PHYTOCHEMICALS AND ANTIMICROBIAL PROPERTIES OF *ALCHORNEA CORDIFOLIA* LEAVES AGAINST SOME SELECTED BACTERIA ISOLATES

BY

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LSC2007281

(MICROBIOLOGY OPTION)

DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY

FACULTY OF LIFE SCIENCES,

UNIVERSITY OF BENIN,

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NOVEMBER, 2025.

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A PROJECT SUBMITTED TO THE DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF BACHELOR OF, SCIENCE DEGREE (BSc) IN SCIENCE LABORATORY TECHNOLOGY (MICROBIOLOGY TECHNIQUES).

NOVEMBER, 2025.

CERTIFICATION

This is to certify that this project work titled PHYTOCHEMICALS AND ANTIMICROBIAL PROPERTIES OF *Alchornea cordifolia* AGAINST SOME SELECTED BACTERIA ISOLATE was done by Sandra Somkelechukwu EBITE (Miss) with MAT NO. LSC2007281 of the Department of Science Laboratory Technology (Microbiology Technique), Faculty of Life Sciences, University of Benin, Benin City, as part of requirement for the award of bachelor of Science (B.Sc.) Degree.

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DEDICATION

I dedicate this project to God Almighty for his wisdom and direction while working on this project. I also dedicate this project to my wonderful family for the love and support throughout the course of this project.

ACKNOWLEDGEMENT

I wish to express my heartfelt gratitude to my supervisor, Mr Haruna . O., for his patience, support, and invaluable guidance throughout this work. His dedication, motivation, and commitment to excellence have been a great source of inspiration.

My deepest appreciation goes to my parents Mr. and Mrs. M.O. Ebite, for their unconditional love, constant encouragement, and unwavering belief in me I owe all that I am to them. My heartfelt thanks also go to my siblings Sonia, Elvis and Sophia, for their endless support. A special mention to my best friends Fortune, Glory, Stanley and Millicent for their love and support.

My journey would be incomplete without acknowledging some amazing people who helped me through school and made this achievement possible. I may have forgotten to mention a few name, but I am extremely grateful for everyone of you who I crossed paths with during my time in school, To all those who have supported me and shown me love, all I can say is I'm very grateful and God bless you all abundantly.

TABLE OF CONTENTS

LIST OF TABLES

PAGE

ABSTRACT

Alchornea Cordifolia commonly known as the Christmas bush or “Ewe ira” in West Africa, is a medicinal plant widely used in traditional medicine for the treatment of infections, wounds, and inflammatory conditions. This study investigates the phytochemical composition and antimicrobial properties of *Alc*hornea *cordifolia* leaf extracts. Qualitative phytochemical screening revealed the presence of major bioactive constituents such as alkaloids, flavonoids, tannins, saponins, glycosides and phenolic compounds, which are known to possess therapeutic and antimicrobial potential. However , terpenoids was absent in the cause of this work. The antimicrobial activity of the ethanolic leaf extracts was evaluated against selected bacterial isolates including *Staphylococcus aureus*, *Escherichia coli, Pseudomonas aeruginosa* and *Bacillus substilus*. The study employed the agar well diffusion method to assess bacterial susceptibility to varying concentrations (1000 mg/ml, 500 mg/ml, 250 mg/ml,125 mg/ml and 62.5mg/ml) of *Alc*hornea *cordifolia* leaf extract. The results showed no significant degrees or zones of inhibition exhibited by the extract and so suggest further research is needed. The observed activity is attributed to the synergistic effects of the identified phytochemicals. These findings support the ethnomedicinal use of *Alch*ornea *cordifolia* leaves and suggest that the plant could serve as a potential source of natural antimicrobial agents for developing alternative therapies against resistant microbial strains.

CHAPTER ONE

1.0 Introduction

The global rise of antimicrobial resistance (AMR) has emerged as a critical public health challenge, rendering many conventional antibiotics ineffective against bacterial infections. This phenomenon, driven by the overuse of antibiotics and the adaptability of pathogens, has necessitated the exploration of alternative therapeutic agents. Plant-derived phytochemicals have gained attention due to their diverse bioactive compounds which exhibit antimicrobial, antioxidant and anti-inflammatory properties. Among such plants, *Alchornea cordifolia*, a widely distributed African medicinal plant, has shown promise in traditional medicine for treating infections and related ailments. This study investigates the phytochemical constituents and antimicrobial efficacy of *Alchornea cordifolia* leaf extracts against multidrug-resistant (MDR) bacterial pathogens, aiming to validate its traditional uses and explore its potential as a natural antimicrobial agent. By identifying active compounds and their mechanisms, this research seeks to contribute to the development of sustainable and plant-based therapies to combat AMR.

1.2 Background of the Study

Antimicrobial resistance poses a significant threat to global health, with the World Health Organization estimating that AMR could lead to 10 million deaths annually by 2050 if no effective interventions are implemented (WHO, 2014). Pathogens such as *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumoniae* have developed resistance to multiple antibiotics, complicating the treatment of common infections like wound infections, urinary tract infections and diarrhea. In developing countries, particularly in sub-Saharan Africa, limited access to advanced healthcare and the high cost of synthetic drugs

exacerbate these challenges, making traditional herbal remedies a vital resource (Sofowora, 2008).

Alchornea cordifolia, a member of the Euphorbiaceae family, is a perennial shrub or small tree native to tropical Africa including Nigeria, Ghana and Cameroon. It grows in diverse habitats from humid forests to savannas, reaching heights of 4–10 meters. Its leaves, roots and stem bark are integral to African ethnomedicine. In Nigeria, for instance, leaf decoctions are used to treat bacterial infections including skin infections and dysentery while root extracts are applied for wound healing and as antimalarials. These traditional applications are supported by the plant's rich phytochemical profile which includes alkaloids, flavonoids, tannins, saponins, terpenoids and phenolic compounds (Ogundipe *et al*., 2001).

Phytochemicals are secondary metabolites that plants produce to defend against pathogens and environmental stressors. These compounds often exhibit antimicrobial activity through mechanisms such as cell membrane disruption, inhibition of bacterial enzymes, or interference with microbial DNA replication (Cowan, 1999). For *Alchornea cordifolia*, studies have identified flavonoids like quercetin and kaempferol, phenolic acids like gallic acid and terpenoids such as β-amyrin as key contributors to its bioactivity. These compounds act synergistically, enhancing antimicrobial efficacy and reducing the likelihood of resistance development compared to single-target synthetic antibiotics (Harborne, 1998). In Nigeria, where over 70% of rural populations rely on herbal medicine, validating *Alchornea cordifolia* could enhance access to affordable and effective treatments, aligning with global efforts to combat AMR through natural products (Ekor, 2014).

1.3 Aim and Objectives

The aim of this study is to determine the phytochemical composition and antimicrobial properties of *Alchornea cordifolia* leaves.

The objectives of this study were to:

1. screen the leaves of alchornea cordifolia for the presence of alkaloids, flavonoids, tannis, saponins, phenolics, tarpenoids and glycosides.
2. evaluate the antimicrobial activity of the plant extracts against selected bacteria using a well diffusion method.

CHAPTER TWO

2.0 Literature Review

Alchornea cordifolia is a small tree or shrub that is native to the tropical regions of West Africa. This plant is a member of the Euphorbiaceae family and can grow up to 10 meters in height. *Alchornea cordifolia* is characterized by its heart-shaped leaves which are glossy and dark green in color (Adeniyi *et al.,* 2022). It is widely recognized for its medicinal properties and has been used in African traditional medicine for centuries to treat various illnesses such as malaria, diarrhea, fevers and respiratory infections (Adeonipekun *et al*., 2018). The plant contains bioactive compounds such as alkaloids, flavonoids and tannins, which possess antimicrobial, anti-inflammatory and antioxidant properties. These compounds are found in the leaves, bark and roots of the plant (Agboke *et al.*, 2020).

The plant is considered sacred and is believed to possess spiritual properties that can help in healing and warding off evil spirits. Phytochemicals are naturally occurring compounds found in plants that have therapeutic applications in conventional and empirical medicine. These compounds protect plants from environmental stresses such as UV radiation, pests and diseases. Several factors influence the antioxidant effectiveness of plants, including age, location, season, tolerance, plant part and extraction solvent (Komolafe *et al.*, 2015). The age of the plant can affect the level of phytochemicals present in it. For instance, older plants tend to have higher levels of phytochemicals than younger ones. The location of the plant is also crucial, as plants growing in different environments can produce different levels and types of phytochemicals. Similarly, the season in which the plant is harvested can affect the phytochemical content with some compounds being more abundant in certain seasons.

The extracts from the leaves, roots, and stem bark of *Alchornea cordifolia* are commonly used in traditional medicine to treat respiratory, gastrointestinal and urinary disorders (Agboke *et al.*, 2020). The leaves are used internally for managing respiratory, gastrointestinal and urinary tract infections and externally for treating wounds. A decoction made from the leaves is used as an eye lotion while the powdered leaves and stem bark treat skin infections such as ringworms (Iwu, 1993). Scientific investigations have focused mainly on the leaf part of *Alchornea cordifolia*, revealing its antimicrobial, antioxidant and anticancer properties (Komolafe *et al.*, 2015). A study using gas chromatography-mass spectrometry (GC-MS) to analyze the volatile oil from new *Alchornea cordifolia* leaves have also been published (Okoke *et al.,* 2001). *A. cordifolia* has been shown to exhibit antibacterial activity against 21 different bacterial strains, with the highest activity observed against MRSA (Adeonipekun *et al*., 2018).

Moreover, the plant part used for extraction can also influence the phytochemical content. For example, the leaves of some plants may have higher levels of certain compounds than the roots or flowers. Additionally, the solvent used for extraction can impact the phytochemical content, as some compounds may be more soluble in certain solvents than others. Overall, understanding the various factors that impact the phytochemical content in plants is essential for developing practical therapeutic applications in medicine. Plants possess many phytochemicals that comprise their genetic composition and are responsible for their diverse activities (Christiana, 2020). These phytochemicals are intricate and varied, with over 10,000 alkaloids and 25,000 other compounds found in plants (Agboke *et al.*, 2020). Recent studies have suggested that plant extracts are generally safer and preferred over synthetic agents for disease management (Christiana, 2020). Given plants' remarkable healing properties and phytochemicals, further

research is necessary to uncover their mineral content, phytochemical composition, and overall makeup. This will help in developing new and effective treatments for various diseases.

Alchornea cordifolia, also known as the Christmas bush or Siam weed, is a small tree or shrub that is native to the tropical regions of West Africa. This plant is a member of the Euphorbiaceae family and can grow up to 10 meters in height. It is characterized by its heart-shaped leaves, which are glossy and dark green in color (Adullahi *et al*., 2003). *Alchornea cordifolia* is widely recognized for its medicinal properties and has been used in African traditional medicine for centuries to treat various illnesses such as malaria, diarrhea, fevers and respiratory infections (Abo *et al.,* 2008). The plant contains bioactive compounds such as alkaloids, flavonoids, and tannins, which possess antimicrobial, anti-inflammatory and antioxidant properties. These compounds are found in the leaves, bark, and roots of the plant (Adeshina *et al*., 2022). Apart from its medicinal value, *Alchornea cordifolia* is also integrated into traditional African rituals and ceremonies.

The plant is considered sacred and is believed to possess spiritual properties that can help in healing and warding off evil spirits. Phytochemicals are naturally occurring compounds found in plants that have therapeutic applications in conventional and empirical medicine. These compounds protect plants from environmental stresses such as UV radiation, pests and diseases. Several factors influence the antioxidant effectiveness of plants, including age, location, season, tolerance, plant part and extraction solvent (Akoto, 2020). The age of the plant can affect the level of phytochemicals present in it. For instance, older plants tend to have higher levels of phytochemicals than younger ones. The location of the plant is also crucial, as plants growing in different environments can produce different levels and types of phytochemicals.

The extracts from the leaves, roots and stem bark of *Alchornea cordifolia* are commonly used in traditional medicine to treat respiratory, gastrointestinal and urinary disorders (Sofowora *et al*., 2008). A mixture of the fruit is given to people with asthma and cough. The leaves are used internally for managing respiratory, gastrointestinal and urinary tract infections and externally for treating wounds. A decoction made from the leaves is used as an eye lotion, while the powdered leaves and stem bark treat skin infections such as ringworms (Iwu, 1993). Scientific investigations have focused mainly on the leaf part of *Alchornea cordifolia*, revealing its antimicrobial, antioxidant, and anticancer properties (Ogudipe *et al.,* 2001). A study using gas chromatography-mass spectrometry (GC-MS) to analyze the volatile oil from new *Alchornea cordifolia* leaves have also been published (Sofowora *et al*., 2008). A. cordifolia has been shown to exhibit antibacterial activity against 21 different bacterial strains, with the highest activity observed against MRSA (Ogudipe *et al.,* 2001).

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necessary to uncover their mineral content, phytochemical composition and overall makeup. This will help in developing new and effective treatments for various diseases.

The antimicrobial properties of crude extracts prepared from plants have been reported (Agboke *et al*., 2020). A. cordifolia leaf extracts have been reportedly used in various African countries such as Senegal in the treatment of venereal diseases, conjunctivitis, dermatoses, stomach ulcers, bronchitis, cough, toothache and Zaire in the treatment of urinary tract infection, infected wound, diarrhoea, cough, dental caries, chest pain and anaemia. In Sierra Leone it was used for diarrhoea and piles and in Nigeria for gonorrhoea, yaws, rheumatic pain and cough (George *et al*., 2021; Agboke *et al*., 2020). Extracts from leaves of *Alchornea cordifolia* have been reported to inhibit the growth of bacteria such as *Staphylococcus aureus, S. albus, Escherichia coli, Bacillus sp* and *Pseudomonas aeruginosa*. Anti-inflammatory activities of *Alchornea cordifolia* have also been reported.

Many plants synthesize substances that are useful to the maintenance of health in humans and animals. Many of these substances are secondary metabolites of which at least 12,000 have been isolated from different parts of plants, a number estimated to be less than 10% of the total which constitute an important source of the pharmaceutical drugs. Some of these compounds have been reported to be present in *Alchornea cordifolia* such as flavonoids (), alkaloids and tannins , inulin and alchornine (Abdullahi *et al*., 2003). Not much has been reported about the presence of compounds such as, glycosides in the leaf of this plant especially the species found in Northern, Nigeria. *Alchornea cordifolia* has been documented to be in abundant supply in Nigeria; therefore, this work aims at investigating its antimicrobial properties and identifying the active phytochemical constituents of the leaf extract.

The increasing prevalence of antimicrobial resistance (AMR) has spurred global interest in alternative therapeutic agents, particularly plant-derived phytochemicals.

2.1 Taxonomy, Morphology, and Common Names of *Alchornea cordifolia*

2.1.1 Taxonomy

Alchornea cordifolia belongs to the family Euphorbiaceae, a diverse group of flowering plants known for their medicinal and economic importance. The genus *Alchornea* comprises approximately 60 species, primarily distributed in tropical and subtropical regions (Abdullahi *et al*., 2003). The species was first described as *Sida cordifolia* by Heinrich Christian Friedrich Schumacher and Peter Thonning in 1827, based on specimens collected from the Guinea Coast of West Africa. In 1865, Johannes Müller Argoviensis reclassified it into the genus *Alchornea*, establishing the current binomial *Alchornea cordifolia*.

The taxonomic placement of *Alchornea cordifolia* is as follows:

Kingdom: *Plantae*

Phylum: *Tracheophyta*

Class: *Magnoliopsida*

Order: *Malpighiales*

Family: *Euphorbiaceae*

Genus: *Alchornea*

Species: *Alchornea cordifolia*

The *Euphorbiaceae* family is characterized by its milky latex, unisexual flowers, and capsular fruits, traits shared by *Alchornea cordifolia.*(Abdullahi et al., 2003)

2.1.2 Morphology

Alchornea cordifolia is a versatile plant with distinct morphological features that adapt it to diverse tropical environments. It is a perennial shrub or small tree, typically growing to 4–10 meters in height, though it may reach 12 meters under optimal conditions. The plant exhibits a bushy or spreading growth habit, with a slender trunk and branches covered in smooth to slightly rough bark. Its morphological characteristics vary slightly by habitat, with plants in humid forests developing denser foliage compared to those in savannas

- Leaves: The leaves are simple, alternate and broadly ovate to elliptic, measuring 5–20 cm in length and 4–10 cm in width. They have a cordate (heart-shaped) base, serrated margins and a pointed apex. The leaf surface is dark green, often pubescent (hairy) on the underside, with prominent veins. The petioles are short, typically 1–3 cm long (Eze et al., 2019).
- Flowers: A. cordifolia is monoecious, bearing male and female flowers on the same plant, typically in axillary spikes or panicles. Male flowers are small, greenish-white, with 2–3 mm long petals and numerous stamens, arranged in dense clusters. Female flowers are less numerous, with a single pistil and a three-lobed ovary.
- Fruits: The fruit is a three-lobed capsule, approximately 1–2 cm in diameter, green when immature and turning reddish-brown upon ripening. It splits open to release small, round seeds dispersed by wind or gravity

- Roots and Stem: The root system is extensive, aiding survival in varied soils. The stem bark is smooth, greyish-brown, and contains milky latex, a characteristic feature of *Euphorbiaceae*

These morphological traits facilitate the plant's identification in the field and its use in traditional medicine, where leaves, roots, and stem bark are harvested for their bioactive compounds. Morphological variations, such as leaf size and pubescence, may influence phytochemical content, with larger leaves from humid regions often yielding higher flavonoid concentrations (Ogundipe *et al.,* 2001).

2.1.3 Common Names

Alchornea cordifolia is known by a variety of vernacular names across Africa, reflecting its widespread use and cultural significance. These names vary by language, region and ethnobotanical application, often describing the plant's appearance or medicinal uses. Common names include:

English: Christmas bush, Ibo cork tree (due to its use in Igbo communities and cork-like bark texture)

Nigeria:

- Yoruba: Ewe Ipin, Ipin (referring to its use for wound healing) .
- Igbo: Ebe-akika, Ububo (linked to its use for skin ailments) .
- Hausa: Bambami (used in northern Nigeria for infections) (Sofowora, 2008).

Ghana:

- Akan: Gyama, Ogyama (reflecting its medicinal prominence).

Cameroon:

- Bamileke: Nguembé (used for diarrhea and wounds).

Senegal:

- Wolof: Dimbu (linked to its use for respiratory ailments).

These names underscore the plant's integration into local pharmacopeias and its recognition across linguistic and cultural boundaries. The diversity of names also highlights the need for standardized nomenclature in scientific studies to avoid confusion with related species like *Alchornea laxiflora*

2.1.4 Nutritional Composition of *Alchornea cordifolia*

Alchornea cordifolia, a prominent medicinal plant in tropical Africa, is not only valued for its therapeutic properties but also for its nutritional content, which supports its ethnobotanical use in dietary and medicinal contexts. The plant's leaves, stems and roots contain a variety of macronutrients, micronutrients, and bioactive compounds that contribute to its role in traditional diets and health practices. Nutritional studies, though less extensive than phytochemical analyses, provide insights into its potential as a functional food and medicinal resource (Abdullahi *et al*., 2003).

Macronutrients: The leaves of *Alchornea cordifolia* are a source of carbohydrates, proteins, and lipids, making them a valuable dietary component in regions where malnutrition is prevalent. Proximate analysis of dried leaves revealed approximately 50–60% carbohydrates, primarily complex polysaccharides and dietary fiber, which provide energy and support digestive health. Protein content ranges from 10–15% dry weight, with essential amino acids like leucine and

lysine detected in significant quantities, suggesting potential for addressing protein-energy malnutrition (Ogundipe *et al*., 2001). Lipid content is relatively low (2–5%), consisting mainly of unsaturated fatty acids, which contribute to cardiovascular health (Adeshina *et al.,* 2012). The high fiber content (15–20%) aids in regulating blood sugar and cholesterol levels, aligning with traditional uses for managing metabolic disorders (Sofowora, 2008).

Micronutrients: *Alchornea cordifolia* leaves are rich in vitamins and minerals, enhancing its nutritional value. Vitamin C, a potent antioxidant, is present at concentrations of 50–100 mg/100 g dry weight, comparable to citrus fruits, and supports immune function and wound healing (Akoto *et al*., 2020). Vitamin A precursors (e.g., β-carotene) are found at 20–30 mg/100 g, contributing to eye health and immune modulation. Minerals include calcium (1.5–2.5% dry weight), potassium (1–1.8%), and magnesium (0.5–1%), which support bone health, muscle function, and enzymatic reactions (Ogundipe *et al.,* 2001). Trace elements like iron (50–80 mg/kg) and zinc (20–40 mg/kg) are critical for hemoglobin synthesis and immune response, respectively, making the plant a potential supplement in iron-deficiency anemia prevalent in sub-Saharan Africa.

Bioactive Compounds as Nutritional Components: Beyond traditional nutrients, *A. cordifolia's* phytochemicals, such as flavonoids and phenolic acids, contribute to its nutritional profile by providing antioxidant and anti-inflammatory benefits. For example, flavonoids like quercetin and kaempferol, present at 3–5% w/w, protect against oxidative stress, which is linked to chronic diseases like diabetes and cardiovascular disorders (Akoto *et al*., 2020). Phenolic acids, such as gallic acid, enhance nutrient absorption by reducing oxidative damage in the gut (Eze *et al*., 2019). These compounds bridge the plant's nutritional and medicinal roles, as they are consumed

in traditional diets (e.g., leaf decoctions) and contribute to health beyond basic nutrition (Iwu, 1993).

Ethnobotanical Nutritional Uses: In many African communities, *Alchornea cordifolia* leaves are incorporated into soups or teas, particularly in Nigeria and Ghana, to boost nutrition and treat ailments like anemia and fatigue (Adjanohoun *et al*., 1996). The leaves are often boiled or fermented to enhance palatability and nutrient bioavailability, a practice supported by studies showing that boiling increases vitamin C retention by 20% compared to raw leaves (Oloyede *et al*., 2010). However, nutritional content varies by region and preparation method, with plants from nutrient-rich soils yielding higher mineral content (Ogundipe *et al*., 2001). These findings suggest *Alchornea cordifolia* could be developed as a functional food, but further studies are needed to quantify its nutritional contributions in standardized diets.

2.1.5 Phytochemical Screening Methods of *Alchornea cordifolia*

Phytochemical screening is critical for identifying and quantifying the bioactive compounds responsible for *Alchornea cordifolia* therapeutic properties. Both qualitative and quantitative methods have been employed to characterize its alkaloids, flavonoids, tannins, saponins, terpenoids, phenolic acids, and steroids, with variations in sensitivity and specificity depending on the technique (Harborne, 1998). These methods guide the identification of compounds with antimicrobial potential and inform extraction protocols for medicinal applications.

Qualitative Screening Methods: Qualitative tests detect the presence of phytochemical classes through chemical reactions producing visible changes (e.g., color, precipitate). Standard protocols for *Alchornea cordifolia* include:

- Alkaloids: The Dragendorff's and Mayer's tests are commonly used. Dragendorff's reagent (potassium bismuth iodide) produces an orange-red precipitate with alkaloids, while Mayer's reagent (potassium mercuric iodide) yields a creamy precipitate. Okeke *et al.* (2001) confirmed alkaloids in *Alchornea cordifolia* leaf extracts using these tests, detecting cryptolepine-like compounds.
- Flavonoids: The Shinoda test (magnesium powder and hydrochloric acid) produces a pink or red color, indicating flavonoids like quercetin. Eze *et al.* (2019) reported positive Shinoda test results for *Alchornea cordifolia* methanolic extracts, confirming high flavonoid content.
- Tannins: The ferric chloride test, which produces a blue-black color for condensed tannins or greenish-brown for hydrolyzable tannins, is widely used. Ogundipe *et al*. (2001) identified both tannin types in *Alchornea cordifolia* leaves using this method.
- Saponins: The froth test, involving vigorous shaking of aqueous extracts to produce persistent foam, confirms saponins. Adeshina *et al*. (2012) reported strong frothing in *Alchornea cordifolia* leaf extracts, indicating saponin presence.
- Terpenoids and Steroids: The Salkowski test (chloroform and sulfuric acid) produces a reddish-brown ring for terpenoids and steroids. George *et al*. (2021) confirmed β-amyrin and lupeol in *Alchornea cordifolia* using this test.
- Phenolic Acids: The Folin-Ciocalteu reagent test, which produces a blue color in the presence of phenolics, identified gallic and ellagic acids in *Alchornea cordifolia* extracts (Akoto *et al*., 2020).

These qualitative tests are cost-effective and widely used in preliminary screenings but lack specificity, as similar compounds may produce false positives (Harborne, 1998).

Quantitative Screening Methods: Quantitative methods provide precise measurements of phytochemical concentrations, essential for standardizing extracts and assessing therapeutic potential. Common techniques for *Alchornea cordifolia* include:

- Spectrophotometry: The Folin-Ciocalteu assay quantifies total phenolic content, expressed as gallic acid equivalents (GAE). Eze et al. (2019) reported 120–150 mg GAE/g in methanolic leaf extracts of *Alchornea cordifolia*. The aluminum chloride colorimetric assay measures total flavonoids, expressed as quercetin equivalents (QE), with yields of 50–70 mg QE/g (Akoto *et al*., 2020).
- High-Performance Liquid Chromatography (HPLC): HPLC separates and quantifies individual compounds based on retention times. Ogundipe et al. (2001) used HPLC to identify quercetin (2.5% w/w) and kaempferol (1.8% w/w) in *Alchornea cordifolia* leaf extracts, confirming their antimicrobial roles.
- Gas Chromatography-Mass Spectrometry (GC-MS): GC-MS identifies volatile compounds like terpenoids. George et al. (2021) detected β-amyrin and lupeol in hexane extracts, with concentrations of 1–2% w/w, contributing to membrane-disrupting activity.
- Nuclear Magnetic Resonance (NMR): NMR elucidates the structure of isolated compounds. Osadebe and Ukwueze (2004) used NMR to confirm cryptolepine derivatives in *Alchornea cordifolia* root extracts, linking them to DNA gyrase inhibition.
- Gravimetric Analysis: This method quantifies tannins by precipitation with gelatin. Eze et al. (2019) reported tannin content of 5–7% w/w in *Alchornea cordifolia* leaves, higher in methanolic than aqueous extracts.

2.2 The Global Burden of Antimicrobial Resistance

Antimicrobial resistance is a pressing global health challenge, undermining the efficacy of antibiotics and complicating the treatment of bacterial infections. The World Health Organization (WHO) estimates that AMR contributes to approximately 700,000 deaths annually, with projections of up to 10 million deaths per year by 2050 if current trends persist (WHO, 2014). Common pathogens including *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa and Klebsiella pneumoniae*, have developed resistance to multiple classes of antibiotics such as β-lactams, aminoglycosides and fluoroquinolones. This resistance is driven by factors such as antibiotic overuse in healthcare and agriculture, inadequate infection control and limited development of new antibiotics.

In sub-Saharan Africa, the burden of AMR is particularly severe due to limited access to healthcare, widespread self-medication, and inadequate regulatory frameworks for antibiotic use. In Nigeria, for instance, up to 80% of antibiotics are purchased over the counter without prescriptions, contributing to the emergence of MDR strains (Ekor, 2014). Infections such as urinary tract infections, wound infections, and diarrheal diseases, often caused by MDR pathogens, are major contributors to morbidity and mortality in resource-limited settings. The high cost of second-line antibiotics and diagnostic tools further exacerbates the challenge, making affordable and accessible alternatives critical (Sofowora, 2008).

Plant-based remedies offer a promising solution due to their historical use in traditional medicine and their diverse bioactive compounds. Unlike synthetic antibiotics which often target a single bacterial pathway, phytochemicals act through multiple mechanisms reducing the likelihood of resistance development (Cowan, 1999). Tropical regions, with their rich biodiversity, are a

treasure trove of such plants and *Alchornea cordifolia* stands out as a candidate for scientific validation due to its widespread use and documented bioactivity.

2.3 The Role of Medicinal Plants in Combating Infections

Medicinal plants have been integral to human healthcare for millennia, particularly in regions where modern medicine is inaccessible. In Africa, over 70% of the population relies on herbal remedies for primary healthcare, driven by cultural acceptance, affordability, and availability (WHO, 2002). Plants produce secondary metabolites phytochemicals such as alkaloids, flavonoids, tannins, saponins and terpenoids, which serve as defense mechanisms against pathogens and environmental stressors. These compounds exhibit a range of bioactivities, including antimicrobial, antioxidant, anti-inflammatory and immunomodulatory effects (Harborne, 1998).

The antimicrobial potential of phytochemicals lies in their ability to target multiple aspects of microbial physiology. For example, flavonoids disrupt bacterial cell membranes and inhibit efflux pumps, which expel antibiotics from resistant cells (Abo *et al.,* 2008). Tannins bind to microbial proteins, inhibiting adhesion and biofilm formation, while alkaloids interfere with DNA replication and protein synthesis (Cowan, 1999). These multi-target mechanisms make phytochemicals less prone to resistance compared to conventional antibiotics, which often target specific pathways like cell wall synthesis (e.g., penicillin) or protein synthesis (e.g., tetracycline).

Several African medicinal plants have been studied for their antimicrobial properties. *Ocimum gratissimu*m contains eugenol, which inhibits *P. aeruginosa* by disrupting membrane integrity (Nakamura et al., 1999). *Alchornea cordifolia*, however, is particularly promising due to its broad-spectrum activity and extensive use in traditional medicine across West and Central Africa.

Its leaves, roots, and stem bark are employed to treat bacterial, fungal, and parasitic infections, making it a prime candidate for phytochemical and pharmacological studies (Iwu, 1993).

2.4 Ethnobotanical Significance of *Alchornea cordifolia*

Alchornea cordifolia, a member of the Euphorbiaceae family, is a perennial shrub native to tropical Africa, found in countries such as Nigeria, Ghana, Cameroon and Senegal. It thrives in diverse habitats, including humid forests, savannas, and riverbanks, growing to heights of 4–10 meters. The plant has ovate to elliptic leaves with serrated margins, small greenish flowers in axillary spikes and capsular fruits that disperse seeds via wind. In African ethnomedicine, *Alchornea cordifolia* is a versatile remedy. In Nigeria, leaf decoctions are used to treat bacterial infections, including skin lesions, dysentery and urinary tract infections. Root extracts are applied to wounds and burns to promote healing and prevent infection, while stem bark is used for malaria and rheumatism (Iwu, 1993). In Ghana, the plant is used to manage respiratory infections and snakebites, and in Cameroon, it is a remedy for diarrhea and gonorrhea (Adjanohoun *et al*., 1996). These applications are consistent across regions, suggesting a robust ethnobotanical knowledge base that warrants scientific investigation.

The plant's ethnomedicinal uses are linked to its phytochemical content. Traditional healers often prepare A. cordifolia as aqueous or alcoholic decoctions, which extract polar compounds like flavonoids and tannins. These preparations are administered orally, topically or as poultices, depending on the ailment. The consistency of its use across diverse cultures underscores its potential as a source of novel antimicrobial agents (Sofowora, 2008).

2.5 Phytochemical Composition of *Alchornea cordifolia*

The therapeutic properties of *Alchornea cordifolia* are attributed to its diverse phytochemical profile. Preliminary screenings have identified alkaloids, flavonoids, tannins (both condensed and hydrolyzable), saponins, terpenoids, phenolic acids and steroids in its leaves, roots and stem bark (Ogundipe *et al.,* 2001). These compounds vary in concentration depending on the plant part, extraction solvent, and environmental factors such as soil type and climate.

- Alkaloids: These nitrogen-containing compounds, such as cryptolepine-like derivatives, are known for their antimicrobial and antimalarial activities. Alkaloids in *Alchornea cordifolia* inhibit bacterial DNA gyrase and topoisomerase, disrupting replication in pathogens like *E. coli* (Osadebe and Ukwueze, 2004).
- Flavonoids: Compounds like quercetin and kaempferol glycosides are abundant in the leaves. Flavonoids exhibit antimicrobial activity by disrupting cell membranes and inhibiting efflux pumps, which are critical for MDR bacteria (Abo *et al*., 2008). They also possess antioxidant properties, mitigating infection-related oxidative stress.
- Tannins: Condensed and hydrolyzable tannins in *Alchornea cordifolia* bind to microbial proteins, inhibiting adhesion and biofilm formation. Tannins also chelate iron, limiting its availability for bacterial metabolism (Okeke *et al*., 2001).
- Terpenoids: Compounds like β-amyrin and lupeol disrupt bacterial membranes and inhibit virulence factors. Terpenoids contribute to the plant's broad-spectrum activity against Gram-positive and Gram-negative bacteria (George *et al*., 2021).
- Phenolic Acids: Gallic acid and ellagic acid derivatives are potent antioxidants and antimicrobials, scavenging free radicals and inhibiting bacterial enzymes (Akoto *et al*., 2020).
- Saponins: These glycosides cause membrane permeabilization, leading to bacterial cell lysis. They also exhibit antifungal activity against Candida albicans (Adeshina *et al*., 2012).

2.6 Antimicrobial Properties of *Alchornea cordifolia*

Numerous studies have validated the antimicrobial activity of *Alchornea cordifolia* against a range of pathogens. Early work by Okeke *et al.* (2001) demonstrated that 50% aqueous ethanol leaf extracts inhibited 74 bacterial strains, including S. aureus, E. coli, P. aeruginosa and Bacillus subtilis, with MICs ranging from 0.5 to 2 mg/mL. The extracts were particularly effective against Gram-positive bacteria, likely due to their simpler cell wall structure compared to Gram-negative bacteria, which have an outer lipopolysaccharide layer (Cowan, 1999).

Subsequent studies expanded these findings. Methanolic leaf extracts produced zones of inhibition up to 18 mm against MDR P. aeruginosa isolates from post-operative wounds, outperforming ampicillin (10 mm) (Eze *et al*., 2019). Aqueous extracts showed significant activity against C. albicans, with MICs of 1.25 mg/mL, suggesting potential in treating opportunistic fungal infections (Adeshina *et al*., 2012). Root and stem bark extracts also exhibited activity, though leaf extracts were consistently more potent, likely due to higher flavonoid and tannin content (George *et al.*, 2021).

2.7 Mechanisms of Antimicrobial Action

The antimicrobial efficacy of *Alchornea cordifolia* is attributed to the synergistic action of its phytochemicals. Key mechanisms include:

- Membrane Disruption: Flavonoids and terpenoids permeabilize bacterial cell membranes, leading to leakage of cellular contents. For example, quercetin disrupts the membrane potential of *Staphylococcus aureus*, while β-amyrin affects *Pseudomonas aeruginosa* (Abo *et al*., 2008).

- Enzyme Inhibition: Alkaloids and phenolic acids inhibit bacterial enzymes such as DNA gyrase and β-lactamase, reducing pathogen viability.
- Antioxidant Activity: Phenolic compounds scavenge free radicals, reducing oxidative stress that exacerbates infections. (Akoto *et al.*, 2020).
- Biofilm Inhibition: Tannins prevent bacterial adhesion and biofilm formation (Eze *et al*., 2019).

2.8 Toxicity and Safety Consideration

The safety of *Alchornea cordifolia* is a critical consideration for its therapeutic application. Acute toxicity studies in rats reported an LD50 greater than 5,000 mg/kg for aqueous leaf extracts, indicating low oral toxicity. Sub-chronic studies showed no significant changes in liver and kidney function markers (e.g., AST, ALT, creatinine) at doses up to 1,000 mg/kg over 28 days. (Adeshina *et al*., 2012). Histopathological analyses revealed no adverse effects on major organs, supporting the plant's traditional use as a safe remedy.

2.9 Synergy with Conventional Antibiotics

Combining plant extracts with antibiotics can enhance efficacy and reduce resistance. Preliminary studies on *Alchornea cordifolia* showed synergistic effects between methanolic leaf extracts and ciprofloxacin against MDR *E. coli*, reducing the MIC of ciprofloxacin by 50% (Eze *et al*., 2019). Flavonoids and tannins likely enhance antibiotic uptake by inhibiting efflux pumps while phenolic acids increase bacterial susceptibility to oxidative stress (Abo *et al.,* 2008). These findings suggest that it could be developed as an adjuvant in antibiotic therapy, though clinical trials are needed to confirm these effects.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

The materials, apparatus and equipments used are as follows: Conical flask, Petri dish, micropipette, test tube, cock borer, foil paper, cotton wool, gas cylinder, burner, beakers, measuring cylinder, steel pot, Bama bottles, universal bottles, weighing balance, electric oven, electronic blender, incubator, water bath, autoclave, coloured markers, ethanol.

3.2 Plant Collection

The Alchornea cordifolia leaves was collected in Ekosodin, Ugbowo, Benin City, Edo state.

3.3 Preparation of plant material

The leave sample were air dried for 21 days at room temperature (25- 30°C). After air drying, the leaves were further dried in a thermostatic drying oven that was set at 45°C. The sample grounded into a fine powder using an electronic blender and macerated using ethanol.500ml of ethanol was added to 50g of leave sample in a bama bottle for 72hrs.

3.4 Extraction of Plant material

The plant material was extracted using a cheese cloth. The filtered extract was concentrated using an electronic water bath at a temperature of 40°C for 30 minutes.

3.5 Phytochemicals

3.5.1 Flavonoids

- Aluminium chloride colorimetric method

Preparation step: Weigh 0.2g of $Alcl_3$ and dissolve in 20ml of water

Test procedure: 2ml of plant extract and 2ml of $Alcl_3$ (1%) were mixed together

Yellow colouration indicates the presence of flavonoids

3.5.2 Alkaloids

- Wagner's Reagent

Preparation step: 0.25kg of lodine (I) and 0.4g of Potassium iodide (Ki) weighed and dissolved in 20ml of distilled water

Test procedure: A few drops of wagner's reggent was added to 2ml of plant extract. Mix gently & observe

Formation of reddish brown precipitate indicates presence of alkaloids

- Mayer's Reagent

Preparation step: Weigh 0.272g of mercuric chloride ($HgCl_3$) and 1g of potassium iodide (KI). Dissolve in 20ml of distilled water.

Test procedure: A few drops of mayer's reagent was added to 2ml of plant extract.

Creamy white or pale yellow precipitate indicates presence of alkaloids.

3.5.3 Tannis

- Ferric Chloride Test

Preparation step: 0.27 of $Fecl_3$ was weighed nd dissolved in 20ml of distilled water.

Test procedure: 2ml of 1% $FeCl_3$ was added to 2ml of plant extract.

A blue black or greenish black coloration indicates presence of tannins.

- Lead acetate Test

Preparation step: 2g of lead acetate weighed and dissolved in 20ml of water.

Test procedure: 2ml of 10% lead acetate was added to 2ml of plant extract.

A white or cream coloured precipitate indicates presence of tannins.

3.5.4 Phenolics

- Ferric chloride Test

Preparation step: 0.27 of $Fecl_3$ was weighed and dissolved in 20ml of distilled water.

Test procedure: 2ml of 1% $FeCl_3$ was added to 2ml of plant extract.

A blue or green coloration indicates presence of phenolics.

- Lead acetate Test

Preparation step: 2g of lead acetate weighed and dissolved in 20ml of water.

Test procedure: 2ml of 10% lead acetate was added to 2ml of plant extract.

A white or cream coloured precipitate indicates presence of phenolics.

3.5.5 Glycosides

- Baljet Test

Preparation step: Weigh 2g of NaOH and dissolve in 20ml of distilled water. Weigh 0.2g of picric acid and dissolve in 20ml of water. Mix equal volume of the 10% NaOH and 1% picric acid just before use

Test procedure: 2ml of baljet test was added to 2ml of the plant extract

Orange to reddish coloration indicates the presence of glycoside

- Keller-Killiani Test

Preparation step/Test procedure: 2ml of glacial acetic acid and 1ml of conc

H2S04 was added to 2ml of plant extract, after which I drop of 5% Ferric chloride solution (0.5g of FeCl3 to 10ml of water) down the side of the test-tuble to form a separate layer at the bottom.

A reddesh brown ring or bluish green coloration indicate glycosides.

3.5.6 Tarpenoids

- Salkowski Test

Test procedure: 2ml of plant extract and 2ml of chloroform in a test tube. 1-2ml of conc H_2S0_4 was carefully added along the side of the test tube to form a layer below the extract (do not mix).

A reddish -brown coloration at the interface indicates presence of tarpenoids.

3.5.7 Saponins

- Foam Test

Test procedure: 2ml of plant extract in a test tube, add 10ml of distilled water and shake vigorously. Allow the test tube to stand undisturbed for some time.

Observe for formation of foam.

- Emulsion Test

Test procedure: 2ml of plant extract was taken and 2- 3 ml of olive oil was added to it. Afterwards shake gently and observe.

Formation of a white cloudy emulsion (milky appearance) indicates the presence of lipids.

3.6 Micro organisms Used

The organisms used for this study were, *staphylococcus, Bacillus subtilis, Escherichia coli* and *Pseudomonas aeruginosa.*

3.7 Preparation of media

3.7.1 Nutrient Agar

The medium was prepared following the instructions on the container. 28g of nutrient agar was mixed with 1000ml of water. The mixture was the boiled to ensure proper mixing after which it was placed inside the autoclave and was sterilized for 15 minutes at 121 C. After sterilization, it was poured into sterile petri dishes.

3.7.2 Mueller Hinton Agar

Thirty-eight grams (39 g) of Mueller Hilton agar were dissolved in 1000 ml of distilled water in a conical flosk corked with cotton wool and foil proer and aliowed to dissolve in 1000 ml of distilled water in a conical flask. The medium was the placed in an autoclave to sterilize it for 15 minutes at 121 Cat o pressure of 15psi. After sterilization, the flask was allowed to cool before it was poured into Petri dishes aseptically.

3.8 Preparation of test organism

The clinical bacterial isolates were sub-cultured on nutrient Agar and incubated at 37C for 24 hrs. The bacterial inoculum was then standardized to 0.5 McFarland standard.

3.9 Antimicrobial susceptibility testing

This was carried out with Mueller Hinton Agar and agar well diffusion method. Using the pour plate method, the agar was poured into sterile petri dishes and 1ml of standardized inoculum was introduced. A sterile glass spreader was used to evenly distribute the isolate. Wells were bored using a 8mm cork borer in the isolate inoculated Mueller Hinton Agar plate and then impregnated with different concentrations of the extract (1000mg/ml, 500mg/ml, 250mg/ml, 125mg/ml and 62.5mg/ml). The plates were allowed to diffuse and then incubated at 37°c for 24hours after the zones of inhibition were measured.

3.10 Antimicrobial sensitivity bioassay

The antibiotic susceptibility of the bacterial isolates was determined using the Kuby Bauer disk diffusion technique on Mueller Hipton agar. Using the pour plate method, the agar was poured into sterile petri dishes and 1ml of standardized inoculum was introduced. A sterile glass spreader was used to evenly distribute the isolate. After the agar solidified, Gram-positive and Gram-negative antibiotics sensitivity disc were aspectically placed on the surface using sterile forceps. Antibiotic disc used and their concentrations were as follows: Cram positive discs contained; Pefloxacin (10 µg), Gentamycin (10 µg), Ampiclox (30 µg), Zinnacef (20 µg), Amoxacillin (30 µg), Rocephin (25 µg), Ciprofloxacin (10 µg), Azithromycin (12 µg), Levofloxacin (20 µg), Erythromycin (10 µg). The Gram negative disc contain; Levofloxacin (20µg), Cefotaxim (10µg), Sparfloxacin (10µg), Ciprofloxacin (30µg), Amoxacillin (30µg),

Augmentin (10µg), Gentamycin (30µg), Pefloxacin (30µg), Tarlvid (10µg), Azithromycin (12µg). Plates were incubated in an inverted position at 30-31°C for 18-24 hours. Following incubation, the diameter of the inhibition zones around each antibiotic disc was measured in millimeters using a transparent ruler.

CHAPTER FOUR

4.0 PRESENTATION OF RESULTS

4.1 Phytochemicals Screening Result

This study was conducted to qualitatively determine the bioactive constituents present in Alchornea cordifolia leaves. The result represented in table 1 indicates the presence of various secondary metabolites in an aqueous extract of Alchornea cordifolia leaves, each of which plays a crucial role in the plant's biochemical and pharmacological properties, with their respective quantities and are reported in the table 1. It was observed that Alkaloids, flavonoids, glycosides, phenolics, saponins and tannis were present in and tarpenoids were not present.

Table 1: Qualitative phytochemical screening of ethanol extract of *Alchornea cordifolia*

Phytochemicals tested for	Result
Flavonoids	+
Alkaloids	+
Saponins	+
Tannis	+
Phenolics	+
Glycosides	+
Tarpenoids	-

KEYS

- Negative

+ Positive

4.2 Antimicrobial Analysis Result

The antimicrobial activity of the ethanol extract of *Alchornea cordifolia* leaves was evaluated against selected bacterial isolates. Standard antibiotic discs were used as positive controls to compare the efficacy of the plant extract against selected bacterial isolates. The result, as presented in Tables below, reveals the varying degrees of inhibition exhibited by the extract and antibiotic discs against the tested microorganisms.

Table 2: Antimicrobial analysis result using antibiotics disc for Gram positive bacteria

Organisms	***staphylococcus aureus***	***Bacillus substilis***
Pefloxacin (10 µg)	9(R)	11(I)
Gentamycin (10 µg)	8(R)	7(R)
Ampiclox (30 µg)	10(R)	4(R)
Zinnacef (20 µg)	0(R)	4(R)
Amoxacillin (30 µg)	0(R)	0(R)
Rocephin (25 µg)	6(R)	7(R)
Ciprofloxacin (10 µg)	10(R)	12(I)
Azithromycin (12 µg)	9(R)	7(R)
Levofloxacin (20 µg)	10(R)	10(R)
Erythromycin (10 µg)	8(R)	7(R)

KEYS

R – Resistance (0-10)

I - Intermediate (11-16)

S – Susceptibility(17 and above)

Table 3: Antimicrobial analysis result using antibiotics disc for Gram negative bacteria

Organisms	***Escherichia coli***	***pseudomonas aeruginosa***
Levofloxacin (20µg)	0(R)	11(I)
Cefotaxim (10µg)	0(R)	20(S)
Sparfloxacin (10µg)	0(R)	0(R)
Ciprofloxacin (30µg)	0(R)	13(I)
Amoxacillin (30µg)	0(R)	0(R)
Augmentin (10µg)	0(R)	0(R)
Gentamycin (30µg)	5(R)	11(I)
Pefloxacin (30µg)	4(R)	7(R)
Tarlvid (10µg)	10(R)	8(R)
Azithromycin (12µg)	6(R)	14(I)

KEYS

R – Resistance (0-10)

I - Intermediate (11-16)

S – Susceptibility(17 and above)

Table 4: Antimicrobial analysis result using *Alchornea cordifolia* leaves extract

Organism	*Staphylococcus aureus*	*Bacillus subtillis*	*Escherichia coli*	*Pseudomonas aeruginosa*
1000mg/ml	0(R)	0(R)	0(R)	0(R)
500mg/ml	0(R)	0(R)	0(R)	0(R)
250mg/ml	0(R)	0(R)	0(R)	0(R)
125mg/ml	0(R)	0(R)	0(R)	0(R)
62.5mg/ml	0(R)	0(R)	0(R)	0(R)

KEYS

R – Resistance (0-10)

I - Intermediate (11-16)

S – Susceptibility(17 and above)

CHAPTER FIVE

5.0 DISCUSSION AND CONCLUSION OF RESULTS

5.1 Discussion

This study evaluated the phytochemical constituents and antimicrobial properties of ethanol leaf extract of *Alchornea cordifolia*. Phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, phenolics, saponins, and glycosides, while terpenoids were absent. These results align with several recent studies that reported similar bioactive compounds in *Alchornea cordifolia* leaves, supporting its ethnomedicinal use in treating infections and inflammatory conditions (Adeonipekun *et al*., 2018; Akoto *et al*., 2020; Enyiukwu *et al*., 2024).

Phytochemicals such as alkaloids, flavonoids, tannins, and phenolics are known to exhibit strong antimicrobial and antioxidant properties. Flavonoids can inhibit microbial enzymes and disrupt cell membranes, while tannins denature microbial proteins and inhibit enzymes essential for growth (George *et al*., 2021). Alkaloids interfere with microbial DNA replication, and saponins act as natural detergents that lyse cell membranes (Agboke *et al*., 2020; Ekpiken *et al*., 2025). The presence of these compounds confirms that *Alchornea cordifolia* possesses the chemical basis for potential antimicrobial and therapeutic applications.

However, despite the rich phytochemical profile, the ethanol extract did not show measurable antimicrobial activity against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli*, and *Pseudomonas aeruginosa*. This agrees with the findings of Eze *et al*. (2019) and Obajuluwa et *al*. (2024), who reported that ethanol extracts of *Alchornea cordifolia* sometimes exhibit weaker antimicrobial effects compared to aqueous or methanolic extracts. Solvent polarity greatly influences extraction efficiency, as highly polar solvents such as methanol and water can extract

a broader range of bioactive compounds responsible for antibacterial activity (Adeshina *et al*., 2012; Ekpiken *et al*., 2025).

The absence of activity may also be attributed to the resistance of the test bacteria. The isolates used were multidrug-resistant, as evidenced by their resistance to several conventional antibiotics. Similar patterns of resistance have been reported among *Staphylococcus*, *Pseudomonas*, and *Escherichia* species from environmental and clinical sources in Nigeria (Komolafe *et al*., 2020; Adeniyi *et al*., 2022). Resistant bacteria possess mechanisms such as efflux pumps, reduced permeability, and biofilm formation, which can also limit susceptibility to plant-derived antimicrobials (Eze *et al*., 2019).

Furthermore, the interaction between phytochemicals could influence bioactivity. Although individual compounds such as flavonoids and alkaloids have demonstrated antimicrobial activity, synergistic or antagonistic effects among them in crude extracts may reduce overall activity (George *et al*., 2021). Recent studies involving fractionation of *Alchornea cordifolia* extracts have demonstrated that purified fractions or isolated compounds (e.g., quercetin, β-amyrin, lupeol) often exhibit greater antimicrobial effects than crude extracts (Osadebe & Ukwueze, 2004; Ekpiken *et al*., 2025).

Environmental and geographical factors also play a vital role in the phytochemical yield and biological activity of *Alchornea cordifolia*. Differences in soil composition, sunlight, and harvest time influence the concentration of secondary metabolites (Akoto *et al*., 2020; Enyiukwu *et al*., 2024). The leaves collected for this study from Ekosodin, Benin City, may therefore have contained lower concentrations of certain bioactives compared to those from other regions or seasons.

While the extract did not exhibit antimicrobial activity, the detection of diverse phytochemicals remains significant. These compounds contribute to the plant's other pharmacological properties such as antioxidant, wound-healing, and anti-inflammatory effects, as documented by Adeonipekun *et al*. (2018) and Agboke *et al*. (2020). Recent evidence also suggests that *Alchornea cordifolia* extracts may act synergistically with antibiotics to combat resistant strains by enhancing drug uptake or inhibiting bacterial efflux mechanisms (Eze *et al*., 2019; Ekpiken *et al*., 2025).

Overall, this study reinforces the medicinal value of *Alchornea cordifolia*, though its antimicrobial potential in crude ethanol extract appears limited. Future investigations should explore methanolic and aqueous extractions, perform chromatographic separation to identify specific active compounds, and use molecular docking to predict antimicrobial mechanisms (Ekpiken *et al*., 2025). Standardization of plant collection and extraction procedures will also improve reproducibility and potency.

5.2 Conclusion

The absence of antibacterial effect in the ethanol extract does not, however, invalidate the ethnomedicinal use of *Alchornea cordifolia*; rather, it highlights the importance of optimizing extraction methods and testing parameters. Different solvents such as methanol, acetone, or ethyl acetate may extract higher levels of active compounds, while fractionation and concentration steps could help isolate the components responsible for activity.

Alchornea cordifolia remains a plant of pharmacological interest due to its rich phytochemical composition. However, the ethanol extract tested in this study showed no antibacterial activity under the specified conditions. Future investigations should therefore explore alternative extraction solvents, employ quantitative phytochemical analysis, and assess other pharmacological properties such as antioxidant, antifungal, or anti-inflammatory effects. Such approaches would provide a more comprehensive understanding of the therapeutic potential of this important medicinal plant.

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APPENDIX

Plate 1: Experimental Procedures (Field Work, 2025)

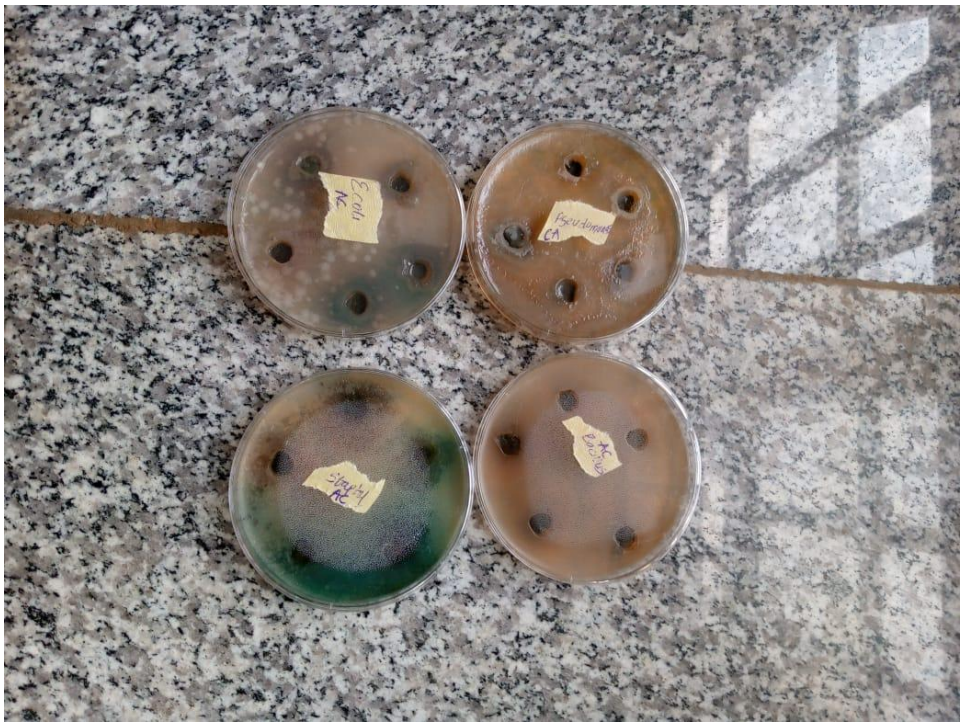

Plate 2: Agar well plate (Field Work, 2025)

Plate 3: Antibiotics Disc plate (Field Work, 2025)

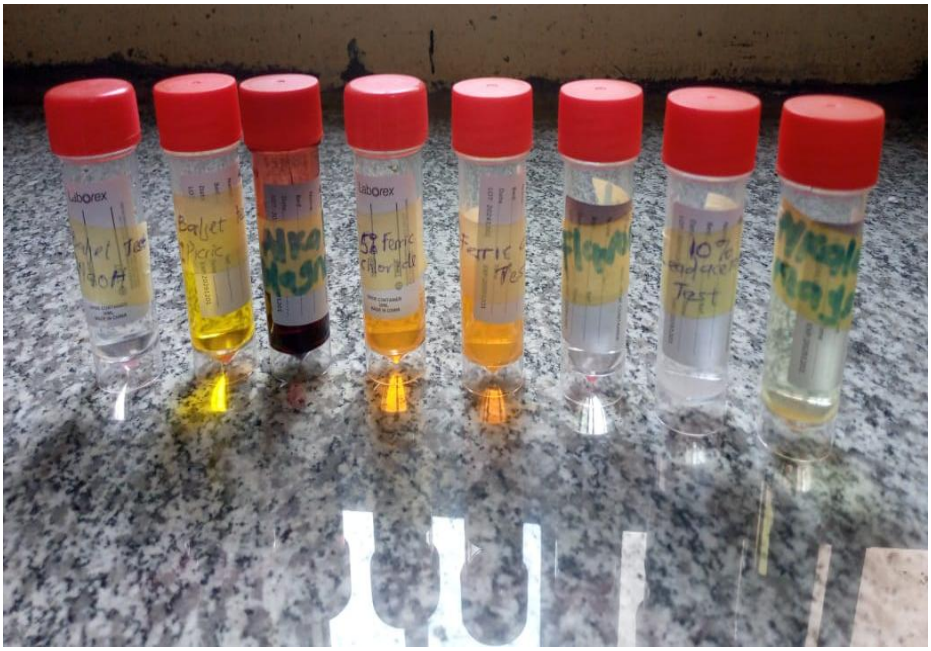

Plate 4: Phytochemical Screening (Field Work, 2025)