriaceae* (CRE)

Carbapenem-Resistant *Enterobacteriaceae* (CRE) refers to a group of bacteria within the *Enterobacteriaceae* family that have developed resistance to carbapenem antibiotics, which are considered some of the last-resort antibiotics for treating serious bacterial infections (Okwu, 2024). *Enterobacteriaceae* includes well-known pathogens like *Escherichia coli* and *Klebsiella* species. *Carbapenem-Resistant Enterobacteriaceae* has developed resistance to carbapenems primarily through the production of carbapenemase enzymes (Prabhu

et al., 2021). These enzymes can break down and inactivate carbapenem antibiotics, rendering them ineffective. Carbapenem-Resistant *Enterobacteriaceae* encompasses various bacterial species within the *Enterobacteriaceae* family, including *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter* species, and others (Preethi *et al.*, 2021). These bacteria can cause a range of infections, including urinary tract infections, bloodstream infections, and respiratory infections (Rabelo *et al.*, 2021).

Clostridium botulinum

Clostridium botulinum is another antibiotic resistant bacteria, the neurotoxin produced by *C. botulinum* is responsible for food-borne botulism which causes illness mostly by the consumption of unhygienic processed seafood's. The bacterium produces several serologically classified toxins of which A, B, E, and F cause human food-borne botulism. Temperature abuse, inadequate preservation process, and the absence of oxygen during storage (e.g. vacuum packaging, canning) are the major causes of food-borne illness in consumers of seafood products (Russell and Jarvis, 2023).

Mechanism of Bacterial Resistance to Antimicrobial Agents (Antibiotics)

After being exposed to antimicrobial agents, bacteria may develop drug resistance mechanisms or, in some cases, be intrinsically resistant to certain antimicrobials, and these resistance determinants reduce the antimicrobial activities of the drugs (Suree and Pana, 2021). There are four primary molecular mechanisms of bacterial drug resistance.

Efflux Pumps

Efflux pumps are the resistance mechanisms used in several bacteria for some antibiotics from different classes (Gandra *et al.*, 2020). The efflux pumps evolved as a way for bacteria to interact with the environment, their ubiquitous presence in nature allowed them to survive in their ecological niches, protecting them from toxic compounds produced by other species of bacteria or by the host, antimicrobial molecules, reactive oxygen species (ROS), and toxic byproducts of biochemical degradation pathways (Effraim *et al.*, 2018). Efflux pumps can be specific for only one substrate or can export a range of molecules. This difference in substrate selection is directly associated with the physicochemical characteristics of the substrate and the molecular interactions between the substrate, protein binding site, and environment, possibly reflecting the ability of the efflux pumps to capture their substrates (Lino and Deogracious, 2020). The diagram showing the mechanism of efflux pump is presented in figure 2.1.

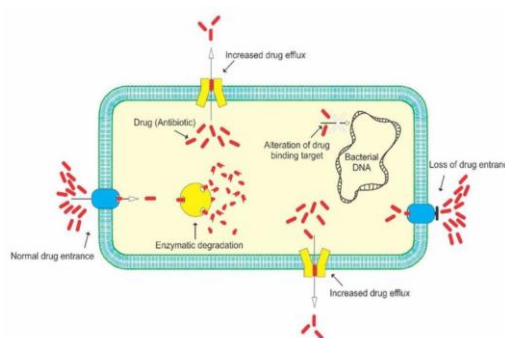


Fig. 2.1: Diagram and mechanism of efflux pump

Source: (Matasyoh *et al.*, 2018).

Enzymatic Hydrolysis

Enzymatic hydrolysis is a process in which enzymes break down substances by adding water to the molecular bonds between atoms, and some bacteria have developed resistance to antibiotics through the production of enzymes that can hydrolyze or break down specific types of antibiotics before they can kill the bacterial cells. For example, one common mechanism of antibiotic resistance is the production of beta-lactamase enzymes by some bacteria (Matasyoh *et al.*, 2018). Beta-lactamase enzymes break down a class of antibiotics called beta-lactams, which are commonly used to treat bacterial infections such as penicillins and cephalosporins. When bacteria produce beta-lactamase, it can inactivate these antibiotics by cleaving the beta-lactam ring in their chemical structure. Other examples of enzymes produced by bacteria that can hydrolyze antibiotics include aminoglycoside-modifying enzymes (AMEs) and chloramphenicol acetyltransferases (CATs). These enzymes can modify or inactivate certain antibiotics, making them ineffective against the bacteria (Nwinyi *et al.*, 2019).

Bacteria that produce these types of enzymes can be highly resistant to antibiotics, making it more difficult to treat bacterial infections. Researchers continue to study these mechanisms of antibiotic resistance in bacteria in order to develop new strategies for combating drug-resistant infections (Matasyoh *et al.*, 2018). One approach involves designing new antibiotics that are less susceptible to enzymatic hydrolysis, while another approach focuses on developing drugs that target the production of resistance enzymes directly (Nwosu and Okafor, 2020).

Alteration of Target Membrane

Alterations of the target membrane of bacteria is another mechanism by which bacteria can develop resistance to antibiotics. In this mechanism, bacteria modify their cell membranes in a way that makes it harder for antibiotics to bind to and disrupt them. There are several ways in which these alterations can occur (Obi and Onuoha, 2022). One common way is through mutations or changes in genes that encode proteins involved in membrane synthesis or structure. These mutations can lead to changes in the composition or organization of the membrane, making it more resistant to antibiotic action (Okigbo and Mmekaka, 2023).

Another way alterations may occur is through the acquisition of new genes via horizontal gene transfer from other microorganisms, for example, some bacteria can obtain plasmids that contain genes encoding enzymes that can modify or enhance cell membrane function, thus conferring resistance to certain antibiotics (Okwu, 2022). Some specific examples of alterations to the target membrane that confer antibiotic resistance include changes in porin channels; some bacteria can alter the size or number of membrane channels that allow antibiotics to pass into cells, and this alterations in porins can make it more difficult for antibiotics to enter bacterial cells and exert their effects (Ram *et al.*, 2018).

Modification of lipopolysaccharides are components of the outer membrane of Gram-negative bacteria, and they play an important role in immune recognition and protection. Modifications to LPS structures can reduce the susceptibility of bacteria to some antibiotics (Saad *et al.*, 2021). Bacteria can form biofilms on surfaces, which provide a protective barrier against antibiotics and other antimicrobial agents and biofilm-forming bacteria have been shown to be more resistant to antibiotic treatment than planktonic (free-floating) bacteria (Ram *et al.*, 2018). However, alterations in target membranes represent a significant challenge for clinicians trying to treat bacterial infections. Researchers are working on developing new strategies for overcoming these mechanisms of resistance while minimizing unnecessary use of antibiotics (Tenover, 2019).

Target Modification

Target modification is a mechanism by which bacteria can develop resistance to antibiotics. In this mechanism, bacteria modify the target of an antibiotic, making it less susceptible to the drug's action. The target may be an enzyme, protein, or other molecule that the antibiotic normally binds to and inhibits (Zeedan *et al.*, 2023). There are several ways in which target modification can occur. One common way is through mutations or changes in genes that encode the target molecule. These mutations can alter the structure or function of the molecule in a way that reduces its binding affinity for the antibiotic, rendering the bacterium resistant to the drug (Ram *et al.*, 2018).

Another way targets may be modified is through enzymatic modification, some bacteria produce enzymes that can modify the target molecule, preventing the antibiotic from binding to it effectively, for example, some bacteria produce beta-lactamases that can hydrolyze (break down) certain types of antibiotics like penicillin and cephalosporins, rendering them ineffective (Agbafor *et al.*, 2023). Modification of ribosomal proteins; antibiotics like macrolides and tetracyclines bind to ribosomal proteins to inhibit protein synthesis in bacteria. Mutations in these proteins can reduce the binding affinity of the drug and lead to resistance. Modification of DNA gyrase/topoisomerase, fluoroquinolones inhibit bacterial DNA replication by targeting DNA gyrase/topoisomerase enzymes (Abdullahi, 2017). Mutations in these enzymes can reduce their susceptibility to fluoroquinolone antibiotics. Beta-lactams like penicillin and cephalosporins target enzymes involved in bacterial cell wall synthesis. Enzyme modifications such as beta-lactamase production can render these antibiotics ineffective. Target modification is a significant challenge for clinicians trying to treat bacterial infections. Researchers are working on developing new strategies for overcoming these mechanisms of resistance while minimizing unnecessary use of antibiotics (AlMasaudi *et al.*, 2024).

Plant Extract

Plant extracts are concentrated substances obtained from various parts of plants such as leaves, stems, roots, flowers, and seeds through methods that isolate their active compounds (Adegbola *et al.*, 2022). These extracts have been used for centuries across different cultures for their medicinal, culinary, cosmetic, and industrial properties (Chovanová *et al.*, 2023). The process of extraction involves removing the desired phytochemicals from the plant matrix using solvents like water, ethanol, methanol, or supercritical CO₂, which selectively dissolve bioactive components (Agbafor *et al.*, 2023). The resulting extracts contain a wide range of

compounds, including alkaloids, flavonoids, terpenes, phenolic acids, and essential oils, each contributing to the plant's unique therapeutic and sensory properties (Eja *et al.*, 2020). The importance of plant extracts spans multiple disciplines. In traditional medicine, they have been the cornerstone of herbal remedies, used to treat ailments ranging from common colds to chronic inflammatory diseases. For example, extracts from plants like Echinacea, ginger, and turmeric are known for their immunomodulatory, anti-inflammatory, and antioxidant effects (Gheldof *et al.*, 2021).

Phytochemical Constituent of Plant Extracts

Phytochemical constituents refer to the diverse array of naturally occurring chemical compounds found in plants, and they are primarily responsible for the biological activities and therapeutic properties of plant extracts (Akinjogunla *et al.*, 2019). These constituents, which include alkaloids, flavonoids, terpenoids, phenolic acids, glycosides, saponins, and tannins, are synthesized by plants as secondary metabolites (Okwu, 2022). One of the major classes of phytochemicals is alkaloids, nitrogen-containing compounds that often exhibit potent pharmacological activities, examples include morphine from opium poppy and quinine from cinchona bark (Patel, 2021). Alkaloids typically have complex ring structures and can interact strongly with biological receptors, making them useful as pain relievers, stimulants, and antimicrobials. Flavonoids, another significant group, are polyphenolic compounds known for their antioxidant properties (AlMasaudi *et al.*, 2024). They are abundant in fruits, vegetables, and teas, and include compounds like quercetin and kaempferol (Okwu, 2022). These molecules help neutralize free radicals and reduce oxidative stress, which is linked to aging and chronic diseases (Rabelo *et al.*, 2021).

Terpenoids, derived from five-carbon isoprene units, represent the largest class of secondary metabolites and include essential oils, carotenoids, and resin acids (Carnwath *et al.*, 2020). Their wide range of functions from serving as natural fragrances and flavors to exhibiting anti-inflammatory and anticancer properties demonstrates their versatility (Rabelo *et al.*, 2021). Phenolic acids, such as caffeic and ferulic acids, are also prevalent in plant extracts and contribute not only to the plants' structural integrity but also to their defense mechanisms. These compounds exhibit antimicrobial, anti-inflammatory, and antioxidant activities, which are beneficial in preventing oxidative damage and in modulating immune responses (Okwu, 2022).

Glycosides, compounds in which sugars are bound to non-carbohydrate moieties (aglycones), play a crucial role in plant metabolism and defense. Cardiac glycosides, for example, are used therapeutically to treat heart conditions by influencing the contractility of cardiac muscle (Chovanová *et al.*, 2023). Saponins, another diverse group of glycosides, are known for their ability to form soap-like foams in aqueous solutions (Shaista *et al.*, 2019). They have been shown to lower cholesterol, exhibit antimicrobial effects, and boost the immune system. Tannins, high-molecular-weight polyphenols, are responsible for the astringency in many fruits and teas, and possess the ability to precipitate proteins (Dash *et al.*, 2020). Their antioxidant and antimicrobial properties contribute to the preservation of plant tissues and provide protective benefits in medicinal applications (Effraim *et al.*, 2018). More so, the process of extraction and subsequent analysis of these phytochemical constituents is critical for standardizing plant extracts and ensuring their efficacy and safety in various applications (Rabelo *et al.*, 2021).

Methods of Obtaining Plant Extracts

Steam distillation: Steam distillation is suitable for extracting essential oils from aromatic plants like lavender or peppermint.

Solvent extraction: Organic solvents (e.g., ethanol, hexane) are used to dissolve and extract specific compounds (Effraim *et al.*, 2018). This method is common for isolating various plant constituents, including phytochemicals and bioactive compounds. Cold pressing; Used for extracting oils from citrus fruits like oranges and lemons (Effraim *et al.*, 2018).

Maceration: Maceration involves soaking plant material in a solvent, typically alcohol or oil, to extract compounds over time (Rabelo *et al.*, 2018).

Infusion and decoction: Infusion and decoction is often used for water-soluble compounds, where plant material is steeped in hot water for a period (infusion) or boiled (decoction).

After extraction, the mixture is often filtered to separate the liquid extract from any solid plant material or particulates (Matasyoh *et al.*, 2017). In some cases, the obtained extract may be concentrated to increase the potency of the desired compounds. This can be achieved through processes like evaporation or freeze-drying (Matasyoh *et al.*, 2017).

***Vernonia Amygdalina* (Bitter Leaf)**

According to Agbafor *et al.*, (2023), report showed that bitter leaf (*Vernonia amygdalina*), is a medicinal and nutritional plant widely grown and consumed in many African countries, especially Nigeria. It belongs to the family *Asteraceae* and is recognized for its characteristic bitter taste, which is mainly due to the

presence of phytochemicals such as alkaloids, saponins, tannins, flavonoids, and sesquiterpene lactones (Effraim *et al.*, 2018). The plant grows as a small shrub or tree and thrives well in tropical climates with adequate rainfall and sunlight. Bitter leaf is commonly cultivated around homes, farms, and gardens because of its importance as both a vegetable and a medicinal herb (Eja *et al.*, 2020).

The leaves of *Vernonia amygdalina* are dark green, elliptical in shape, and have a rough surface, although naturally bitter, the leaves are usually washed or squeezed repeatedly with water during food preparation to reduce the bitterness before cooking (Gheldof *et al.*, 2021). In many Nigerian homes, bitter leaf is used in the preparation of traditional soups such as bitter leaf soup, which is highly valued for its taste and nutritional content. Apart from its culinary use, the plant has long been used in traditional medicine for the treatment of various illnesses (Ishiwu *et al.*, 2021).

The medicinal value of *Vernonia amygdalina* has been widely recognized in African traditional medicine. Okigbo *et al.* (2023), reported that different parts of the plant, especially the leaves and roots, are used in the treatment of several health conditions. In their findings, it was reported that traditionally, bitter leaf extracts have been used for the management of malaria, fever, stomach upset, diarrhea, intestinal worms, and skin infections. Suree and Pana, (2021), in their study, also noted that plant extract possess antimicrobial, antifungal, antiparasitic, antioxidant, and anti-inflammatory properties. More so, several studies have supported some of these traditional claims by demonstrating that extracts of the plant can inhibit the growth of certain bacteria and fungi (Kotloff *et al.*, 2020).

Bitter leaf is also associated with blood sugar regulation and is sometimes used traditionally in the management of diabetes. Justina and Solomon, (2021), suggested in their report that compounds present in the plant extracts may help lower blood glucose levels and improve insulin sensitivity. In addition, to the previous findings, Ishiwu *et al.* (2021), also noted that the antioxidant compounds found in the leaves help neutralize harmful free radicals in the body, thereby reducing oxidative stress and protecting cells from damage. This has drawn attention to the possible role of bitter leaf in preventing chronic diseases linked to oxidative damage. The diagram showing the Bitter leaf plant is presented in figure 2.2.



Fig. 2.2: *Vernonia amygdalina* (Bitter leaf)
Source: (Oladokun, 2024)

Antimicrobial Properties of Bitter Leaf Extract

According to Agbafor *et al.* (2023), the major active compounds responsible for the antimicrobial activity of bitter leaf is flavonoids. In their study, they noted that flavonoids are natural phenolic compounds known for their antioxidant and antimicrobial effects. Matasyoh *et al.* (2018), reported that flavonoids act by damaging microbial cell membranes, interfering with enzyme activity, and disrupting the metabolism of microorganisms. Flavonoids may also inhibit nucleic acid synthesis in bacterial cells, preventing their growth and multiplication (Ladipo *et al.*, 2022). However, another important group of active compounds present in bitter leaf is alkaloids. Alkaloids possess strong antimicrobial properties and can interfere with DNA replication and protein synthesis in microorganisms (Lai *et al.*, 2022). These compounds may alter the permeability of microbial cell membranes, leading to leakage of cellular contents and eventual cell death. Alkaloids are also believed to contribute to the bitter taste associated with the plant (Lino *et al.*, 2020).

Tannins are also present in bitter leaf extracts and play a role in antimicrobial activity, they are polyphenolic compounds capable of binding to proteins on microbial cell walls and membranes (Lopez *et al.*, 2019). This interaction can disrupt the structural integrity of microorganisms and inhibit essential metabolic activities. Tannins may also form complexes with microbial enzymes, reducing their ability to function properly (Matasyoh *et al.*, 2018). More so, saponins are another class of phytochemicals found in bitter leaf, these compounds possess surface-active properties and can interact with the lipid components of microbial cell membranes (Nwinyi *et al.*, 2019). This interaction increases membrane permeability, causing leakage of important intracellular materials. Saponins have shown antibacterial, antifungal, and antiparasitic activities in several laboratory studies (Nwosu and Okafor, 2020). Phenols are known for their ability to denature proteins and damage microbial membranes, while terpenoids may interfere with microbial respiration and energy production. Sesquiterpene lactones, which are characteristic compounds in *Vernonia amygdalina*, are particularly associated with antimicrobial and anti-inflammatory activities (Obi and Onuoha, 2022).

Combined Effect of Antibiotics and Bitter Leaf Extracts on *Escherichia coli*

Bitter leaf includes a wide spectrum of bioactive phytochemicals, and these compounds have been demonstrated to possess antibacterial effects against numerous pathogenic microbes (Okwu, 2022). Though, when used alone, bitter leaf extracts can inhibit the growth of *Escherichia coli*, although the level of inhibitory activity may depend on factors such as extraction method, solvent used, and concentration (Omoya and Akharaiyi, 2022). However, when combined with conventional antibiotics such as ciprofloxacin a stronger antibacterial effect is often observed (Orafidiya *et al.*, 2022). The synergistic effect occurs when the interaction between bitter leaf extract and antibiotics produces a greater antibacterial activity than either agent used alone, in some cases, this interaction may reduce bacterial growth significantly, increase the size of inhibition zones in culture media, or lower the minimum inhibitory concentration (MIC) of antibiotics (Patel *et al.*, 2021). This suggests that plant extracts can enhance the effectiveness of existing antibiotics against resistant strains of *Escherichia coli*. One of the main mechanisms behind this synergistic effect is the ability of bitter leaf phytochemicals to disrupt bacterial cell structures, compounds such as saponins and flavonoids can damage the outer membrane of Gram-negative bacteria like *Escherichia coli*, increasing cell permeability (Prabhu *et al.*, 2021). This disruption allows antibiotics to penetrate the bacterial cell more easily and reach their target sites in higher concentrations. The outer membrane of *Escherichia coli* normally acts as a barrier to many antibiotics, so weakening this structure significantly improves drug entry and effectiveness (Preethi *et al.*, 2021).

Some *E. coli* strains produce enzymes such as beta-lactamases that break down antibiotics, making them ineffective (Rabelo *et al.*, 2021). Certain compounds in bitter leaf extracts may interfere with these enzymes or reduce their activity, thereby restoring the effectiveness of antibiotics. Moreover, plant metabolites may inhibit efflux pumps, which are protein systems used by bacteria to expel antibiotics from the cell (Ram *et al.*, 2018). When efflux pumps are blocked, antibiotics remain inside the bacterial cell for longer periods, increasing their antibacterial effect (Russell and Jarvis, 2023). The combination of bitter leaf extracts and antibiotics has also been shown to affect bacterial metabolism. Alkaloids and tannins in the extract may interfere with protein synthesis, DNA replication, or energy production in bacterial cells (Shaista *et al.*, 2019). When these metabolic processes are weakened, bacteria become more vulnerable to antibiotic action, and this multi-target approach reduces the ability of bacteria to survive or develop resistance quickly (Tenover, 2019). Common antibiotics tested in combination with bitter leaf extracts include tetracycline, ampicillin, ciprofloxacin, gentamicin, and chloramphenicol, and in laboratory experiments, the agar well diffusion or disc diffusion method is often used to compare the zones of inhibition produced by antibiotics alone and in combination with plant extracts (Adegbola and Adewoye, 2022). A significant increase in inhibition zone diameter or a reduction in MIC values indicates synergism (Dash *et al.*, 2020).

Table 1: Comparing the degree of antibiotic resistance against different plant extracts

Plant extract	Test organism	Zone of inhibition (mm)	Degree of Resistance
Bitter leaf	<i>E. coli</i>	8mm	High resistance
Carica papaya leaf	<i>Staphylococcus aureus</i>	14mm	Moderate resistance
Neem extract	<i>Pseudomonas aeruginosa</i>	18mm	Low resistance
Scent leaf	<i>Klebsiella pneumoniae</i>	12mm	Moderate resistance
Ginger extract	<i>Escherichia coli</i>	20mm	Low resistance

Source: (Matasyoh *et al.*, 2018).

III. MATERIALS AND METHODS

Study Area

The study was carried out in the Niger Delta Development Commission (NDDC) Hostel's waste water dump site in Rivers State University, Port Harcourt City Local Government Area, Rivers State.

Sample Collection

Wastewater samples were collected from the NDDC Hostel wastewater dump site using sterile, screw-capped sample containers. The samples were aseptically collected to prevent external contamination and were properly labelled before being transported immediately to the Microbiology Laboratory for analysis.

Materials and Apparatus

The materials and apparatus used in this study included sterile containers for sample collection, sterile gloves, laboratory coats, face mask and ice packs with a cooler for transportation of samples. In the laboratory, the equipment used comprised Petri dishes, sterile test tubes, conical flasks, and beakers, micropipettes with sterile tips, wire loops, glass spreaders, and measuring cylinders.

Other apparatus included an incubator, autoclave, analytical balance, refrigerator, and a bright-field microscope with glass slides, cover slips, and immersion oil. Additional items used for aseptic procedures and safety included Bunsen burners, spirit lamps, disinfectants (70% ethanol and sodium hypochlorite), sterile water, biohazard disposal bags, masking tape and marker pens for labeling samples and plates.

Reagents and Media

The media and reagents used for microbial isolation and antibiotic sensitivity testing included normal saline for serial dilution procedure, Nutrient Agar (NA) for total heterotrophic bacteria, and Eosin Methylene Blue (EMB) Agar for the isolation and differentiation of coliforms.

Sterilization of Glass-wares and Working Benches

Materials such as glasswares and media were sterilized before use. The glasswares including pipettes, test tubes, beakers, measuring cylinder, bijou/MacCartney bottles etc, were washed thoroughly with detergent, rinsed thoroughly with clean tap water and allowed to air-dry before sterilizing in the laboratory hot air oven at 160°C for 1 hour. The media were sterilized by autoclaving at 121°C for 15 minutes. Bench working areas were swabbed with dry cotton wool soaked in ethanol to sterilize before any microbiological analysis were carried out to avoid contamination. Sterile disposable hand gloves were worn and changed after each procedures to ensure aseptic conditions/techniques.

Preparation of *Vernonia amygdalina* Leaf Extract

Fresh leaves of *Vernonia amygdalina* were washed thoroughly with distilled water to remove dirt and debris, air-dried for approximately two weeks, and ground into a fine powder. 100 g of the powdered leaves was soaked in 100 mL of ethanol and allowed to stand for 48 hours with intermittent shaking to facilitate extraction. The mixture was then filtered using Whatman No. 1 filter paper to obtain the filtrate.

The filtrate was concentrated by evaporating the solvent in a hot air oven at 74°C until a dry crude extract was obtained. The dried crude extract was weighed and stored in a sterile airtight container until required for analysis.

For the preparation of extract concentrations, 2 g of the crude extract was dissolved in 5 mL of dimethyl sulfoxide (DMSO) to obtain the stock solution. A two-fold serial dilution was subsequently carried out using sterile DMSO to produce different concentrations of the extract. Equal volumes of the stock solution were transferred into successive tubes containing sterile DMSO to obtain concentrations of 400mg/mL, 200 mg/mL, 100 mg/mL, 50 mg/mL, and 25 mg/mL, which were used for the antibacterial susceptibility tests.

Preparation of Standard Bacteria Suspension

A standardized bacterial suspension was prepared following the McFarland turbidity standard method. Pure colonies from 24-hour-old bacterial cultures were picked using a sterile loop and emulsified in 5 mL of sterile normal saline. The turbidity of the suspension was adjusted visually to match the 0.5 McFarland standard, which corresponds to approximately 1.5×10^8 CFU/ml. The suspension was thoroughly mixed to ensure uniformity and was used immediately for antimicrobial susceptibility testing. Standardization ensured that the bacterial inoculum used in the sensitivity assay was consistent and reproducible (CLSI, 2021).

Preparation of 0.5ml McFarland turbidity standard

The 0.5 McFarland turbidity standard was prepared by mixing 0.05 mL of 1.175% (w/v) barium chloride dihydrate ($\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$) with 9.95 mL of 1% (v/v) sulfuric acid (H_2SO_4). The reaction between barium chloride and sulfuric acid produces a fine precipitate of barium sulfate (BaSO_4), which gives the solution a turbidity equivalent to approximately 1.5×10^8 CFU/mL of bacterial suspension. The prepared standard was stored in a tightly sealed glass tube, protected from light, and mixed thoroughly before each use to ensure even turbidity. The turbidity of bacterial inocula was visually compared to this standard to achieve consistency in antimicrobial susceptibility testing (Andrews, 2001).

Antibiotic Susceptibility Testing by Agar Well Diffusion Method

Antibacterial susceptibility testing was carried out using the agar well diffusion method on Nutrient Agar (NA). A 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL) was prepared from 18–24-hour pure bacterial cultures and evenly lawn-streaked onto the surface of sterile Nutrient Agar plates using a sterile cotton swab. The inoculated plates were allowed to stand for approximately 15 minutes to permit absorption of the inoculum. Using a sterile cork borer, wells of approximately 6 mm in diameter were aseptically made in the agar. The wells were filled with predetermined volumes of *Vernonia amygdalina* leaf extract, ciprofloxacin solution, and a combination of *Vernonia amygdalina* extract and ciprofloxacin. Care was taken to prevent overflow and cross-contamination between wells.

The plates were allowed to stand for approximately 30 minutes at room temperature to facilitate diffusion of the test agents into the agar before incubation.

The plates were then incubated in an inverted position at $35 \pm 2^\circ\text{C}$ for 18–24 hours. Following incubation, the diameters of the zones of inhibition around each well were measured to the nearest millimetre using a transparent ruler. The antibacterial activities of the extract, ciprofloxacin, and their combination were compared based on the observed zones of inhibition (Perez *et al.*, 2016).

IV. RESULTS

Bacterial Population

Table 4.2 shows the microbial population of the wastewater samples. Total Heterotrophic Bacteria (THB) count ranged from $1.1 \pm 0.13 \times 10^7$ to $3.2 \pm 0.26 \times 10^7$ CFU/ml with W2 having the higher counts. Total Fecal Coliform ranged from $1.5 \pm 0.14 \times 10^5$ to $2.9 \pm 0.26 \times 10^5$ CFU/ml with W2 also having higher counts, while Total Coliform Counts ranged from $8.0 \pm 0.10 \times 10^3$ to $11.0 \pm 0.23 \times 10^3$ with W2 also having higher counts. Sample W2 recorded the highest total heterotrophic bacterial (THB) count ($3.2 \pm 0.26 \times 10^7$ CFU/ml), the highest total coliform count (TCC) ($2.9 \pm 0.26 \times 10^5$ CFU/ml), and the highest total faecal coliform count (TFC) ($11.0 \pm 0.23 \times 10^3$ CFU/ml), compared with sample W1, which recorded THB, TCC, and TFC values of $1.1 \pm 0.13 \times 10^7$, $1.5 \pm 0.14 \times 10^5$, and $8.0 \pm 0.10 \times 10^3$ CFU/ml, respectively. Overall, both wastewater samples contained heterotrophic bacteria, total coliforms, and faecal coliforms, indicating microbial contamination of the wastewater, although higher bacterial counts were observed in sample W2.

Biochemical Tests

The biochemical and morphological characteristics of the bacterial isolates are presented in Table 4.2. All isolates (W1M1, W1E1, W2M1, W2E1, and W2E2) were Gram-negative rods and exhibited similar biochemical characteristics. The isolates were catalase positive, oxidase negative, indole positive, motile, citrate negative, methyl red positive, and Voges–Proskauer negative. They also fermented glucose, lactose, mannitol, and sucrose with the production of acid and gas (AG). The observed morphological and biochemical characteristics are consistent with those of *Escherichia coli*, indicating that all the isolates were identified as *Escherichia coli*.

Antimicrobial Susceptibility Results

The susceptibility results revealed considerable variation in the antibacterial activity of ciprofloxacin, *Vernonia amygdalina* extract, and their combinations against the wastewater isolates. Ciprofloxacin alone produced little inhibition (12–16 mm) against some isolates, suggesting reduced susceptibility or possible resistance, although some isolates had increased zones of inhibition (18mm). On the other hand, the plant extract alone exhibited weak antibacterial activity (0–30 mm) at varying concentrations. However, the ciprofloxacin–plant extract mixtures produced markedly larger zones of inhibition, reaching 32 mm in isolate E3 and 22 mm in isolate E4. These findings suggest that combining ciprofloxacin with *Vernonia amygdalina* extract enhanced antibacterial activity against some isolates, indicating a possible synergistic interaction between the antibiotic and the phytochemicals present in the plant extract.

Table 1: Bacterial Counts of Wastewater Sample

Samples	THB ($\times 10^7$ CFU/ml)	TCC ($\times 10^5$ CFU/ml)	TFC ($\times 10^3$ CFU/ml)
W1	1.1 ± 0.13^a	1.5 ± 0.14^a	8.0 ± 0.10^a
W2	3.2 ± 0.26^a	2.9 ± 0.26^a	11.0 ± 0.23^a

THB- Total Heterotrophic Bacteria

TCC- Total Coliform Count

TFC- Total Fecal Coliform

Isolate	MORPHOLOGICAL							PHYSIOLOGICAL							SUGER FERMENTATION				Suspecte d organism	
	Gram reaction	Shape	Elevation	Color	Shape	Margin	Texture	Size	Catalase	Oxidase	Indole	Motility	Citrate	MR	VP	Glucose	Lactose	Mannitol		Sucrose
W1M1	-	R o d s	Rai sed	Pin k	R ou nd	Enti re	Moi st	3 m m	+	-	+	-	-	+	-	A G	A G	A G	A G	Escherichia coli
W1E1	-	R o d s	Rai sed	Sh een gre en	R ou nd	Enti re	Moi st	2 m m	+	-	+	-	-	+	-	A G	A G	A G	A G	Escherichia coli
W2M1	-	R o d s	Rais ed	Pin k	Ro un d	Entir e	Mois t	2 m m	+	-	+	-	-	+	-	AG	A G	A G	A G	Escherichia coli
W2E1	-	R o d s	Rai sed	She en gre en	Ro un d	Entir e	Mois t	1 . 8 m m	+	-	+	-	-	+	-	A G	A G	A G	A G	Escherichia coli
W2E2	-	R o d s	Rai sed	She en gre en	Ro un d	Entir e	Mois t	2 m m	+	-	+	-	-	+	-	A G	A G	A G	A G	Escherichia coli

IDENTIFICATION

MR- Methyl Red

VP- Vogues Proskauer Test

Table 3: Susceptibility of the *Escherichia coli* to Ciprofloxacin-plant-extract Mixtures with *Vernonia amygdalina* (bitter leaf)

Organism	Zone of inhibition (mm)						
	400mg/ml	200mg/ml	100mg/ml	50mg/ml	12.5mg/ml	CPX	CPX-VA
E1	25mm	20mm	15mm	0mm	0mm	12mm	26mm
E2	24mm	23mm	20mm	12mm	0mm	16mm	30mm
E3	30mm	25mm	20mm	15mm	8mm	18mm	32mm
E4	27mm	20mm	17mm	13mm	0mm	18mm	22mm
E5	25mm	24mm	21mm	13mm	0mm	18mm	28mm

E1: Isolate 1, E2: Isolate 2, E3: Isolate 3, E4: Isolate 4, E5: Isolate 5

CPX: Ciprofloxacin, CPX-VA: Ciprofloxacin and *Vernonia amygdalina* extract mixture

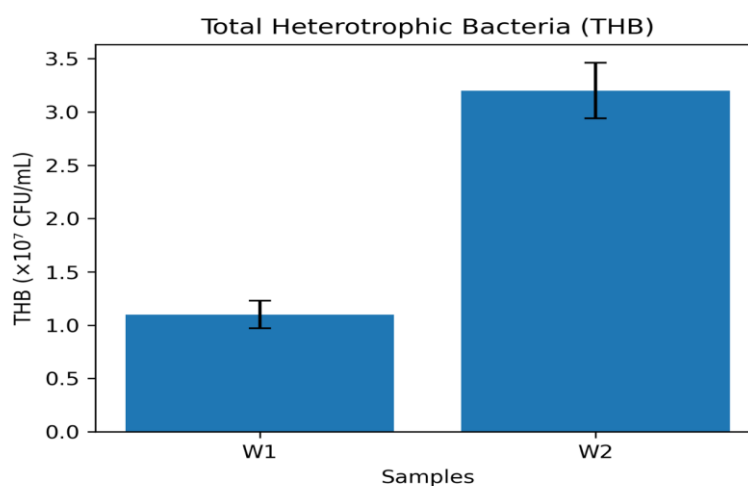


Figure 1: Total Heterotrophic Bacterial Count (THB) in wastewater samples W1 and W2 ($\times 10^7$ CFU/mL).

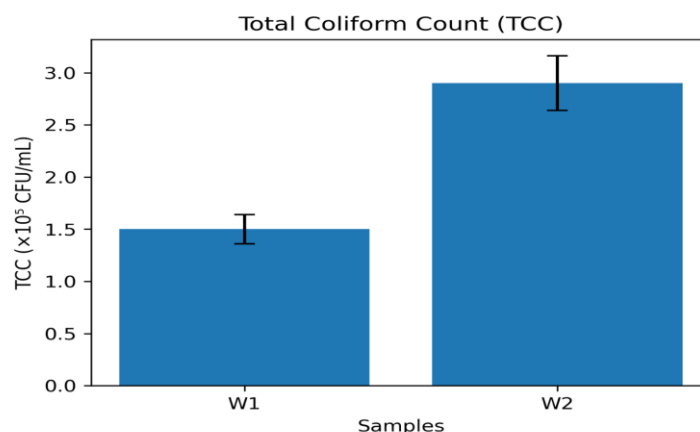


Figure 2: Total Coliform Count (TCC) in wastewater samples W1 and W2 ($\times 10^5$ CFU/mL).

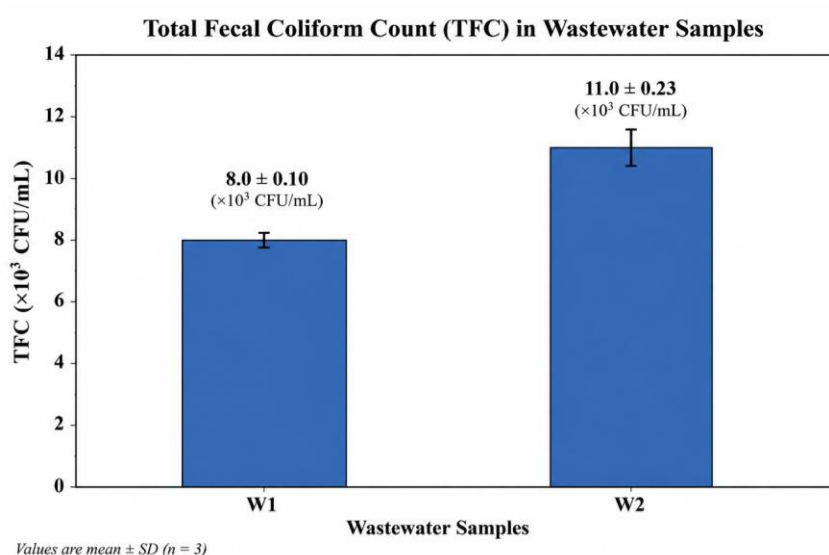


Figure 3: Total Fecal Coliform Count (TFC) in wastewater samples W1 and W2 ($\times 10^3$ CFU/mL)

V. DISCUSSION

Microbial Population

The isolation of *Escherichia coli* from the wastewater samples suggests faecal contamination of the wastewater sources. *Escherichia coli* is universally recognized as an indicator organism for faecal pollution because it inhabits the gastrointestinal tract of humans and warm-blooded animals (Lopez *et al.*, 2019). Its detection therefore indicates possible contamination with pathogenic enteric microorganisms. This agreed with the findings of Kotloff, *et al.* (2020), who reported a high bacteria load from a wastewater source. The relatively high microbial counts observed suggest that the wastewater environment provides favourable conditions for the survival and multiplication of enteric bacteria (Nwosu *et al.*, 2020). These findings agree with reports of Chuka *et al.* (2022), that wastewater systems commonly harbour large populations of *Escherichia coli* and other enteric bacteria due to continuous discharge of domestic sewage, hospital effluents and industrial wastes. Wastewater environments serve as important reservoirs for microbial pathogens and contribute significantly to environmental dissemination of faecal bacteria (Okigbo and Mmeka, 2023).

The biochemical characterization generally supported the identification of Gram-negative enteric bacteria (Okwu, 2022). The positive indole and methyl red reactions obtained for most isolates are consistent with characteristics commonly associated with *Escherichia coli*. This findings agreed with the study of Lai *et al.* (2022).

Antimicrobial Susceptibility

The antimicrobial susceptibility results indicate that ciprofloxacin alone demonstrated little inhibitory activity against the isolates, while *Vernonia amygdalina* (bitter leaf) extract alone also produced relatively small inhibition zones. This observation suggests that many of the wastewater isolates possess varying degrees of

resistance to ciprofloxacin (Agbafor *et al.*, 2023). Interestingly, the combination of ciprofloxacin with *Vernonia amygdalina* extract resulted in substantially larger inhibition zones against several isolates, indicating possible synergistic antibacterial activity. This agreed with findings of Abdullahi, (2017). Plant secondary metabolites such as flavonoids, alkaloids, tannins, saponins and phenolic compounds present in *Vernonia amygdalina* may enhance bacterial cell permeability or inhibit bacterial resistance mechanisms, thereby improving the effectiveness of ciprofloxacin (Dash *et al.*, 2020). These findings are consistent with the work of Eja *et al.* (2020), who reported that *Vernonia amygdalina* extracts exhibited antibacterial activity against *Escherichia coli*, *Klebsiella* species and *Staphylococcus* species, with stronger activity observed when appropriate extraction methods were used.

Similarly, recent investigations by Chovanová *et al.* (2023), reports has shown that wastewater environments are important reservoirs of antimicrobial-resistant *Escherichia coli*, including isolates resistant to ciprofloxacin and other fluoroquinolones. Their studies demonstrated that wastewater systems promote the persistence and dissemination of resistant bacteria because of continuous exposure to antibiotics and other selective agents. The reduced susceptibility of some isolates to ciprofloxacin observed in this present study therefore supports previous reports that environmental wastewater serves as a significant source of antimicrobial-resistant bacteria, posing considerable risks to environmental and public health.

VI. Conclusion

This study investigated the synergistic antimicrobial effect of *Vernonia amygdalina* (bitter leaf) extract and ciprofloxacin against *Escherichia coli* isolated from wastewater samples. The antimicrobial susceptibility test revealed that ciprofloxacin alone exhibited limited activity against several isolates, while *Vernonia amygdalina* extract alone produced weak to moderate antibacterial effects. However, the combination of ciprofloxacin and the plant extract resulted in larger zones of inhibition against several *Escherichia coli* isolates, demonstrating an enhanced antimicrobial effect compared with either agent used individually. This finding suggests a possible synergistic interaction between ciprofloxacin and the bioactive phytochemicals present in *Vernonia amygdalina*, including flavonoids, alkaloids, tannins, saponins and phenolic compounds, which may enhance antibiotic activity or help overcome bacterial resistance mechanisms. Similar studies have shown that *Vernonia amygdalina* possesses antibacterial properties and can enhance the effectiveness of conventional antibiotics against pathogenic bacteria. More so, the study demonstrates that combining *Vernonia amygdalina* extract with ciprofloxacin may represent a promising strategy for improving the treatment of *Escherichia coli* infections, particularly those involving antibiotic-resistant strains.

VII. Recommendations

1. The use of plant–antibiotic combination therapy should be further explored as a strategy for combating antimicrobial resistance, particularly against multidrug-resistant *Escherichia coli* and other clinically important bacterial pathogens.
2. Further studies should be conducted to determine the Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC), and Fractional Inhibitory Concentration Index (FICI) of the combination of *Vernonia amygdalina* extract and ciprofloxacin. These tests will provide stronger evidence for confirming the synergistic interaction between the plant extract and the antibiotic.
3. Advanced phytochemical and molecular studies should be carried out to isolate, identify and characterize the bioactive compounds in *Vernonia amygdalina* responsible for the observed antimicrobial activity and to investigate their mechanisms of action against *Escherichia coli*.
4. In vivo studies and toxicity evaluations should be undertaken to assess the safety, efficacy and pharmacological properties of the combined use of *Vernonia amygdalina* extract and ciprofloxacin before considering clinical application in the treatment of bacterial infections.

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