PHYTOCHEMICAL COMPOSITION AND ANTIBACTERIAL ACTIVITY OF *Cinnamomum tamala* EXTRACT AGAINST URINARY ISOLATES FROM UBTH, EDO STATE

BY

OVIGUEROYE OGHENEYOMA PROMISE

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DEPARTMENT OF MEDICAL LABORATORY SCIENCE
SCHOOL OF BASIC MEDICAL SCIENCES
COLLEGE OF MEDICAL SCIENCES
UNIVERSITY OF BENIN
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THIS PROJECT IS SUBMITTED TO:
THE DEPARTMENT OF MEDICAL LABORATORY SCIENCE,
SCHOOL OF BASIC MEDICAL SCIENCES
UNIVERSITY OF BENIN IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF BACHELOR OF MEDICAL LABORATORY SCIENCE DEGREE

SUPERVISOR:

DR.(MRS) N.A. OLISE

NOVEMBER, 2025

CERTIFICATION

This is to certify that this project work was satisfactory carried out by **OVIGUEROYE OGHENEYOMA PROMISE (MR)** with matriculation number: **BMS2001202** in Department of Medical Laboratory Science, University of Benin, Benin City, under my supervision in partial fulfillment for the award of Bachelor of Medical Laboratory Science (BMLS) Degree.

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(Head of Department)

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PROF. OMORUYI PIUS OMOSIGHO **DATE**
EXTERNAL EXAMINER

DEDICATION

This work is dedicated, first and foremost, to my Heavenly Father, the source of all wisdom, knowledge, and understanding, whose grace has sustained me throughout this journey. It is also lovingly dedicated to my beloved parents, Chief and Mrs. Godwin Ovigueroye, whose sacrifices, encouragement, and unwavering support have been my strongest foundation**.**

ACKNOWLEDGEMENTS

I acknowledge the Almighty God, the all-wise and all-knowing, from whom wisdom, knowledge, and strength come. I wish to express my profound gratitude to my supervisor, Dr. (Mrs.) Olise, for the professional guidance, patience, and encouragement given to me throughout the course of this work. My heartfelt thanks also go to the Head of Department, Dr. (Mrs.) Z. Omoruyi, for providing a conducive environment for academic excellence. To all my lecturers in the Department, it has indeed been a privilege to learn under your tutelage, and I am grateful for the knowledge imparted to me. I also appreciate the Department of Pharmaceutical Microbiology for granting me access to the laboratory facilities used in this research. My sincere appreciation goes to Mr. John Uwadia, who assisted and guided me during the laboratory aspect of this research. Special gratitude goes to my beloved parents, Chief And Mrs. Godwin Ovigueroye, for their unwavering support, sacrifices, encouragement, and prayers throughout my academic pursuit. I also extend my heartfelt thanks to my siblings: Ovigueroye Sophia, Ovigueroye Anita, Ovigueroye Destiny, and Ovigueroye Faith, for their constant encouragement, understanding, and love. I owe a very special appreciation to Jane Ovwigho, whose patience, guidance, and constant readiness to assist me provided clarity and encouragement during the writing of this work. Her contributions hold a truly valued place in my journey. I am equally grateful to Peace Kadiri and Anthony Michael Ikpeminogena, whose support, kindness, and willingness to help me in moments of confusion contributed greatly to the successful completion of this research. I also deeply appreciate my friends Ebenezer, Praise, Endurance, Raphael, Perfect, and Prosper for their steadfast support, encouragement, and companionship throughout this journey. Finally, my gratitude goes to everyone who, in one way or another, contributed to my academic journey and supported me throughout my stay in school up to the completion of this project.

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ABSTRACT

Urinary tract pathogens are increasingly resistant to conventional antibiotics, prompting interest in plant-derived bioactive agents. This study evaluated the phytochemical profile and antibacterial potential of ***Cinnamomum tamala*** bark extracts against selected clinical isolates. Dried bark samples were subjected to aqueous and ethanolic extraction, followed by phytochemical screening using GC–MS analysis. Antimicrobial activity was carried out using ditch plate and agar well diffusion methods, while minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values were determined via agar dilution techniques. The ethanolic extract demonstrated concentration-dependent inhibition, with zones of inhibition ranging from 8.25 ± 4.8 mm at 50 µg/mL to 21.75 ± 2.93 mm at 800 µg/mL, showing significant differences across concentrations ($p = 0.034$). The aqueous extract exhibited no effect at low concentrations but was active at higher concentration, producing inhibition zones up to 6.50 ± 3.77 mm, significantly different across groups ($p < 0.001$). MIC results indicated stronger activity for the ethanolic extract, particularly against ***E. coli*** (12.5 µg/mL), compared to the aqueous extract, which required higher concentrations (100–200 µg/mL) across organisms. Similarly, ethanolic MBC values ranged between 25–100 µg/mL, significantly lower than the consistent 200 µg/mL required for the aqueous extract. Phytochemical screening revealed alkaloids, flavonoids, tannins, terpenoids, and phenols in both extracts, while saponins and glycosides were exclusive to the aqueous extract, and steroids and resins were unique to the ethanolic extract. GC–MS analysis identified major constituents including Squalene (21.13%), 9-Octadecenoic acid (17.62%), and 13-Octadecenal (16.89%) in the ethanolic extract, while the aqueous extract was dominated by 9-Borabicyclo[3.3.1]nonane (28.24%) and Cyclopropane derivatives (17.04%). These findings highlight the potent antibacterial efficacy of ***C. tamala*** ethanolic extract, particularly against ***E. coli***, with activity linked to its terpenoid and fatty acid constituents. The results suggest that ***C. tamala*** may serve as a promising source of natural antimicrobials.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background To The Study

Antimicrobial resistance (AMR) has emerged as one of the most critical challenges confronting global health systems. The World Health Organization warns that, without effective interventions, drug-resistant infections may surpass all other causes of mortality worldwide by 2050 (WHO, 2019). This crisis is especially pronounced in Nigeria, where research has consistently documented high resistance rates among common urinary tract infection (UTI) pathogens such as *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*, many of which display multidrug resistance (MDR) (Onanuga *et al*., 2019). For instance, a 2024 investigation in Ado Ekiti found that over 90% of *E. coli* and *K. pneumoniae* isolates were resistant to amoxicillin–clavulanate, although all remained sensitive to imipenem (AO *et al*., 2024). UTIs rank among the most common bacterial infections in both primary care and hospital environments. Globally, uropathogenic *E. coli* accounts for 70–90% of these cases. In resource-limited locations such as Benin City, the increasing occurrence of MDR strains contributes to extended illness duration, higher treatment costs, and greater risk of complications (Onanuga *et al*., 2019). Given these concerns, there is heightened interest in identifying plant-derived antimicrobials as either replacements for or adjuncts to conventional antibiotics. Medicinal plants synthesize a diverse range of secondary metabolites including alkaloids, flavonoids, tannins, saponins, and phenolic acids that have demonstrated notable antibacterial properties under laboratory conditions (Abdulkadir *et al*., 2015). A phytochemical assessment of 50 medicinal plant species in Nigeria revealed that over half could inhibit the growth of pathogens such as *E. coli* and *Staphylococcus aureus*, largely due to these bioactive

constituents (Kubmarawa *et al*., 2007). *Cinnamomum tamala* (Buch.-Ham.) T. Nees and Eberm., commonly called Indian bay leaf and a member of the Lauraceae family, is native to the Indian subcontinent and certain Himalayan regions. The species has a long history of use in Ayurvedic and other traditional healing systems. Various plant parts including the leaves, bark, and essential oils are valued for medicinal purposes. The bark, in particular, is aromatic and has been traditionally applied as a stimulant, carminative, and antimicrobial agent. Its notable phytochemical profile includes cinnamaldehyde, eugenol, and tannins, compounds associated with both antibacterial and antioxidant effects (Hassan and Kazmi, 2015). While research has primarily focused on the essential oil and leaf extracts of *C.tamala*, recent studies have underscored the pharmacological promise of the bark. One investigation in the northwestern Himalayas identified cinnamaldehyde and eugenol as predominant bark constituents, with strong inhibitory activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Bacillus subtilis* (Heer *et al*., 2016). Another study demonstrated that both aqueous and ethanolic bark extracts were capable of suppressing the growth of *E. coli*, *S. aureus*, and *Salmonella typhi*, though resistance patterns varied across bacterial strains (Bharadwaj *et al*., 2022). Although these findings suggest that *C.tamala* possesses considerable antimicrobial potential, there remains limited targeted research on the efficacy of its bark extracts particularly aqueous and ethanolic preparations against clinical urinary isolates in African contexts such as Nigeria. This research will therefore address this gap by analyzing the phytochemical composition and antibacterial activity of *C.tamala* bark extracts against bacterial pathogens recovered from urine samples obtained at the University of Benin Teaching Hospital (UBTH), Edo State, Nigeria.

1.2 Statement of the Problem

Urinary tract infections (UTIs) are among the most frequently encountered bacterial infections worldwide, affecting individuals in both hospital and community settings. In Nigeria, uropathogens such as *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* are commonly implicated. For example, a study conducted in South West Nigeria analyzing 200 urine samples reported that *Klebsiella* species accounted for 40% of the isolates, followed by *E. coli* and *Staphylococcus aureus* at 25% each. Other identified pathogens included *Proteus* species (4 %), *Pseudomonas aeruginosa* (2.5 %), and *Enterococcus faecalis* (3.5 %) (*Antibiotic Resistance Pattern of Uropathogenic Escherichia coli in South West Nigeria*, 2011). Similarly, research conducted in Calabar revealed that *Klebsiella pneumoniae* was the predominant isolate at 23.1%, followed by *Staphylococcus* species (16.9%) and *Escherichia coli* (12.3%) (Bassey *et al.*, 2025). Numerous studies have highlighted alarming resistance rates among these pathogens to commonly prescribed antibiotics. In Ibadan, one study showed that 97% of uropathogens were resistant to ampicillin, while 98% were resistant to co-trimoxazole. Even more potent antibiotics such as ciprofloxacin, ceftriaxone, and amikacin showed only 64 to 75 percent effectiveness against the isolates (Dada-Adegbola *and* Muili, 2010). In Port Harcourt, multidrug-resistant *E. coli* strains were found to carry genes encoding extended-spectrum beta-lactamases (ESBLs) and quinolone resistance, with these resistance determinants detected in up to 88 percent of the isolates (Onanuga *et al.*, 2019). Similar findings have been reported in other parts of Nigeria, including Lafia, Enugu, and Calabar, confirming widespread resistance in both community and hospital environments (Bassey *et al.*, 2025). The growing ineffectiveness of conventional antibiotics, especially in urban tertiary healthcare institutions like the University of Benin Teaching Hospital (UBTH), emphasizes the urgent need

to explore alternative antimicrobial strategies. Medicinal plants are increasingly recognized for their potential in this regard, due to the presence of bioactive compounds such as flavonoids, alkaloids, tannins, and saponins, which have demonstrated antimicrobial activity in laboratory studies. *Cinnamomum tamala* , commonly known as Indian bay leaf, is one such medicinal plant. It has been used traditionally for various therapeutic purposes and has shown antibacterial activity in vitro (Heer *et al.*, 2016). However, despite its pharmacological promise, there is limited research assessing the antibacterial efficacy of *C.tamala*, particularly its aqueous and ethanolic bark extracts, against urinary tract pathogens isolated from patients in Nigeria. This lack of localized, clinical data hampers the development of plant-based therapeutic alternatives tailored to regional resistance patterns. This study seeks to address this critical knowledge gap by evaluating the antibacterial activity of aqueous and ethanolic bark extracts of *C.tamala* against multidrug-resistant urinary isolates obtained from patients at UBTH. The findings are expected to provide scientific evidence for the potential integration of *C.tamala* as a plant-based therapeutic option in the treatment of UTIs, especially in settings where conventional antibiotics are becoming increasingly ineffective.

1.3 Justification of the Problem

Urinary tract infections (UTIs) remain among the most common bacterial infections affecting individuals of all age groups (Barber *et al.*, 2013). However, the rising resistance of uropathogenic bacteria to frequently prescribed antibiotics has significantly complicated treatment strategies. A 2011 study conducted in southwestern Nigeria reported that 100% of *Escherichia coli* urinary isolates were resistant to amoxicillin-clavulanate, cotrimoxazole, and amoxicillin. Alarmingly high resistance was also observed against ofloxacin (70%), gentamicin (92%), nalidixic acid (96%), and tetracycline (88%) (*Antibiotic Resistance Pattern of*

Uropathogenic Escherichia coli in South West Nigeria, 2011). This increasing resistance contributes to longer illness durations, elevated healthcare costs, and greater risks of complications, especially in tertiary health institutions where multidrug-resistant organisms are prevalent. As the effectiveness of synthetic antibiotics continues to decline, there is increasing interest in exploring natural therapeutic alternatives that are affordable, locally available, and less likely to contribute to antimicrobial resistance. Medicinal plants have a long-stand ing role in traditional medicine and have been used for generations to manage infections due to the presence of bioactive compounds with antimicrobial potential. *Cinnamomum tamala* , commonly referred to as Indian bay leaf, is one such plant with documented therapeutic applications and reported antibacterial properties (Rasna *et al.*, 2023). While the plant's leaves and essential oil have been the focus of most research, the bark also contains important secondary metabolites with demonstrated antimicrobial activity. Despite this pharmacological potential, *C.tamala* bark has not been widely investigated against clinical urinary pathogens, particularly in Nigerian healthcare settings. This study is therefore justified as it seeks to evaluate the phytochemical constituents and antibacterial activity of aqueous and ethanolic bark extracts of *Cinnamomum tamala* against urinary bacterial isolates obtained from a tertiary healthcare facility. The results of this investigation could contribute meaningful scientific evidence to support the development of accessible, plant-based interventions for managing resistant urinary tract infections, especially in environments where conventional treatment options are increasingly compromised.

1.4 Aim of Study

The aim of this study is to evaluate the phytochemical composition and antibacterial activity of aqueous and ethanolic bark extracts of *Cinnamomum tamala* against selected urinary bacterial isolates obtained from a tertiary healthcare institution in Benin City, Edo State.

1.5 Specific Objectives

The specific objectives of this study are to:

1. Conduct a qualitative phytochemical screening of aqueous and ethanolic bark extracts of *Cinnamomum tamala* .
2. Perform a quantitative phytochemical analysis of the extracts using Gas Chromatography-Mass Spectrometry (GC-MS).
3. Evaluate the antibacterial activity of the extracts using the agar well diffusion method (Zo ne of Inhibition).
4. Determine the Minimum Inhibitory Concentration (MIC) of the extracts against the selected urinary bacterial isolates.
5. Determine the Minimum Bactericidal Concentration (MBC) of the extracts against the selected urinary bacterial isolates.

1.6 Research Questions

This study seeks to answer the following questions:

1. What phytochemical constituents are present in the aqueous and ethanolic bark extracts of *Cinnamomum tamala* ?
2. What is the quantitative composition of the major phytochemicals in the extracts as identified by GC-MS analysis?

3. Does the stem bark extracts have antibacterial activity against the selected urinary bacterial isolates?
4. What are the MIC values of the extracts against the test organisms?
5. What are the MBC values of the extracts against the test organisms?

1.7 Research Hypotheses

1.7.1 Null Hypotheses

1. *Cinnamomum tamala* stem bark extracts do not contain phytochemical compounds of antibacterial significance.
2. GC-MS analysis will not identify any major bioactive compounds in the extracts.
3. The extracts do not exhibit significant antibacterial activity against urinary bacterial isolates.
4. There is no significant difference in the MIC and MBC values of aqueous and ethanolic *Cinnamomum tamala* stem bark extracts on the test organisms.

1.7.2 Alternate Hypotheses

1. *Cinnamomum tamala* stem bark extracts contain phytochemical compounds with antibacterial potential.
2. GC-MS analysis will identify major bioactive phytochemicals with potential pharmacological relevance.
3. The extracts exhibit significant antibacterial activity against urinary bacterial isolates.
4. There is a significant difference in the MIC and MBC values of aqueous and ethanolic *Cinnamomum tamala* stem bark extracts on the test organisms.

CHAPTER TWO

2.0. LITERATURE REVIEW

2.1. Overview of Medicinal Plants in Infectious Disease Management

Medicinal plants are prolific producers of secondary metabolites which are organic compounds that, while not essential for plant growth, serve as chemical defenses against pests, pathogens, and environmental stressors. These metabolites, broadly classified into terpenoids, phenolics, alkaloids, saponins, and essential oils, account for approximately 90% of known secondary plant metabolites and are well recognized for their antimicrobial activity (Gorlenko *et al*., 2020). The use of plant-based therapies is particularly widespread in Africa and Asia. According to the World Health Organization, up to 80% of people in these regions rely on traditional medicine as their primary healthcare resource (Oyebode *et al*., 2016). In Nigeria, traditional medicine remains the principal source of healthcare for nearly 80% of the rural population. Similarly, it is estimated that around 85% of people across Sub-Saharan Africa regularly use indigenous plant-based remedies for health purposes (Adebayo, 2022). This reliance on traditional medicine is largely driven by factors such as accessibility, affordability, and cultural familiarity especially in areas with poor access to modern medical care. For instance, in Nigeria, the doctor-to-patient ratio ranges from 1:4,000 to 1:5,000, which is significantly lower than the WHO recommendation of 1:600 (Okeme *et al*., 2024). Such gaps in health infrastructure contribute to the persistent use of medicinal plants as a viable and trusted alternative. The global pharmaceutical relevance of plant-based compounds is also well established. Approximately 11% of the 252 drugs classified as essential by the WHO are derived exclusively from flowering plants, underscoring the continued importance of botanicals in drug discovery and healthcare

(Porras *et al*., 2020). In the face of increasing antimicrobial resistance, natural products from medicinal plants offer promising avenues for developing new antibacterial agents. A key advantage of plant-derived antimicrobials lies in their multi-target mechanisms. These compounds are capable of disrupting microbial membranes, inhibiting DNA and protein synthesis, chelating essential metal ions, and inducing oxidative stress. Such complex modes of action not only increase antimicrobial effectiveness but also make it more difficult for pathogens to develop resistance (Pérez-Flores *et al*., 2025). In many cases, these phytochemicals act synergistically, producing additive or enhanced therapeutic effects through simultaneous biological interactions. Moreover, medicinal plant-based agents tend to be more biodegradable and environmentally sustainable than synthetic drugs. When properly stand ardized, they are also generally associated with fewer adverse effects, offering a safer profile for long-term and integrative use (ALrawashdeh *et al*., 2019).

2.2 Botanical Profile of *Cinnamomum tamala*

2.2.1 Taxonomy

Cinnamomum tamala is an aromatic evergreen tree belonging to the Lauraceae family and is taxonomically classified as follows:

Kingdom: Plantae

Phylum (Division): Streptophyta

Class: Magnoliopsida (also referred to as Equisetopsida in some taxonomic systems)

Subclass: Magnoliidae

Order: Laurales

Family: Lauraceae

Genus: Cinnamomum

Species*: Cinnamomum tamala* (Buch.-Ham.) T. Nees and Eberm.

This classification is recognized across major taxonomic repositories, including the National Center for Biotechnology Information (NCBI Taxonomy, n.d.).

2.2.2 Morphological Description

Cinnamomum tamala is a medium-sized, evergreen tree that typically grows to a height of 8–20 m, with a trunk diameter of up to 20 cm. The bark is greyish-brown, becoming rough, fissured, and scaly as the tree matures. When crushed, it releases a strong aromatic scent that characterizes members of the genus *Cinnamomum.* The leaves are simple and arranged alternately (occasionally sub opposite in younger shoots). They are elliptic-lanceolate in shape, measuring approximately 7.5–20 cm in length and 2.5–8 cm in width. The upper leaf surface is glossy green, while the underside is paler. A distinguishing morphological trait is the triplinerved venation pattern, where three prominent veins arise from the base (Pyakurel *et al*., 2020). The flowers are small (3–6 mm in diameter), greenish-yellow, and borne in axillary or terminal panicles. Flowering generally occurs in early spring, from March to April (Li and Wu, 2025).

2.2.3 Geographical Distribution

The major area of the production of *Cinnamomum tamala* is Sri Lanka along with Seychelles, Madagascar and India 14. The best quality of *Cinnamomum* stem bark is mainly as quills. It is produced in Sri Lanka in approx. 24000 ha and 3400 ha area. The species is also distributed in Laos, Vietnam, and southwestern China, particularly in Yunnan Province (Pyakurel *et al*.,

2020).This species thrives in moist evergreen and semi-evergreen forests, typically growing on hilly plateaus and mountain slopes at elevations ranging from 300 to 2,400 m above sea level (ALrawashdeh *et al*., 2019). It prefers shaded, well-drained soils with moderate humidity. Due to its economic and medicinal value, *C.tamala* is also cultivated in other parts of South and Southeast Asia, including Sri Lanka, Indonesia, and Malaysia, primarily for its aromatic bark and leaves used in cooking, traditional medicine, and essential oil extraction (Journal of Medicinal Plants Studies, 2018)

Figure 2.1: Dried bark of *Cinnamomum tamala* , illustrating the rolled structure and aromatic-rich texture used in this study (Mohamed *et al*., 2020)

2.3 Phytochemical Constituents of *Cinnamomum tamala*

Phytochemicals are naturally occurring chemical compounds synthesized during the normal metabolic processes of plants, many of which contribute significantly to their therapeutic properties (Krishnaraju, 2005). *Cinnamomum tamala* is recognized as a rich reservoir of diverse phytochemicals, which underlie its broad pharmacological potential. Qualitative phytochemical screenings of its bark and leaves have consistently revealed the presence of major classes such as flavonoids, phenolic acids, tannins, alkaloids, saponins, steroids, terpenoids, and glycosides compounds well-documented for their antioxidant, anti-inflammatory, and antimicrobial activities (P and P, 2011). A detailed GC–MS analysis of *C.tamala* bark essential oil from southern India identified thirty-one volatile constituents, collectively accounting for nearly 100% of the oil's detectable content. Cinnamaldehyde was the most abundant compound (approximately 44.9%), followed by trans-cinnamyl acetate (25.3%), ascabin (15.2%), eugenol (0.08%), linalool (0.44%), α-pinene, and β-caryophyllene (Kumar *et al.*, 2012). Regional chemotypic variation has also been reported: bark oils from the Himalayan region are typically cinnamaldehyde-rich, while leaf oils from northern India contain high levels of methyl eugenol (~46.7%) and eugenol (~26.7%). In contrast, leaf oils from southern India are more cinnamaldehyde-dominant (Agarwal *et al.*, 2011). These chemical differences are crucial in explaining the variation in biological activity between regional chemotypes. Quantitative phytochemical studies have also highlighted the efficiency of different solvents in extracting bioactive constituents. Ethanol has consistently proven more effective than water. For example, one study reported that ethanolic bark extracts contained a total phenolic content of 226 mg gallic acid equivalents per gram (GAE/g) and a flavonoid content of 186 mg quercetin equivalents per gram (QE/g), significantly surpassing those of aqueous extracts (Sasidharan *et al.*,

2010). Similarly, methanolic extracts of the leaves yielded 44.5 mg GAE/g total phenolics and 23.8 mg QE/g total flavonoids, reinforcing *C.tamala*'s rich polyphenolic composition and its therapeutic promise (S *et al.*, 2023). The therapeutic potential of *C.tamala* is strongly attributed to these phytochemical groups, each contributing distinct biological effects relevant to antimicrobial action. A brief account of the most important constituents is presented below:

Flavonoids and Phenolic Acids: *C.tamala* contains abundant flavonoids such as quercetin and kaempferol, as well as phenolic acids like gallic acid. These compounds act as potent antioxidants, neutralizing free radicals and modulating inflammatory pathways. They also exhibit antibacterial properties by inhibiting bacterial enzymes and disrupting cellular metabolism, effectively reducing pathogen viability (Raksha *et al*., 2021).

Cinnamaldehyde and Eugenol

These essential oil constituents have been widely studied for their broad-spectrum antimicrobial activity. Cinnamaldehyde disrupts bacterial cell membranes and interferes with key enzymes, while eugenol contributes to biofilm inhibition and suppression of quorum sensing. Both compounds are effective even against antibiotic-resistant strains.

Linalool

Present in lower concentrations, linalool complements the action of cinnamaldehyde and eugenol. It provides additional antimicrobial and anti-inflammatory effects and is believed to enhance the overall efficacy of *C.tamala* extracts (Upadhyay, 2017).

Tannins

Tannins exert antimicrobial action by precipitating microbial proteins, thereby disrupting

enzymatic activity and cell wall function. They also inhibit bacterial adhesion and colonization by interfering with biofilm formation.

Saponins

Saponins are known to interact with sterols in microbial membranes, increasing permeability and often leading to lysis. When present alongside other antimicrobial phytochemicals, saponins significantly boost the bactericidal potential of plant extracts (Gyanvihar, 2020).

Glycosides and Sterols

found in the bark exhibit significant antioxidant, cardioprotective, and antihyperglycemic effects. While not directly antimicrobial, they support immune function and metabolic resilience which can aid in infection controlantibacterial mechanisms often via interactions with microbial DNA and proteins that can disrupt cell replication and metabolism (Upadhyay, 2017) *C.tamala*'s antibacterial activity can be attributed to the synergistic effects of its diverse phytochemical constituents. The combination of essential oils, polyphenols, saponins, tannins, and alkaloids creates a multifaceted defense against microbial pathogens, supporting the plant's traditional use in managing infections and justifying its exploration in modern antimicrobial studies.

2.4 Pharmacological and Antimicrobial Activities of *Cinnamomum tamala*

Cinnamomum tamala (tejpat/Indian bay leaf), native to the Himalayan region, is recognized for its essential oil-rich leaves containing cinnamaldehyde, eugenol, linalool, furanosesquiterpenoids, flavonoids, tannins, and phenolic compounds (Gyanvihar, 2020). Over 350 bioactive compounds including lignans, phenylpropanoids, alkaloids and sesquiterpenoids have been identified across *Cinnamomum* species; in *C.tamala*, eugenol, cinnamaldehyde, sabinene, β-

caryophyllene, germacrene D, and furanogermacrene predominate in its essential oil (Wu *et al*., 2021).

Antibacterial Effects

One of the most well-validated effects of C.*tamala* bark is its antibacterial activity

A study was done on the stem-bark methanolic and aqueous extracts against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Pseudomonas aeruginosa*. They observed inhibition zones of 11–14 mm, confirming strong bactericidal activity (Tyagi, 2019). In a comparative study, butanol extracts showed similarly potent antimicrobial activity against *K. pneumoniae*, *E. coli*, *S. aureus*, and *P. aeruginosa* and also displayed immunomodulatory effects (Jeyasree and Dasarathan, 2012). The antimicrobial action is attributed to cinnamaldehyde and eugenol: lipophilic molecules that destabilize microbial membranes, inhibit respiratory enzymes, and disrupt quorum sensing thereby preventing biofilm formation (Mohan *et al*., 2012).

Antifungal Activity

Although fewer studies have directly assessed the antifungal efficacy of *Cinnamomum tamala* bark, research involving essential oils rich in cinnamaldehyde and eugenol major constituents also found in the bark have demonstrated potent antifungal effects. These essential oils significantly inhibited the growth of common pathogenic fungi such as *Cand ida albicans* and *Aspergillus niger*, indicating a strong likelihood that bark extracts could offer similar antifungal potential (Tyagi, 2019).

Anti-Inflammatory

The anti-inflammatory properties of *C.tamala* bark have been validated through experimental models involving carrageenan-induced paw edema in Swiss albino mice. In this study, the mice were divided into four groups of six animals each. Group I served as the negative control (administered carrageenan only), while Group II received a stand ard dose of ibuprofen (10 mg/kg). Groups III and IV were treated orally with *C.tamala* bark extract at doses of 250 mg/kg and 500 mg/kg body weight, respectively. Paw volume was measured both before and three hours after carrageenan injection. Results indicated a significant, dose-dependent reduction in paw edema, with 66.75% inhibition observed at 250 mg/kg and 73.71% at 500 mg/kg. These values closely approximated those of the stand ard anti-inflammatory drug. The anti-inflammatory effect is attributed to the modulation of inflammatory mediators such as prostagland ins and cytokines by cinnamaldehyde and eugenol present in the bark. (Thamizhselvam *et al*., 2017).

Antipyretic Activity

Fever was induced using a 20% aqueous suspension of Brewer's yeast. After inducing pyrexia, the *C.tamala* bark extract (administered orally at 250 and 500 mg/kg) significantly reduced rectal temperature over a 3-hour observation period. The reduction in body temperature was statistically significant ($p < 0.01$) when compared to the control group, showing the extract's efficacy in fever management, possibly by inhibiting prostagland in E2 synthesis in the hypothalamus. (Gyanvihar, 2020)

Pain-relief (analgesic) activity:

In antinociceptive assays (hot-plate and acetic acid-induced writhing models), *C.tamala* bark extract produced significant, dose-dependent pain relief. Treated animals exhibited prolonged reaction times on the hot-plate test and fewer writhing episodes in the acetic acid model consistent with modulation of both central and peripheral pain pathways (Annegowda *et al*., 2012)

Antioxidant Activity

The antioxidant potential of *Cinnamomum zeylanicum* bark was comprehensively evaluated using three stand ardized in-vitro assays: Total Phenolic Content (TPC), Total Flavonoid Content (TFC), and DPPH free radical scavenging activity. These assays assess the plant's capacity to mitigate oxidative stress, a major contributor to aging and several chronic illnesses including cardiovascular disease, diabetes, and neurodegenerative disorders (Nawaz *et al*., 2023).

Total Phenolic Content (TPC).Phenolic compounds are potent antioxidants that act as reducing agents, hydrogen donors, and metal chelators. The Folin–Ciocalteu method revealed that the methanolic extract of *C. zeylanicum* bark had a significantly higher TPC value (341.88 ± 0.31 mg GAE/100 g) compared to its nanosuspension (39.51 ± 0.008 mg GAE/100 g) (Chahardehi *et al*., 2009). This pronounced difference suggests a loss or reduced availability of phenolic compounds during nanoformulation, possibly due to changes in solubility, binding, or degradation.

Total Flavonoid Content (TFC)

Flavonoids are known for their ability to scavenge reactive oxygen species and prevent lipid peroxidation. Using the $AlCl_3$ colorimetric method, the total flavonoid content in the extract was found to be 429.19 ± 0.07 mg CE/100 g, while the nanosuspension contained 239.26 ± 3.89 mg CE/100 g (Sahar *et al*., 2022). This difference again reinforces the superior retention of antioxidant compounds in the crude extract, possibly due to less processing and fewer structural alterations compared to nanosuspension preparation.

DPPH Free Radical Scavenging Activity

The DPPH assay is a widely accepted method to assess the hydrogen-donating ability of antioxidants. The methanolic extract of *C. zeylanicum* exhibited a DPPH scavenging activity of 27.3 ± 1.35%, significantly higher than the 10.6 ± 1.35% recorded for the nanosuspension (Hussain *et al*., 2018). This suggests that the extract contains higher concentrations of bioactive compounds capable of neutralizing free radicals. The lower activity in the nanosuspension could be attributed to encapsulation-related hindrance in compound release or reduced molecular interaction with DPPH radicals. The significant levels of phenolics and flavonoids observed in *C. zeylanicum* bark extract directly correlate with its higher antioxidant potential, as confirmed by DPPH radical scavenging. These phytochemicals are well-documented for their roles in inhibiting oxidative chain reactions and protecting against cellular damage (Chahardehi *et al*., 2009; Sahar *et al*., 2022; Hussain *et al*., 2018). While nanoformulation may offer improved stability or bioavailability in some contexts, the process may also reduce immediate antioxidant performance due to limited release or chemical alteration of active compounds.

Antidiabetic Activity

Enzyme inhibition: Methanolic and aqueous extracts of *C.tamala* bark have demonstrated strong α-amylase inhibition up to 97.5% for methanol and 93.8% for water extracts with IC_{50} values of 1.80 µg/mL and 5.53 µg/mL, respectively, indicating potent inhibition of carbohydrate-digesting enzymes in vitro (Sriramavaratharajan and Murugan, 2018)

In vivo glucose reduction: In streptozotocin (STZ)-induced diabetic rats, oral administration of *C.tamala* oil at 100–200 mg/kg significantly lowered fasting blood glucose and increased hepatic glycogen stores. This antidiabetic action is likely driven by cinnamaldehyde, the extract's principal active component (~45%) (Kumar *et al*., 2012) .

Hypolipidemic Effects

Animal model outcomes: In hyperlipidemic rabbits given a high-fat diet, supplementation with *Cinnamomum* bark *C. zeylanicum*, closely related to *C.tamala* at 500 mg/kg/day for 10 days resulted in marked reductions: total cholesterol (–53%), triglycerides (–38%), and LDL (–50%), along with a notable rise in HDL (+42%) (Javed *et al*., 2012)

Hypolipidemic trends in rabbits: Another study using the same *Cinnamomum* species confirmed similar lipid-lowering effects: reductions of total cholesterol (–64%), triglycerides (–53%), LDL (–59%), and increases in HDL (+48%), outperforming stand ard statins in some measures (Javed *et al*., 2012)

Hepatoprotective Activity

In experimental animal models, the bark extract of *Cinnamomum tamala* has shown remarkable hepatoprotective potential against chemically induced liver damage. When rats were pre-treated

with *C.tamala* bark extract at doses ranging from 200 to 400 mg/kg before exposure to hepatotoxins such as paracetamol or carbon tetrachloride (CCl_4), there was a significant restoration of liver function. Serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) which typically rise in response to hepatic injury were effectively reduced to near-normal values, suggesting a protective biochemical effect (Somani *et al.*, 2011).

In addition to biochemical normalization, the extract significantly enhanced the activity of key hepatic antioxidant enzymes. Levels of glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT) were markedly elevated following treatment, indicating a reduction in oxidative stress and protection against lipid peroxidation and free radical-induced cellular injury (Eswaran *et al.*, 2010).

Histological examination further confirmed the protective effects of the extract. Liver tissue from untreated toxin-exposed animals displayed marked inflammation, necrosis, and fatty degeneration. In contrast, tissues from rats treated with *C.tamala* extract exhibited preservation of normal hepatic architecture, with minimal pathological changes.

Taken together, these findings indicate that *C.tamala* bark extract confers hepatoprotection by restoring enzymatic balance, enhancing antioxidant defenses, and maintaining structural integrity of liver tissue in toxic injury models.

Gastroprotective Activity

Direct studies on bark are scarce, but evidence from related *Cinnamomum* species and leaf extracts supports its gastroprotective potential:

Leaf extract evidence: Methanolic extracts of *C.tamala* leaves (50–200 mg/kg) have shown protective effects in rodent models of ethanol-induced ulcers, cold-restraint stress, and pylorus ligation. These effects were demonstrated by decreased ulcer indices, reduced gastric juice volume and acidity, enhanced mucus secretion, and improved antioxidant enzyme profiles (e.g., reduced lipid peroxidation, increased SOD and CAT) (Eswaran *et al*., 2010)

Cinnamomum bark findings (other species): In Ceylon *Cinnamomum* powder-fed mice (approx. 100 mg/g diet), cinnamaldehyde significantly reduced gastric lesions in stress, ethanol, HCl, and aspirin models through mechanisms thought to involve cytoprotection and mucosal defense enhancement(Tankam *et al*., 2012).

2.5 Mechanisms of Action of Plant-Based Antibacterial Agents

Plant-derived antibacterial compounds act on multiple bacterial targets, making them promising alternatives and complements to traditional antibiotics. A 2023 review highlights four major mechanisms: disruption of cell walls/membranes, inhibition of protein synthesis, nucleic acid interference, and membrane alteration (Woo *et al*., 2023)

Disruption of Cell Wall and Membrane Integrity: Phytochemicals such as phenolics (eugenol, carvacrol) and flavonoids (quercetin, kaempferol) compromise bacterial envelopes through multiple well-documented mechanisms. One key mechanism involves binding to peptidoglycan layers. These compounds interact with peptidoglycan, a major structural polymer in Gram-positive bacterial cell walls, thereby disrupting its integrity and weakening the structural support of the cell wall (Kiymaci and Kaskatepe, 2022). Although specific peptidoglycan-binding studies on eugenol remain limited, its lipophilic character allows it to embed within membrane structures, indirectly destabilizing the cell walls of Staphylococcus

aureus and other related organisms (Yadav *et al*., 2015). Another important mechanism is integration into lipid bilayers, which leads to increased membrane permeability. Due to their hydrophobic properties, compounds such as *eugenol* and *carvacrol* incorporate themselves into bacterial membranes, causing leakage of essential ions like potassium (K^+) and calcium (Ca^{2+}), along with cytoplasmic contents such as ATP and nucleic acids. This disruption has been associated with up to a 50, 90% increase in membrane permeability in organisms like *Listeria monocytogenes*, *Proteus mirabilis*, and methicillin-resistant *Staphylococcus aureus* (Lyu *et al.*, 2017). Furthermore, cinnamaldehyde, a major constituent of *Cinnamomum tamala* bark, plays a significant role in disrupting bacterial membrane integrity. It strongly interacts with membrane lipids, altering membrane surface potential and disrupting fatty acid composition, specifically reducing levels of C16:0 and C18:1 fatty acids. These alterations lead to membrane depolarization and the collapse of proton gradients essential for ATP synthesis. Such effects have been observed across both Gram-negative and Gram-positive bacteria, including *Shewanella*, *Salmonella*, and *Escherichia coli* (Lyu *et al.*, 2017).

Inhibition of Protein Synthesis

Plant-derived phytochemicals disrupt bacterial protein synthesis by targeting various stages of the translation machinery, thereby impairing essential cellular functions required for bacterial survival and replication. One key mode of action involves binding to the 30S ribosomal subunit. Flavonoids such as *epigallocatechin gallate* (EGCG) and certain tannins mimic the activity of aminoglycoside antibiotics by attaching to the 30S subunit, where they interfere with the attachment of aminoacyl-tRNA. This disruption inhibits the formation of the ribosomal initiation complex and consequently halts protein synthesis at its earliest phase (Anand abaskar, 2021). In addition, terpenoids such as ursolic acid act by inhibiting essential elongation factors EF-Tu

and EF-G. These GTP-binding proteins are responsible for facilitating the entry of aminoacyl-tRNA into the A-site of the ribosome (EF-Tu) and for mediating the translocation of tRNA and mRNA during elongation (EF-G). Interference with these factors interrupts peptide chain elongation and causes premature termination of protein synthesis, effectively disabling bacterial growth and viability (Helgstrand *et al.*, 2006). By simultaneously targeting ribosomal subunits and their associated elongation factors, plant-based compounds mount a dual assault on bacterial protein production, contributing significantly to their antibacterial efficacy.

Interference with Nucleic Acid Synthesis

Plant-derived phytochemicals disrupt bacterial DNA and RNA functions through two principal mechanisms that impair replication, transcription, and overall genomic integrity. One mechanism involves intercalation into DNA, as seen with isoquinoline alkaloids such as *sanguinarine*. These compounds insert themselves between the base pairs of DNA, distorting the double helix structure and obstructing the action of DNA polymerase and other replication enzymes. This interference hampers both replication and transcription, ultimately inhibiting bacterial proliferation. Studies have demonstrated that *sanguinarine* forms stable complexes with both DNA and RNA, contributing to the suppression of essential nucleic acid-dependent processes (Basu and Kumar, 2016). Another mechanism is the inhibition of DNA gyrase and topoisomerase, which are enzymes vital for relieving torsional strain during DNA replication and transcription. Ellagitannins such as *punicalagin*, widely present in pomegranate and related plant species, target these enzymes by blocking their activity. This disruption leads to excessive DNA supercoiling and fragmentation, thereby impeding the progression of the replication fork and essential cellular processes (Li *et al.*, 2023).

Disruption of Enzymatic and Metabolic Pathways

Plant-derived compounds often inhibit essential bacterial enzymes and metabolic pathways, thereby undermining microbial growth and survival. One notable example is allicin, the active compound found in garlic, which targets thiol-containing enzymes. Allicin reacts with thiol (–SH) groups in cysteine residues of critical bacterial enzymes such as alcohol dehydrogenase and thioredoxin reductase. This reaction forms thioallyl adducts that irreversibly inactivate these enzymes, leading to the disruption of core metabolic functions including cellular respiration, redox homeostasis, and antioxidant defense mechanisms (Singh and Singh, 1996). Additionally, allicin's hydrophobic properties facilitate its penetration through bacterial membranes, enhancing its ability to reach and disable intracellular targets (Thakur *et al.*, 2024). Furthermore, polyphenolic compounds such as catechins contribute to metabolic disruption by chelating essential metal ions like iron. These metal ions serve as indispensable cofactors for a wide range of bacterial enzymes. By sequestering iron, catechins deprive bacteria of the cofactors required for critical enzymatic reactions, impairing metabolic efficiency and ultimately inhibiting growth. (Kiymaci and Kaskatepe, 2022).

Biofilm Inhibition and Quorum Sensing Disruption

Biofilms provide structural and functional protection to bacterial communities, shielding them from antibiotics and host immune responses (Subhaswaraj *et al.*, 2018). However, several plant-derived compounds can disrupt this defense mechanism by interfering with bacterial communication systems and inhibiting biofilm formation. One major target of such compounds is the quorum sensing system, a bacterial communication mechanism that regulates biofilm formation in response to population density. Phytochemicals such as cinnamaldehyde, curcumin,

and resveratrol have been shown to interfere with these cell-density-dependent signaling pathways. For instance, cinnamaldehyde disrupts the LuxR-mediated AI-2 quorum sensing system, thereby reducing the expression of virulence factors, antibiotic resistance genes, and biofilm-associated genes (Subhaswaraj *et al.*, 2018). In *Vibrio* species, cinnamaldehyde downregulates autoinducer signaling genes and prevents LuxR transcription factors from binding DNA, effectively silencing quorum sensing-mediated responses (Santhakumari and Ravi, 2018). In addition to signal interference, these phytochemicals also block biofilm formation by inhibiting the synthesis of extracellular polymeric substances (EPS), which are essential for maintaining biofilm architecture. By disrupting the signaling cascades required for EPS production, compounds like cinnamaldehyde and curcumin prevent bacterial adhesion and limit the maturation of biofilm structures. (Maggio *et al*., 2024) Together, these mechanisms including quorum sensing inhibition, suppression of EPS production, and impairment of cell-to-cell communication undermine the formation and persistence of biofilms. When combined with other antibacterial actions such as enzymatic inhibition and iron chelation, these effects contribute significantly to the broad-spectrum efficacy of plant-derived antimicrobials, including those from *Cinnamomum tamala* bark.

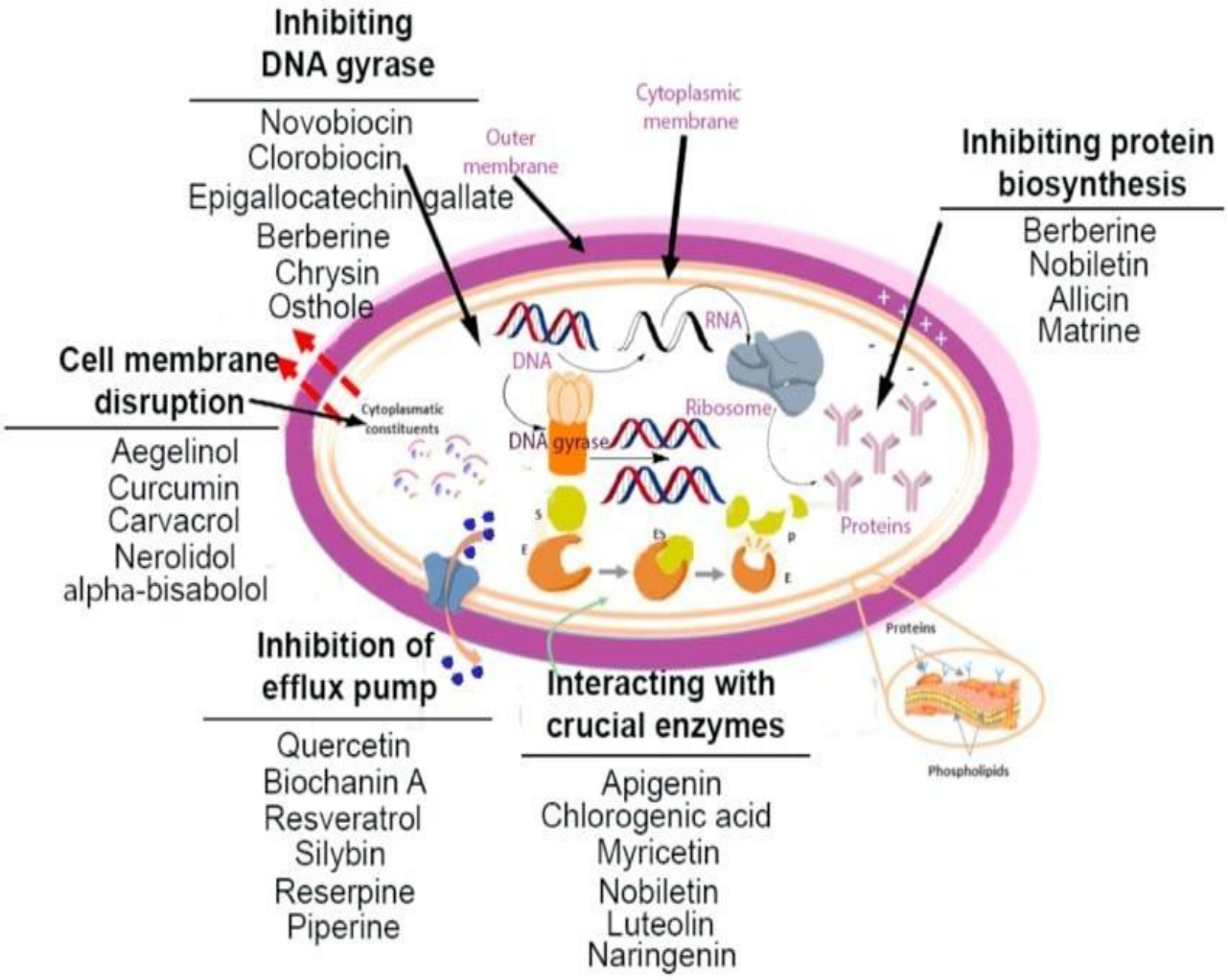

Figure 2.2: Antibacterial mechanism of action of plant-derivative compounds (Khameneh *et al*., 2021).

2.6 Overview of Antimicrobial Resistance

Antimicrobial resistance (AMR) is the phenomenon where microorganisms such as bacteria, viruses, fungi, and parasites evolve to become resistant to the drugs that once effectively treated them. This resistance can render stand ard treatments ineffective, leading to persistent infections and increased risk of disease spread (Chansamouth *et al*., 2021). Antimicrobial resistance (AMR) is a mounting global health crisis in which microorganisms especially bacteria evolve to withstand the effects of drugs designed to kill or weaken them. In 2019, AMR was directly responsible for approximately 1.27 million deaths and contributed to nearly 5 million deaths worldwide (Jinks *et al*., 2016). The Lancet estimates that without urgent intervention, AMR could lead to 8 million deaths per year by 2050 and claim up to 39 million lives cumulatively between 2025 and 2050 (Gordon, 2024).

2.6.1 Drivers of Antimicrobial Resistance

The emergence and spread of antimicrobial resistance (AMR) are fueled by interrelated factors that span human medicine, agriculture, and environmental health.

1. Overuse and Misuse in Human and Veterinary Medicine

Excessive and inappropriate use of antibiotics in both human healthcare and animal husband ry is a primary driver of AMR. In humans, antibiotics are frequently used without prescriptions or for viral infections, promoting the survival of resistant strains. Similarly, in veterinary medicine, antibiotics are often administered prophylactically or via feed without oversight, increasing selective pressure for resistant pathogens (And erson *et al*., 2024). For example, in sub-therapeutic dosing in swine production, antibiotics are added to feed over prolonged periods, enhancing resistance development (Holman and Chénier, 2015). A 2024 review highlighted that

up to 73 % of all antibiotics globally are used in livestock, especially in low- and middle-income countries (LMICs) where regulatory frameworks are weak (Matheou *et al*., 2025).

2. Inappropriate Prescribing Practices

Incorrect prescribing such as sub-therapeutic dosing, overly broad-spectrum antibiotics, or prescriptions without diagnostic confirmation further accelerates resistance. A 2025 Oxford study emphasized widespread unnecessary antibiotic use in both clinical and veterinary settings due to lack of diagnostics and weak stewardship programs (Ehsan, 2025). Overprescription accounts for a significant proportion of antibiotic use in outpatient settings, with up to 50 % of prescriptions deemed inappropriate

3. Widespread Use in Agriculture and Food Production

Antibiotic usage in livestock for growth promotion and disease prevention is a major contributor to AMR. Resistant bacteria from animals can spread to humans through food chains and environment. In many LMICs, 95 % of veterinary antibiotics are obtained over the counter, leading to rampant misuse (Matheou *et al*., 2025). Widespread use in agriculture, especially in poultry and livestock, fosters environmental dissemination of resistance genes via manure, soil, and water (Singh *et al*., 2024)

4. Environmental and Socioeconomic Conditions

Poor sanitation, inadequate infection control, and environmental contamination amplify AMR, especially in LMICs. Studies show that improper disposal of waste including antibiotic residues into water systems and soil creates reservoirs for resistant genes, promoting spread (Musoke *et al*., 2021). Overcrowding, lack of waste management, and open defecation increase infection

rates and antibiotic demand (Kapatsa *et al*., 2025). Environmental pressures like flooding, pollution, and temperature rise also accelerate resistance by fostering horizontal gene transfer among bacteria

5. Weak Healthcare Infrastructure and Socioeconomic Barriers

In resource-limited settings, inadequate healthcare infrastructure scarce diagnostics, poor stewardship, and low awareness drives resistance. Many LMICs suffer from empirical antibiotic prescribing due to scarce diagnostic capacity, leading to misuse. Poverty leads to suboptimal antibiotic use: rationing doses, using leftovers, and self-medication all of which contribute to resistance

2.6.2 Molecular Mechanisms of Resistance

1. Reduced Drug Uptake – Intrinsic Resistance of *Pseudomo*nas aeruginosa

Pseudomonas aeruginosa exhibits intrinsic resistance to most antibiotics due to its uniquely low outer-membrane permeability which is 12 to 100 times lower than that of *Escherichia coli*. This characteristic barrier is primarily responsible for its reduced antibiotic susceptibility compared to other Gram-negative bacteria. (Langendonk *et al*., 2021). The outer membrane acts as a molecular sieve: its size-limited, water-filled porins allow entry of hydrophilic molecules by passive diffusion. However, *P. aeruginosa* expresses 26 different porins, notably OprF, which is predominantly in a closed-channel conformation (~95% closed), severely restricting membrane permeability (Breidenstein *et al*., 2011). As a result, uptake of antibiotics especially those with larger molecular structures (> 400–600 Da) is greatly hindered. Even general porins like OprB (for glucose) and more specific ones like OprD (for basic amino acids and carbapenems) have narrow, selective channels that do not readily permit antibiotic passage (Filloux and Ramos,

2022). This low-permeability feature works synergistically with other mechanisms of resistance (such as efflux pumps and β-lactamases) to maintain high levels of resistance: by slowing antibiotic entry, *P. aeruginosa* allows these secondary defenses time to expel or degrade incoming drugs (Langendonk *et al*., 2021).

2. Target Site Modification

Bacteria can resist antibiotics by altering drug targets, thereby reducing binding affinity. A well-known example is methicillin-resistant *Staphylococcus aureus* (MRSA), which carries the mecA gene encoding PBP2a, a penicillin-binding protein with low affinity for β-lactams. This allows MRSA to continue cell wall synthesis despite the presence of methicillin, penicillin, and most cephalosporins. The structural design of PBP2a, including its concealed active site, enables it to evade inhibition a mechanism termed target bypass. Though rare exceptions like ceftaroline can still bind to PBP2a, resistance generally remains high. The presence of mecA and PBP2a is the key molecular marker for β-lactam resistance in MRSA strains (Belbase *et al*., 2017; Fishovitz *et al*., 2014).

3. Enzymatic Drug Inactivation

One of the most common and clinically significant mechanisms of antimicrobial resistance is the enzymatic inactivation of antibiotics. In this process, bacteria produce enzymes that chemically alter or destroy antibiotic molecules, rendering them ineffective. β-lactamases are a diverse group of enzymes that hydrolyze the β-lactam ring present in penicillins, cephalosporins, monobactams, and carbapenems, thereby neutralizing their antibacterial activity. This mechanism is particularly prevalent in Enterobacteriaceae, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* (Tooke *et al*., 2019). Aminoglycoside-modifying enzymes (AMEs)

deactivate aminoglycosides through chemical modifications such as acetylation, phosphorylation, or adenylation. These modifications impair the antibiotic's ability to bind bacterial ribosomes, effectively abolishing its bactericidal activity. AMEs are commonly encoded on plasmids, facilitating horizontal gene transfer among resistant strains (Labby and Garneau-Tsodikova, 2013). Together, these enzymatic pathways significantly contribute to resistance against broad classes of antibiotics and are often found alongside other resistance genes on mobile genetic elements, accelerating their spread in both clinical and environmental settings.

4. **Active Drug Efflux**

Efflux proteins are membrane-bound transporters that play a critical role in antimicrobial resistance by actively expelling antibiotics and other toxic compounds from bacterial cells. These pumps are major determinants of multidrug resistance (MDR), particularly in Gram-negative bacteria, and enable bacteria to survive antibiotic exposure even at higher drug concentrations (Kumawat *et al*., 2023). Efflux pumps reduce intracellular concentrations of antibiotics to sublethal levels, safeguarding bacteria from drug-mediated toxicity. They are energized either by ATP hydrolysis (ABC transporters) or by the proton/sodium motive force (MFS, RND, SMR, MATE families) (Sinha *et al*., 2024)

2.7 Urinary Tract Infections and Uropathogens

Urinary tract infections (UTIs) represent one of the most common bacterial infections globally, affecting millions of individuals annually across all age groups. They are especially prevalent among women, older adults, and immunocompromised patients (Flores-Mireles *et al.*, 2015). UTIs occur when pathogenic microorganisms colonize and invade any part of the urinary tract, including the urethra, bladder, ureters, or kidneys. Clinically, they are classified as either

uncomplicated, primarily affecting healthy individuals with structurally normal urinary tracts, or complicated, occurring in patients with anatomical or functional abnormalities (Terlizzi *et al.*, 2017). The predominant etiological agent of UTIs is *Escherichia coli*, responsible for approximately 70–90% of community-acquired cases. However, other important uropathogens such as *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* are commonly isolated, particularly in nosocomial and catheter-associated infections (Parish and Holliday, 2012). These organisms possess multiple virulence factors, including adhesins like type 1 fimbriae, hemolysins, siderophores, and the ability to form biofilms, which significantly enhance their capacity to adhere to uroepithelial surfaces and evade host immune responses (Smelov *et al.*, 2016). The rising prevalence of antimicrobial-resistant uropathogens is a serious public health concern. Multidrug-resistant (MDR) strains of *E. coli* and *K. pneumoniae* producing extended-spectrum beta-lactamases (ESBLs), as well as carbapenem-resistant strains of *P. aeruginosa*, have been reported globally, leading to diminished treatment options and increased healthcare costs (Magiorakos *et al.*, 2012). In response to this challenge, plant-derived antimicrobials have gained renewed interest as potential alternative or complementary therapies for UTIs. Medicinal plants such as *Cinnamomum tamala* have demonstrated notable antibacterial properties in various studies. These effects are attributed to their diverse phytochemical composition, which includes bioactive compounds such as flavonoids, tannins, alkaloids, and essential oils (Kumar *et al.*, 2020). Investigating the antibacterial efficacy of such plant extracts against clinically relevant uropathogens is crucial for the development of novel, natural therapeutic agents.

2.7.1 *Escherichia coli*

Escherichia coli is a facultative anaerobic, Gram-negative bacillus commonly found in the gastrointestinal tract of humans and animals. Under normal physiological conditions, it maintains a mutualistic relationship with its host, contributing to intestinal homeostasis and the stability of the gut microbiota (Yan and Polk, 2004). As a commensal organism, *E. coli* typically remains confined to the intestinal lumen and does not cause disease. However, in immunocompromised individuals or when the gastrointestinal barrier is breached, even nonpathogenic strains may become opportunistic and cause infections (Bien *et al*., 2012). Uropathogenic *Escherichia coli* (UPEC) is the primary cause of community-acquired UTIs, accounting for approximately 80–90% of cases (Flores-Mireles *et al.*, 2015). UPEC strains are genetically distinct from commensal *E. coli* and are characterized by the presence of genomic Pathogenicity Island s (PAIs) that encode a variety of virulence factors. These include adhesins (e.g., type 1 and P fimbriae), toxins (e.g., hemolysin), surface polysaccharides, flagella, and iron-acquisition systems such as siderophores (Bien *et al.*, 2012). Based on these traits, UPEC strains are commonly classified into four major phylogroups: A, B1, B2, and D. The pathogenesis of UPEC in urinary tract infections typically follows a multistep process (Hannan *et al.*, 2012):

1. Colonization of the periurethral and vaginal areas, followed by ascension into the urethra.
2. Entry into the bladder, where UPEC replicates as planktonic cells in urine.
3. Adherence to and interaction with the bladder epithelium, including evasion of innate immune defenses.
4. Formation of biofilms that protect bacteria from host responses and antibiotics.

5. Invasion of urothelial cells, where UPEC forms Intracellular Bacterial Communities (IBCs) and quiescent intracellular reservoirs (QIRs), enabling persistence and recurrence.
6. Ascension to the kidneys, causing tissue damage and potentially leading to bacteremia or septicemia.

This intricate infection cycle allows UPEC to persist within the host and evade both immune clearance and antimicrobial therapy, contributing to recurrent UTIs and chronic infections

2.7.2 *Klebsiella pneumonia*

Klebsiella pneumoniae is a Gram-negative, encapsulated, opportunistic bacterium belonging to the Enterobacteriaceae family, recognized as a major cause of healthcare-associated infections. It is a significant pathogen in UTIs, particularly in hospitalized patients, the elderly, and individuals with underlying medical conditions or indwelling catheters (Filev *et al*., 2025). While less common than *E. coli* as a cause of community-acquired UTIs, *K. pneumoniae* often contributes to more severe and complicated urinary tract infections, including pyelonephritis and urosepsis (Snr *et al*., 2025). A critical concern with *K. pneumoniae* is its notorious capacity for developing and disseminating antibiotic resistance, particularly through the production of Extended-Spectrum Beta-Lactamases (ESBLs) and carbapenemases (Abbas *et al*., 2024). Carbapenem-resistant *K. pneumoniae* (CRKP) strains are a global health threat, often resistant to nearly all available antibiotics, making infections extremely difficult, if not impossible, to treat (Shen *et al*., 2024). The presence of mucoid capsules in many *K. pneumoniae* strains also contributes to their virulence and resistance to host defenses and antibiotics (Zierke *et al*., 2025). Given the high prevalence of these resistant strains in hospital settings, especially in

regions like Nigeria, investigations into alternative antibacterial compounds, such as those from natural products, are crucial for combating *K. pneumoniae* infections

2.7.3 *Staphylococcus aureus*

Staphylococcus aureus is a Gram-positive bacterium, a common inhabitant of the human skin and mucous membranes, but also a significant opportunistic pathogen capable of causing a wide range of infections, from superficial skin infections to life-threatening conditions like sepsis and endocarditis (Taylor and Unakal, 2023). While *E. coli* and other Gram-negative bacteria are the primary causes of UTIs, *S. aureus* can also cause UTIs, particularly in specific populations. UTIs caused by *S. aureus* are often associated with catheterization, recent instrumentation of the urinary tract, bacteremia originating from another site of infection, or underlying conditions that compromise the immune system (Bien *et al.*, 2012). The presence of *S. aureus* in urine cultures may sometimes indicate contamination, but when symptomatic or persistently present, it warrants clinical attention. *S. aureus* possesses a wide range of virulence factors that contribute to its pathogenicity. These include surface proteins (e.g., protein A, fibronectin-binding proteins) that facilitate adhesion to host tissues, as well as enzymes such as coagulase, hyaluronidase, and lipases that promote tissue invasion. Additionally, it produces several toxins, including hemolysins and Panton-Valentine leukocidin (PVL), which can lead to cellular destruction and inflammation (Pal *et al*., 2024) The major concern with *S. aureus* infections is the widespread emergence of Methicillin-Resistant *Staphylococcus aureus* (MRSA) MRSA strains are resistant to all beta-lactam antibiotics, including penicillins, cephalosporins, and carbapenems, due to the acquisition of the *mecA* gene, which encodes for a modified penicillin-binding protein (PBP2a) . This resistance significantly limits therapeutic options, often requiring the use of last-resort drugs like vancomycin, against which resistance is also emerging . Hospital-acquired MRSA (HA-

MRSA) is a global issue, posing a substantial burden on healthcare systems (Yasmin *et al*., 2015). Therefore, finding novel antibacterial agents effective against resistant *S. aureus* strains, including those isolated from urinary sources, is of paramount importance

2.7.4 *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is a Gram-negative, motile, non-fermenting rod that is widely recognized as an opportunistic pathogen, particularly in hospital environments (Gellatly and Hancock, 2013). It is a notorious cause of healthcare-associated urinary tract infections (HAUTIs), especially in patients with indwelling urinary catheters, structural abnormalities of the urinary tract, or prolonged hospitalization (Kidd *et al.*, 2015). Its ability to form biofilms on medical devices, such as catheters, contributes significantly to its persistence and resistance to both antibiotics and host immune responses. The virulence of *P. aeruginosa* is multifactorial. Key factors include:

1. Biofilm formation, which enables the bacteria to adhere to catheter surfaces and evade host immunity.
2. Quorum sensing systems, which regulate the expression of virulence genes.
3. Production of elastases, exotoxins (e.g., Exotoxin A), proteases, and pyocyanin, which contribute to host tissue damage and immune evasion
4. Type III secretion system, which injects toxins directly into host cells, disrupting their function. (Moradali *et al.*, 2017).

P. aeruginosa is intrinsically resistant to many antibiotics due to its low outer membrane permeability, efficient efflux pump systems, and chromosomally encoded AmpC beta-lactamase (Yin *et al*., 2022). Furthermore, it readily acquires additional resistance mechanisms,

including carbapenemases (e.g., VIM, IMP, NDM), leading to multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains (Hu and Chua, 2025). Treating *P. aeruginosa* infections, particularly those involving resistant strains, is incredibly challenging and often requires combinations of potent antibiotics, which may have significant side effects (Mohammed *et al*., 2023). The prevalence of resistant *P. aeruginosa* in hospital settings in Nigeria and globally underscores the critical need for novel therapeutic agents to combat these difficult-to-treat urinary infections.

2.8 Methods for Assessing Antibacterial Activity

Assessing the antibacterial activity of various compounds, especially novel therapeutic agents, is a fundamental step in antimicrobial research. Several stand ardized methods are employed to determine the efficacy of an antimicrobial agent against bacterial pathogens. These methods provide quantitative and qualitative data on how well a substance inhibits bacterial growth or kills bacteria, guiding the selection of effective treatments and the development of new drugs.

2.8.1 Zone of Inhibition

The Zone of Inhibition method, commonly executed using an agar diffusion method (such as the Kirby-Bauer disk diffusion test) method, is a qualitative assay used to determine the susceptibility of bacteria to an antimicrobial agent (Reyes, 2009). In this technique, a stand ardized bacterial suspension is uniformly spread over the surface of an agar plate (typically Mueller-Hinton agar). Then, a source of the antimicrobial agent like paper disks impregnated with a known concentration, or wells cut into the agar and filled with a liquid sample is placed on the inoculated agar surface(Violante *et al*., 2012). As the plate is incubated, the antimicrobial agent diffuses radially outwards from its source into the agar, creating a concentration gradient.

If the bacterium is susceptible to the agent, its growth will be inhibited in the area around the source, resulting in a clear, circular area known as the "zone of inhibition". The diameter of this clear zone (measured in millimeters) is indicative of the antimicrobial's effectiveness; larger zones generally suggest greater susceptibility of the bacterium to the agent, while smaller or absent zones imply resistance (Tanner, 2024) . This method is widely adopted in microbiology for its simplicity, cost-effectiveness, and ability to screen multiple agents simultaneously(Kim *et al*., 2024).

2.8.2 Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) is the lowest concentration of an antimicrobial agent that prevents the visible growth of a bacterium after a specified incubation period (typically 18-24 hours) (Wiegand *et al*., 2008). Unlike the qualitative zone of inhibition, MIC provides a quantitative measure of bacterial susceptibility. It's considered the "gold stand ard" for determining the *in vitro* activity of antimicrobial agents (Kowalska-Krochmal and Dudek-Wicher, 2021). MIC is commonly determined using broth microdilution or agar dilution methods. In the broth microdilution method, a series of decreasing concentrations of the antimicrobial agent is prepared in a liquid growth medium (broth) in wells of a microtiter plate. Each well is then inoculated with a stand ardized bacterial suspension. After incubation, the wells are visually inspected for turbidity (bacterial growth). The MIC is the lowest concentration well that remains clear, indicating no visible bacterial growth (Wiegand *et al*., 2008). For agar dilution, varying concentrations of the antimicrobial are incorporated into agar plates, which are then inoculated with bacteria (Schumacher *et al*., 2017).

MIC values are crucial for guiding clinical treatment decisions, as they help clinicians select appropriate antibiotics and dosages. They also play a vital role in research and development, allowing for direct comparison of the potency of different antimicrobial compounds.

2.8.3 Minimum Bactericidal Concentration (MBC)

The Minimum Bactericidal Concentration (MBC) is defined as the lowest concentration of an antimicrobial agent capable of killing 99.9% of a bacterial population, usually after 18 to 24 hours of incubation. This measure reflects a 3-$\log_{10}$ reduction in viable bacterial count and distinguishes agents that are truly bactericidal from those that are merely bacteriostatic. While the Minimum Inhibitory Concentration (MIC) identifies the concentration needed to inhibit visible growth, the MBC confirms actual bacterial death, a distinction that is crucial when treating severe infections or immunocompromised patients (Ramkumar, 2024).MBC determination is typically performed as an extension of the MIC assay. After establishing the MIC using broth dilution techniques, samples from wells without visible growth i.e., at and above the MIC are subcultured onto antibiotic-free

agar plates such as Mueller-Hinton agar. These plates are then incubated under stand ard conditions. The MBC is identified as the lowest concentration at which no bacterial colonies grow or at which there is a ≥99.9% reduction in colony-forming units (Pankey and Sabath, 2004; Makade *et al*., 2024). To interpret the nature of an antimicrobial agent, the MBC/MIC ratio is calculated. A ratio of ≤4 suggests bactericidal activity, while a ratio >4 indicates a bacteriostatic effect (Makade *et al*., 2024). This ratio provides useful guidance in clinical decision-making, particularly when choosing treatments for infections in immune-privileged sites or in patients with compromised immune responses.

Recent studies have introduced simplified and rapid modifications of the MBC procedure, especially for slow-growing or difficult-to-culture organisms such as *Mycobacterium* spp., enhancing the reliability and utility of this method in both clinical diagnostics and antimicrobial research (Batista *et al*., 2025)

CHAPTER THREE

METHODOLOGY

3.1 Study Area

The study was carried in pharmaceutical microbiology laboratory, university of Benin, Edo state.

3.2 Materials

3.2.1 Microbiological Media

Nutrient agar, Nutrient broth and Muller Hinton agar

3.2.2 Equipment and Apparatus

Portable autoclave, weighing balance, hot air oven, mortar, pestle, mechanical grinding machine, micropipette, incubator, Whatman filter paper, cotton wool, pipette tip, a corkborer (10mm in diameter), a transparent millimetre rule, grease pencil, sterile swab sticks, tripod stand , Bunsen burner, foil paper, dried plant material.

3.2.3 Glassware

Conical flask, bottles (MacCartney, universal and Bijou) as well as test tubes, pipettes, glass stirrers, porcelain dish, pestle, maceration jars, glass funnels, beakers, measuring cylinders, and Petri dishes.

3.2.4 Chemicals and Reagents

Tween 80, distilled water. Disinfectant: Purit, soap, detergent.

3.2.5 Clinical Isolates

Clinical isolates include *Klebsiella pneumoniae, Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa*

3.3 Methods

3.3.1 Collection and Identification of Cinnamomum

The back of *Cinnamomum tamala* were purchased from Ring road market in Oredo local government area, Edo State. The plant was officially identified at the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Edo State and , assigned Voucher numbers: UBH-C395

3.3.2 Preparation of Crude Extract

The dried back of *Cinnamomum tamala* that were obtained from the market were dried properly in the laboratory hot air oven at 40°C for 24 hours. The dried plant back were then pulverized using a commercial grinding machine. Seven hundred grams of *Cinnamomum tamala* powder was macerated in 1L of ethanol and six hundred and fifty grams was macerated in 1L of distilled water for 72 hours. They were stirred at intervals to allow for proper permeation of the extraction solvent. A double filtration using Whatman filter paper was carried out, and the filtrate was concentrated in a hot air oven at 40℃. The extracts were weighed and refrigerated at 4℃ in an airtight container.

3.3.3 Qualitative Phytochemical Screening

Simple chemical tests to detect the presence of secondary metabolites were done according to stand ard requirements and procedures (Evans, 2002). Approximately 4g of the crude sample was dissolved in 75ml Ethanol. The solution was filtered hot and allowed to cool. The filtrate

obtained was used to carry out the following tests. The same procedure was carried out for the aqueous extract of the back of *Cinnamomum* plant.

Preparation of *Cinnamomum* bark extract:

The collected spice sample was dried and grinded into a fine powder, which can be used for extraction.

Aqueous extract:

The 10 grams of *Cinnamomum* bark powder was mixed with 100ml of distilled water and kept for 3 days.Then the extract was filtered by whatman filter paper.

Ethanolic extract:

The 10 grams of powdered spice sample was mixed with 100 ml of Ethanol and kept 3 days and filtered by whatman filter paper.

Qualitative phytochemical analysis of the *Cinnamomum* bark extracts as follows,

Test for Saponins

Frothing Test

2mL of the filtrate was diluted with 10mL distilled water and shaken vigorously for one minute.

Expected observation for a positive result: formation of a persistent frothing.

Test for Tannins

Gelatine Test

To 2mL of the filtrate was added 2mL of 1% gelatine solution in 10% NaCl.

Expected observation for a positive result: formation of precipitate.

Test for Phenolic Compounds

Ferric chloride Test

To 2mL of the filtrate was added 5mL distilled water followed by 2 drops of 5% ferric chloride solution. A blank test was carried out by adding 2 drops of 5% ferric chloride solution to 5mL distilled water.

Expected observation for a positive result: formation of intense colouration.

Test for Terpenoids

Salkowski Test

5mL of the filtrate was mixed with 2mL of chloroform then concentrated H_2SO_4 was carefully added (drop wise) to form a layer.

Expected observation for a positive result: formation of reddish brown colouration at the interface on addition of sulphuric acid.

Test for alkaloids:

2 ml of spice extracts was mixed with few ml of dilute Hydrochloric (Hcl) Acid and filtered.The filtrate was added with few drops of Hager's reagent (Aqueous solution of Picric acid). A yellow precipitate indicates the presence of Alkaloids.

Test for glycosides:

2 ml of spice extract was added with glacial acetic acid, ferric chloride (FeC13) and H2So4 of each 1 ml. A green blue colour indicates the presence of Glycosides.

Test for steroids:

2ml of spice extract was mixed with 5 ml of chloroform, 2 ml of acetic anhydride and 1 ml of concentrated H2So4 and the colour changes was observed. Reddish brown colour indicates the presence of Steroids.

Test for flavonoids:

To a 2ml of spice extracts,few drops of NaOH solution was added, a yellow colour solution was formed. Then add few ml of diluted Hydrocholoric (Hcl) Acid which turns yellow colour into colourless solution indicating the presence of flavonoid

Resins test

Take about 5 mL of the ethanolic extract in a clean test tube.

Add distilled water gradually to the extract.

Observe the mixture for any white precipitate formation.

Formation of a white precipitate indicates the presence of resinous material in the plant extract.

3.3.4 Quantitative Phytochemical Analysis

The quantitative phytochemical composition of the aqueous and ethanolic bark extracts of *Cinnamomum tamala* was determined using Gas Chromatography–Mass Spectrometry (GC–MS)

analysis. The procedure was carried out using an Agilent 7890B GC system coupled with a 5977A mass selective detector.

Sample Preparation

Each dried extract was reconstituted in analytical-grade methanol to a concentration of 1 mg/mL. The solutions were sonicated for 10 minutes to ensure complete dissolution and filtered through a 0.22 µm PTFE syringe filter to remove particulate matter before injection into the GC–MS system.

GC–MS Operating Conditions

The GC–MS was equipped with an HP-5MS capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness). Helium was used as the carrier gas at a constant flow rate of 1.0 mL/min. The injector temperature was set at 250 °C, and 1 µL of each sample was injected in splitless mode.

The oven temperature was programmed as follows: initial temperature of 50 °C (held for 2 min), ramped at 10 °C/min to 250 °C, then at 5 °C/min to 300 °C and held for 10 minutes. The MS ion source temperature was set at 230 °C, and the quadrupole temperature was maintained at 150 °C. Electron ionization (EI) was performed at 70 eV, scanning in the mass range of 50–600 m/z.

Identification and Quantification of Compounds

Phytochemical constituents were identified by comparing the mass spectra of detected compounds with those in the NIST/EPA/NIH Mass Spectral Library (NIST 14). Quantification was based on the peak area normalization method, where the relative percentage of each compound was calculated as the ratio of its peak area to the total peak area of all identified compounds in the chromatogram.

3.4.1 Specimen Collection

Microorganisms used in this study were selected bacterial isolates obtained from the University of Benin Teaching Hospital, Benin City, Edo state, Nigeria. They are: *Staphylococcus aureus, Klebsiella pneumonia, Escherichia coli, Pseudomonas aeruginosa*

3.4.2 Preparation of Test Organisms

All test microorganisms were maintained in 20 % glycerol broth and frozen. Prior to use, test microorganisms were sub-cultured from stock into sterile nutrient agar plates and were incubated overnight at 37°C . After incubation colonies from the overnight plates were suspended in sterile broth for 12 hours and adjusted to 0.5 McFarland standard to give an inoculum size of approximately 10^7 CFU/ml.

3.5 Antimicrobial Assays

Antimicrobial Susceptibility Tests

An antimicrobial susceptibility test was carried out using the ditch plate method. A total of 4 different bacterial isolate were used, sterile muller hinton agar was prepared and 30ml was poured into each petri dish and allowed to solidify. The agar surface was dried in a hot air oven

at 40°C for about 5 minutes to remove excess moisture. A ditch of 10mm wide and 50mm long was created in the centre of the agar plate using a sterile scapel blade and the base of the ditch was sealed with drops of molten agar. A concentration of 800mg/ml of the extract was prepared and 1 ml dispensed into the ditch. The bacterial isolates were then streaked outward from the ditch toward the edge of the plate. The plates were incubated at 37℃ for 24 hrs. The growth of the organism was observed to assess its resistance or susceptibility to 800mg/ml of the extract for both aqueous and ethanolic extract

3.6 Determination of Inhibitory Zone Diameter (IZD)

The inhibitory zone diameter was determined using the agar well diffusion method with some modifications. Sterile muller hinton agar was prepared and 30 ml was poured into Petri dishes aseptically and allowed to solidify. The Petri dishes were dried in a hot air oven at 40°C for about 5 minutes. The dried agar surface was then streaked with the test organism using a swab stick aseptically. A sterile cork borer (10mm) was used to bore 5 wells in each agar plate. The base of the wells were sealed with 0.02 ml of molten agar. Five of the wells were filled with 0.25 ml of 100 mg/0.25ml, 50 mg/0.25ml, 25mg/0.25ml, 12.5 mg/0.25ml, 6.25 mg/0.25ml for the ethanolic extract. For the aqueous extract four of the wells were filled with 0.25ml of 400mg,200mg,100mg,50mg, respectively. A standard antibiotic ciprofloxacin at 0.02 mg was used in standardize the work. The plates were incubated at 37°C for 24 hours. The inhibition zone diameters (IZD) were measured using a ruler in millimeters.

3.7 Determination Of Minimum Inhibitory Concentration (MIC)

Agar dilution method of Afoyan and Meyer (1997) was used in this study for the determination of Minimum Inhibitory Concentration (MIC) of the extract. A 2-fold serial dilution of the test

aqueous extract was prepared to give concentrations of 50mg, 100mg, 200mg and 400mg.Double strength agar will be prepared according to the manufacturer's instruction. Calculated volumes of the extract and double strength agar (1gram of extract + 20ml molten agar) was poured into a petri-dish and rocked gently to mix properly and then allowed to set. This was repeated for 100mg,200mg and 400mg plates. The same method was used in the preparation of 100mg,50mg,25mg,12.5mg and 6.25mg for the ethanolic extract. Bacteria prepared to a stand ard concentration was streaked with the aid of a sterile wire loop on well labeled different sections of each plate. The dilution plates were incubated at 37°C for 18-24 hours for the bacteria populations and at room temperature for the fungi. After incubation, the plates were visually examined for growths in the inoculated spots. The lowest concentration of the extract that inhibits growth was considered as the MIC.

3.8 Determination Of Minimum Bactericidal (MBC)

The MBC was determined using agar well dilution method. It was determined from the agar dilution of the MIC tests by sub-culturing into agar plates that did not contain any test extract. The dilution plates was then incubated at 37°C for 18-24 hours for bacteria populations and at room temperature for the fungi. After incubation, the plates was visually examined for growths in the inoculated spots. The lowest concentration of the extract that showed no growth was considered as the MBC in the bacterial and fungal plates respectively.

3.9 Preparation of McFarland Standard

A 0.5 McFarland stand ard solution is prepared by adding 0.5ml of 1.175 % (weight/volume). Barium Chloride dihydrate salt ($BaCl_2.2H_2O$) to 9.95 ml of 1 % Sulfuric acid (H_2SO_4). The two

solutions are mixed completely to form a turbid suspension in a test tube which is then placed in a test tube rack and kept at room temperature before use.

3.10 Data Analysis

The results were expressed as the percentage composition of each identified phytochemical in both aqueous and ethanolic extracts. Compounds of interest such as saponins, tannins, terpenoids, alkaloids, glycosides, steroids, flavonoids, resins, and phenolic derivatives were highlighted, and their relative abundances were compared between extracts.

CHAPTER FOUR

RESULTS

Table 1 Phytochemical Constituents of Cinnamomum tamala Extracts

The qualitative phytochemical screening revealed that both aqueous and ethanolic bark extracts of *Cinnamomum tamala* contained alkaloids, flavonoids, tannins, terpenoids, and phenols. As shown in Table 4.1, saponins and glycosides were present only in the aqueous extract, while steroids and resins were detected exclusively in the ethanolic extract.

The GC–MS analysis of the ethanolic extract revealed a number of bioactive compounds. As presented in Table 4.2, the major constituents included Squalene (21.13%), 9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester (17.62%), and 13-Octadecenal (16.89%), which together accounted for more than half of the chromatographic peak area. Other compounds detected included Carbonic acid, but-2-yn-1-yl eicosyl ester (5.98%) and Bis(tridecyl) phthalate (2.95%), as well as hydrocarbons such as Decane, 1-fluoro- (1.76%) and Eicosane (1.71%).

In the aqueous extract, a different phytochemical profile was observed. As shown in Table 4.3, the most abundant compound was 9-Borabicyclo[3.3.1]nonane, 9-ethyl-1 (28.24%), followed by Cyclopropane, 1,2-dimethyl-3-methylene- (17.04%). Other notable compounds included cis-Vaccenic acid (8.9% and 4.59%), D-Carvone (5.78%), 1-Nonadecene (3.75%), 9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester (3.72%), Carveol (3.2%), and Ethanol, 2-(tetradecyloxy)- (2.65%).

Table 4.1 Qualitative Phytochemical Constituents of the Aqueous and Ethanolic Bark Extracts of *Cinnamomum tamala*

Phytochemical	**Aqueous Extract**	**Ethanolic Extract**
Alkaloids	+	+
Flavonoids	+	+
Tannins	+	+
Saponins	+	–
Glycosides	+	–
Terpenoids	+	+
Phenols	+	+
Steroids	–	+
Resins	–	+

Key: (+) Present; (–) Absent

Table 4.2. Major Compounds Identified by GC–MS in the Ethanolic Bark Extracts of *Cinnamomum tamala*

Compound	RT (min)	Area %	Quality
Squalene	36.276	21.13	91
9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	34.683	17.62	92
13-Octadecenal	32.704	16.89	90
Carbonic acid, but-2-yn-1-yl eicosyl ester	32.909	5.98	96
Bis(tridecyl) phthalate	33.949	2.95	80
Oxalic acid, allyl undecyl ester	8.313	2.45	58
2-Trifluoroacetoxytridecane	8.106	2.3	60
Pentadec-7-ene, 7-bromomethyl-	8.83	2.08	53
Naphthalene, decahydro-, trans-	7.815	2.05	93
Decane, 1-fluoro-	7.94	1.76	49
Eicosane	26.212	1.71	91

Table 4.3. Major Compounds Identified by GC–MS in the Aqueous Bark Extracts of *Cinnamomum tamala*

Compound	RT (min)	Area%	Quality
9-Borabicyclo[3.3.1]nonane, 9-ethyl- 1	13.622	28.24	42
Cyclopropane, 1,2-dimethyl-3-methylene-	13.59	17.04	47
cis-Vaccenic acid	33.744	8.9	90
D-Carvone	13.507	5.78	92
cis-Vaccenic acid	36.04	4.59	90
1-Nonadecene	31.766	3.75	99
9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	36.102	3.72	97
Carveol	13.4	3.2	87
Ethanol, 2-(tetradecyloxy)-	31.925	2.65	84
Isopropyl tetracosyl ether	31.64	2.26	64

4.2 Percentage Yield of Cinnamon Extracts

The percentage yield of cinnamon extracts was determined to evaluate the efficiency of the extraction process using both aqueous and ethanolic solvents. The calculation was based on the weight of the dried extract obtained relative to the initial weight of the powdered plant material, using the formula:

%yield = Weight of dried extract / Weight of initial powdered plant material × 100

As shown in Table 4.4, the aqueous extract produced a yield of 5.66%, while the ethanolic extract yielded 3.84%.

Table 4.4 Percentage Yield of Cinnamon Extracts

Extract Type	Weight of Dried Extract (g)	Initial Plant Material (g)	Percentage Yield (%)
Aqueous Cinnamon Extract	36.8	650	5.66
Ethanolic Cinnamon Extract	26.9	700	3.84

4.3. Zone of Inhibition of Bacteria with Aqueous and Ethanolic bark extracts of Cinnamomum tamala

The ethanolic extract demonstrated antibacterial activity against all test organisms. As presented in Table 4.5, inhibition zones ranged from 15 mm at 50 mg to 28 mm at 800 mg against *Escherichia coli*. *Staphylococcus aureus* also showed inhibition zones up to 27 mm at 400 mg. In contrast, the aqueous extract exhibited limited antibacterial activity. As shown in Table 4.6, inhibition zones of 12 mm and 14 mm were recorded against *S. aureus* at 400 mg and 800 mg respectively, while no inhibition was observed for *K. pneumoniae* and *E. coli*.

Statistical analysis of inhibition zones revealed distinct differences between the two extracts. As presented in Table 4.7, the ethanolic extract showed a steady increase in inhibition with concentration, reaching 21.75 ± 2.93 mm at 800 mg, while the aqueous extract recorded a maximum of 6.50 ± 3.77 mm at the same concentration. The standard drug, ciprofloxacin, produced 23.75 ± 1.70 mm. One-way ANOVA revealed significant differences across concentrations for both extracts (Ethanolic: $p = 0.034$; Aqueous: $p < 0.001$), and Tukey's post hoc test revealed that the standard differed significantly from lower doses ($p < 0.05$).

Table 4.5 Zone of inhibition of Bacteria with Ethanolic extracts of Cinnamomum tamala at different concentrations.

Organism	**Standard**	**50mg**	**100mg**	**200mg**	**400mg**	**800mg**
Staphylococcus aureus	28mm	15mm	19mm	21mm	27mm	25mm
Klebsiella pneumoniae	21mm	NZ	12mm	15mm	17mm	19mm
Pseudomonas aeruginosa	21mm	NZ	11mm	12mm	13mm	15mm
Escherichia coli	25mm	18mm	22mm	24mm	26mm	28mm

NZ = No Zone of Inhibition observed

Standard = Ciproflaxin(0.02mg)

Table 4.6 Zone of inhibition of Bacteria with Aqueous bark extracts of Cinnamomum tamala at different concentrations.

Organism	**Standard**	**50mg**	**100mg**	**200mg**	**400mg**	**800mg**
Staphylococcus aureus	28m	NZ	NZ	NZ	12mm	14mm
Klebsiella pneumoniae	21mm	NZ	NZ	NZ	NZ	NZ
Pseudomonas aeruginosa	21mm	NZ	NZ	NZ	NZ	12mm
Escherichia coli	25mm	NZ	NZ	NZ	NZ	NZ

NZ = No Zone of Inhibition observed

Standard = Ciproflaxin(0.02mg)

Table 4.7. Mean inhibition zones (mm) of ethanolic and aqueous bark extracts of Cinnamomum tamala at different concentrations.

Concentration (mg/mL)	Ethanolic Extract (Mean ± SEM)	Aqueous Extract (Mean ± SEM)
50	8.25 ± 4.8	0.00 ± 0.00
100	16.00 ± 2.68	0.00 ± 0.00
200	18.00 ± 2.73	0.00 ± 0.00
400	19.75 ± 2.93	5.75 ± 3.33
800	21.75 ± 2.93	6.50 ± 3.77
STD	23.75 ± 1.70	23.75 ± 1.70

One-way ANOVA showed significant differences in inhibition zones across concentrations (Ethanolic: $p = 0.034$; Aqueous: $P < 0.001$). Tukey's post hoc revealed that the standard concentration differed significantly from lower doses ($p < 0.05$).

4.4 Minimum Inhibitory Concentration (MIC) Aqueous and Ethanolic bark extracts of Cinnamomum tamala

The inhibitory effect of the aqueous extract is shown in Table 4.8, where MIC values ranged between 100–200 mg/mL, with *S. aureus* inhibited at 100 mg/mL and the remaining organisms at 200 mg/mL. The ethanolic extract, however, presented lower MIC values. As shown in Table 4.9, *E. coli* exhibited an MIC of 12.5 mg/mL, *S. aureus* and *K. pneumoniae* had 25 mg/mL, while *P. aeruginosa* required 50 mg/mL. The overall MIC pattern, summarized in Table 4.10, revealed lower values for the ethanolic extract compared to the aqueous extract. The Kruskal–Wallis test indicated significant differences in MIC values among organisms for both extracts (Ethanolic: $p < 0.001$; Aqueous: $p < 0.001$), and post hoc analysis revealed that *E. coli* had significantly lower MIC values compared to the others ($p \leq 0.016$).

Table 4.8 Minimum Inhibitory Concentration (MIC) of Aqueous Cinnamomum tamala Extract

Organism	**50mg**	**100mg**	**200mg**	**400mg**	**MIC**
Staphylococcus aureus	G	NG	NG	NG	100mg
Klebsiella pneumoniae	G	G	NG	NG	200mg
Pseudomonas aeruginosa	G	G	NG	NG	200mg
Escherichia coli	G	G	NG	NG	200mg

G: Growth

NG:No Growth

Table 4.9 Minimum Inhibitory Concentration (MIC) of Ethanolic Cinnamomum tamala Extract

Organism	**6.25mg**	**12.5mg**	**25mg**	**50mg**	**100mg**	**MIC**
Staphylococcus aureus	G	G	NG	NG	NG	25mg
Klebsiella pneumoniae	G	G	NG	NG	NG	25mg
Pseudomonas aeruginosa	G	G	G	NG	NG	50mg
Escherichia coli	G	NG	NG	NG	NG	12.5mg

G: Growth

NG:No Growth

Table 4.10 Mean Minimum Inhibitory Concentration (MIC) values of bark Cinnamomum tamala extracts against urinary pathogens.

Organism	**Ethanolic MIC (mg/mL)**	**Aqueous MIC (mg/mL)**
Staphylococcus aureus	25	100
Klebsiella pneumoniae	25	200
Pseudomonas aeruginosa	50	200
Escherichia. coli	12.5	200

Kruskal–Wallis test indicated significant differences in MIC values among organisms (Ethanolic: $p < 0.001$; Aqueous: $p < 0.001$). Post hoc analysis showed that E. coli had significantly lower MIC values compared to other organisms ($p \leq 0.016$).

4.5 Minimum Bactericidal Concentration (MBC) of Aqueous and Ethanolic bark extracts of Cinnamomum tamala

The bactericidal activity of the aqueous extract is presented in Table 4.11, where an MBC value of 200 mg/mL was observed uniformly across all organisms tested. In contrast, the ethanolic extract revealed lower MBC values. As shown in Table 4.12, *E. coli* exhibited an MBC of 25 mg/mL, *S. aureus* and *K. pneumoniae* had 50 mg/mL, while *P. aeruginosa* required 100 mg/mL. A summary comparison of the two extracts, presented in Table 4.13, indicated consistently higher MBC values for the aqueous extract than for the ethanolic extract. The Kruskal–Wallis test revealed significant differences among organisms for the ethanolic extract ($H(3) = 23.0$, $p < 0.001$), while no significant differences were observed for the aqueous extract ($H(3) = 0.00$, $p = 1.000$). Post hoc analysis further revealed that *E. coli* had significantly lower MBC values compared to the other organisms ($p < 0.005$).

Table 4.11 Minimum Bactericidal Concentration (MBC) of Aqueous Cinnamomum tamala Extract

Organism	**100mg**	**200mg**	**400mg**	**MBC**
Staphylococcus aureus	G	NG	NG	200mg
Klebsiella pneumoniae	G	NG	NG	200mg
Pseudomonas aeruginosa	G	NG	NG	200mg
Escherichia coli	G	NG	NG	200mg

G: Growth

NG:No Growth

Table 4.12 Minimum Bactericidal Concentration (MBC) of Ethanolic Cinnamomum tamala Extract

Organism	**12.5mg**	**25mg**	**50mg**	**100mg**	**MBC**
Staphylococcus aureus	G	G	NG	NG	50mg
Klebsiella pneumoniae	G	G	NG	NG	50mg
Pseudomonas aeruginosa	G	G	G	NG	100mg
Escherichia coli	G	NG	NG	NG	25mg

G: Growth

NG:No Growth

Table 4.13. Minimum Bactericidal Concentration (MBC) values of bark extracts against urinary pathogens.

Organism	**Ethanolic MBC (mg/mL)**	**Aqueous MBC (mg/mL)**
Staphylococcus aureus	50	200
Klebsiella pneumoniae	50	200
Pseudomonas aeruginosa	100	200
Escherichia. coli	25	200

Kruskal–Wallis test showed significant differences in MBC across organisms for the ethanolic extract ($H(3) = 23.0$, $p < 0.001$), but not for the aqueous extract ($H(3) = 0.00$, $p = 1.000$). Post hoc analysis indicates that E.coli had significantly lower MBC values that the other organisms ($p < 0.005$).

CHAPTER FIVE
5.0 DISCUSSION AND CONCLUSION

5.1 Discussion

The present study demonstrated that both ethanolic and aqueous bark extracts of *Cinnamomum tamala* exhibited antibacterial activity against common urinary pathogens, though the ethanolic extract consistently showed greater potency at lower concentrations. Zones of inhibition recorded for ethanolic extracts ranged from 8.25 ± 4.8 mm at 50 µg/mL to 23.75 ± 1.70 mm at standard concentration, while aqueous extracts were largely inactive at lower concentrations but became effective only at higher doses, achieving inhibition comparable to the ethanolic extract at standard concentration. This dose-dependent antibacterial activity suggests that *C. tamala* contains bioactive metabolites with significant therapeutic potential, but solvent polarity strongly influences their extraction efficiency.

These findings align with earlier reports by Goyal *et al*. (2009), who observed concentration-dependent antibacterial activity of crude *C. tamala* extracts against *Escherichia coli* and *Staphylococcus aureus*. Similarly, Mishra *et al*. (2010) reported significant antibacterial efficacy of *C. tamala* leaf oils and ethanolic extracts, with inhibition zones comparable to standard antibiotics. The present results therefore Is in line with the broad-spectrum antibacterial potential of *C. tamala* documented in prior studies. The ethanolic extract was more effective in terms of both MIC and MBC. *E. coli* showed the lowest MIC (12.5 µg/mL) and MBC (25 µg/mL), while *Pseudomonas* required higher concentrations (MIC: 50 µg/mL; MBC: 100 µg/mL). In contrast, aqueous extracts required markedly higher concentrations (100–200 µg/mL MIC and 200 µg/mL MBC for all organisms). The greater susceptibility of *E. coli* aligns with findings by Kalauni *et al*. (2021), who demonstrated that ethanolic *C. tamala* extracts displayed stronger inhibition against Gram-negative bacteria compared to Gram-positive species. The differences in

susceptibility between pathogens may reflect variations in cell wall architecture. Gram-negative bacteria possess an outer membrane with lipopolysaccharides that typically limit antibiotic entry, but hydrophobic phytochemicals such as terpenoids and flavonoids in ethanolic extracts can penetrate these barriers, explaining their strong activity against *E. coli*.

The aqueous extracts' relatively poor performance parallels results in *C. zeylanicum*, where aqueous bark extracts displayed weaker antibacterial activity than ethanol or methanol extracts (Abdulrasheed and Ibrahim, 2019). This disparity can be attributed to the poor solubility of lipophilic compounds such as steroids, terpenoids, and certain phenols in water, thus reducing the concentration of active molecules in aqueous fractions. Phytochemical screening of *C. tamala* bark revealed the presence of alkaloids, flavonoids, tannins, terpenoids, and phenols in both aqueous and ethanolic extracts. Steroids and resins were detected only in ethanolic extracts, whereas saponins and glycosides were exclusive to aqueous extracts. This distribution of compounds likely explains the higher efficacy of ethanolic extracts. Flavonoids and tannins exert antibacterial action through protein precipitation and disruption of bacterial cell walls, while terpenoids and steroids enhance membrane permeability and cause leakage of cellular contents (Borkataky and Kaushal, 2014). Phenolic compounds also act as proton exchangers, collapsing bacterial proton gradients and inhibiting ATP production (Upadhyay, 2017).

Interestingly, the exclusive presence of steroids and resins in ethanolic extracts may explain their superior activity. Previous reports highlight that steroids possess strong membrane-disrupting properties, enhancing the antibacterial spectrum of plant extracts (Kumar *et al*., 2025). Conversely, while saponins (detected only in aqueous extracts) can form complexes with

membrane sterols, their effectiveness may be limited without synergy from more lipophilic metabolites.

The GC–MS profile further substantiated the phytochemical screening. The ethanolic extract was dominated by terpenoids and fatty acid derivatives such as squalene (21.13%), octadecenoic acid derivatives (17.62%), and aldehydes (16.89%). These compounds have well-documented antimicrobial roles. Squalene disrupts bacterial cell membranes and has been linked to both antibacterial and antioxidant activity (Keloth *et al*., 2018). Similarly, fatty acid esters and aldehydes interfere with membrane integrity and enzyme systems, enhancing bactericidal potential. In contrast, the aqueous extract was enriched in bicyclic hydrocarbons (9-borabicyclo[3.3.1]nonane, 28.24%) and monoterpenes (cyclopropane derivatives, 17.04%). These compounds, while bioactive, are generally less potent antibacterial agents than the terpenoids and steroids found in ethanolic extracts. This compositional difference explains the weaker antibacterial performance of aqueous extracts observed in this study. The results are consistent with antibacterial studies on other *Cinnamomum* species. For instance, ethanolic bark extracts of *C. zeylanicum* demonstrated higher antibacterial activity than aqueous extracts against *E. coli* and *S. aureus* (Varalakshmi *et al*., 2014). Likewise, comparative analyses of *Cinnamomum* species have shown that bark extracts generally possess stronger antimicrobial activity than leaf extracts, due to higher concentrations of terpenoids and essential oils (Keloth *et al*., 2018).

Overall, the findings of this study confirm that ethanolic extracts of *C. tamala* bark have significant antibacterial potential against urinary pathogens, outperforming aqueous extracts due to higher concentrations of bioactive metabolites such as squalene, terpenoids, steroids, and fatty

acid esters. These results corroborate and extend previous research on *C. tamala* and related *Cinnamomum* species, reinforcing the role of extraction solvent in modulating phytochemical yield and antibacterial efficacy.

5.2 Conclusion

This study demonstrated that bark extracts of *Cinnamomum tamala* possess significant antibacterial activity against urinary pathogens, with ethanolic extracts exhibiting stronger and broader efficacy than aqueous extracts. The ethanolic extract achieved lower MIC and MBC values, particularly against *E. coli*, indicating its superior potency at reduced concentrations. Phytochemical screening revealed that both extracts contained alkaloids, flavonoids, tannins, terpenoids, and phenols; however, the ethanolic extract uniquely contained steroids and resins, while the aqueous extract was richer in saponins and glycosides. GC–MS profiling further confirmed that the ethanolic extract was dominated by terpenoids and fatty acid derivatives such as squalene, octadecenoic acid esters, and aldehydes, compounds well known for their antimicrobial properties. In contrast, the aqueous extract was characterized by bicyclic hydrocarbons and monoterpenes, which are less effective antibacterial agents.Collectively, these findings suggest that the solvent used for extraction plays a critical role in determining the phytochemical composition and antibacterial potency of *C. tamala*. The stronger performance of ethanolic extracts highlights their promise as a potential source of plant-based antibacterial agents, especially in the treatment of urinary tract infections caused by multidrug-resistant bacteria. These results also support the ethnomedicinal use of *C. tamala* and provide scientific justification for its continued exploration as a natural therapeutic candidate.

5.3 Recommendations

The findings from the study show that ethanolic extract of *Cinnamomum tamala* bark possesses more bioactive compounds responsible for the antibacterial activity of *Cinnamomum tamala* bark, particularly the terpenoids, fatty acid esters, and steroids identified in the ethanolic extract. Advanced in vivo studies and clinical trials are needed to validate the safety, efficacy, and dosage parameters of these extracts in managing urinary tract infections and other bacterial diseases. Given the higher potency of ethanolic extracts, future work should also explore optimization of extraction techniques, such as supercritical fluid extraction or green solvent methods, to maximize yield of active metabolites. Additionally, synergistic studies combining *C. tamala* extracts with standard antibiotics may provide valuable insight into its potential role in combating multidrug-resistant pathogens. From an industrial perspective, the promising antibacterial properties of *C. tamala* highlight its potential for development into herbal formulations, nutraceuticals, or natural preservatives in the food and pharmaceutical industries. Finally, given its ethnomedicinal relevance, awareness campaigns and collaborative research between traditional practitioners and scientific institutions should be encouraged to ensure sustainable utilization and conservation of this valuable medicinal plant.

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APPENDIX I

Compounds Identified by GC–MS in the Aqueous Bark Extract of *Cinnamomum tamala*

Pk#	RT (min)	Area%	First Compound	Quality
1	5.373	0.1	1-Fluorononane	58
2	5.466	0.14	Benzene, 1-ethyl-2-methyl-	70
3	5.615	0.23	1,3-Cyclohexanediamine	62
4	5.674	0.2	Benzene, 1,2,3-trimethyl-	90
5	11.578	2.2	Cyclohexanol, 5-methyl-2-(1-methylethyl)-	91
6	12.116	0.24	α-Terpineol	50
7	12.266	0.9	Cyclohexanol, 2-methyl-5-(1-methylethenyl)-	94
8	13.023	0.34	2-Cyclohexen-1-ol, 2-methyl-5-(1-methylethylethenyl)-, cis-	95
9	13.4	3.2	Carveol	87
10	13.507	5.78	D-Carvone	92
11	13.59	17.04	Cyclopropane, 1,2-dimethyl-3-methylene-	47
12	13.622	28.24	9-Borabicyclo[3.3.1]nonane, 9-ethyl-	42
13	14.841	0.34	Bicyclo[4.1.0]heptane, 3,7,7-trimethyl-	94
14	16.818	0.1	2-Cyclohexen-1-ol, 2-methyl-5-(1-methylethenyl)-, acetate	99
15	18.215	0.26	Caryophyllene	98
16	22.76	0.22	Hexadecane	97
17	22.901	0.43	Dodecanoic acid	95
18	23.32	0.53	Epicubenol	98
19	29.538	0.43	Hexadecanoic acid, methyl ester	99
20	31.113	0.34	8,11-Octadecadienoic acid, methyl ester	99
21	31.157	2.2	9-Octadecenoic acid (Z)-, methyl ester	99
22	31.342	0.78	Methyl stearate	98
23	31.64	2.26	Isopropyl tetracosyl ether	64
24	31.69	0.73	tert-Hexadecanethiol	96
25	31.766	3.75	1-Nonadecene	99
26	31.868	1.06	cis-Vaccenic acid	83
27	31.925	2.65	Ethanol, 2-(tetradecyloxy)-	84
28	32.913	0.06	1-Docosene	91
29	33.744	8.9	cis-Vaccenic acid	90
30	33.881	0.46	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	97
31	33.899	0.36	9-Octadecenoic acid (Z)-, 2-hydroxyethyl ester	87
32	33.939	1.75	Oleic Acid	91
33	36.04	4.59	cis-Vaccenic acid	90
34	36.056	0.54	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	94
35	36.102	3.72	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	97

Compounds Identified by GC–MS in the Ethanolic Bark Extract of *Cinnamomum tamala*

Pk#	RT (min)	Area%	First Compound	Quality	Column 1
1	5.214	0.43	4-Heptafluorobutyryloxyhexadecane	52	1
2	5.367	0.19	Cyclopropane, octyl-	46	1
3	5.479	0.54	Benzene, 1-ethyl-3-methyl-	64	1
4	5.686	0.36	Benzene, 1,2,3-trimethyl-	90	1
5	5.93	0.39	Cycloheptane, methyl-	76	1
6	6.367	1.54	Decane	95	1
7	6.729	0.65	5-Eicosene, (E)-	72	1
8	6.967	0.76	E-15-Heptadecenal	50	1
9	7.093	0.63	Dichloroacetic acid, 2-pentadecyl ester	64	1
10	7.192	0.49	4-Propylcyclohexanone	55	1
11	7.409	1.54	1-Fluorononane	64	1
12	7.583	0.78	Cyanoacetic acid, tetradecyl ester	86	1
13	7.815	2.05	Naphthalene, decahydro-, trans-	93	1
14	7.94	1.76	Decane, 1-fluoro-	49	1
15	8.106	2.3	2-Trifluoroacetoxytridecane	60	1
16	8.313	2.45	Oxalic acid, allyl undecyl ester	58	1
17	8.672	1.01	1-Nonadecene	47	1
18	8.83	2.08	Pentadec-7-ene, 7-bromomethyl-	53	1
19	9.168	0.87	Undecane	81	1
20	9.408	0.22	Naphthalene, decahydro-2-methyl-	96	1
21	12.09	0.13	Dodecane	93	1
22	14.333	0.04	Heptadecane, 8-methyl-	72	1
23	17.566	0.43	7-Tetradecene, (E)-	49	1
24	17.684	0.2	Tetradecane	97	1
25	20.101	0.1	Heptadecane	72	1
26	20.29	0.54	Hentriacontane	58	1
27	21.234	0.22	Eicosane, 1-iodo-	62	1
28	22.754	0.65	Hexadecane	96	1
29	23.877	0.65	Epinephrine	35	1
30	24.243	0.08	Tetrapentacontane, 1,54-dibromo-	42	1
31	24.4	0.15	Hexadecane, 1-(ethenyloxy)-	48	1
32	24.943	0.38	7-Methyl-Z-tetradecen-1-ol acetate	41	1
33	25.095	0.4	Dodecane, 2-methyl-	83	1
34	25.222	0.87	Heptadecane	91	1
35	25.427	0.26	Oxalic acid, 3,5-difluorophenyl tetradecyl ester	64	1
36	25.542	0.76	Aspidospermidin-17-ol derivative	70	1
37	25.895	0.71	Oleic Acid	55	1
38	26.212	1.71	Eicosane	91	1
39	26.411	0.54	Octadecane	86	1
40	26.497	0.47	1-Octadecanesulphonyl chloride	62	1
41	26.639	1.3	2-Methylhexacosane	58	1

42	26.953	0.76	Heptadecane, 3-methyl-	95	1
43	27.185	1.05	5-Eicosene, (E)-	99	1
44	27.32	1.36	Octadecane	97	1
45	27.477	1.65	5-Octadecene, (E)-	90	1
46	29.151	0.07	Nonadecane	94	1
47	29.391	0.09	Heptadecane	91	1
48	29.574	0.65	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	97	1
49	29.887	0.08	Nonane, 5-butyl-	92	1
50	30.003	0.05	Dibutyl phthalate	52	1
51	30.224	0.87	1-Octadecene	95	1
52	30.399	0.06	E-15-Heptadecenal	95	1
53	31.1	0.37	Heptadecane, 2-methyl-	87	1
54	31.348	0.65	Octadecane, 3-methyl-	90	1
55	31.64	0.59	Cyclotetradecane, 1,7,11-trimethyl-4-(1-methylethyl)-	81	1
56	31.77	0.87	1-Docosene	98	1
57	32.704	16.89	13-Octadecenal, (Z)-	90	1
58	32.909	5.98	Carbonic acid, but-2-yn-1-yl eicosyl ester	96	1
59	33.949	2.95	Bis(tridecyl) phthalate	80	1
60	34.173	1.35	1-Hexacosene	95	1
61	34.683	17.62	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	92	1
62	36.276	21.13	Squalene	91	1

APPENDIX II

PLANT VERIFICATION CERTIFICATE

Prof. Akinnibosun Henry Adewale (FLS, MRSB; London)
Faculty of Life Sciences,
Department of Plant Biology and Biotechnology,
P. M. B. 1154 Ugbowo, 300283 Benin City,
Edo State, Nigeria.

University of Benin

Department of Plant Biology and Biotechnology

Herbarium Unit

Faculty of Life Sciences

University of Benin, Benin City, Edo State

Plant Name: *Cinnamomum tamala* (Buch.-Ham.) T. Ness & C. H. Eberm.

Family: Lauraceae

Common Name: Cinnamon

Voucher Number: UBH-C395

Student Name: Ovigueroye Ogheneyoma Promise

Plant Identification and Voucher Number Issued by:

20/05 /2025

Prof. Akinnibosun Henry Adewale (FLS, MRSB; London, MECOSON, LMBOSON, MAEIAN; MFBAN Nigeria).

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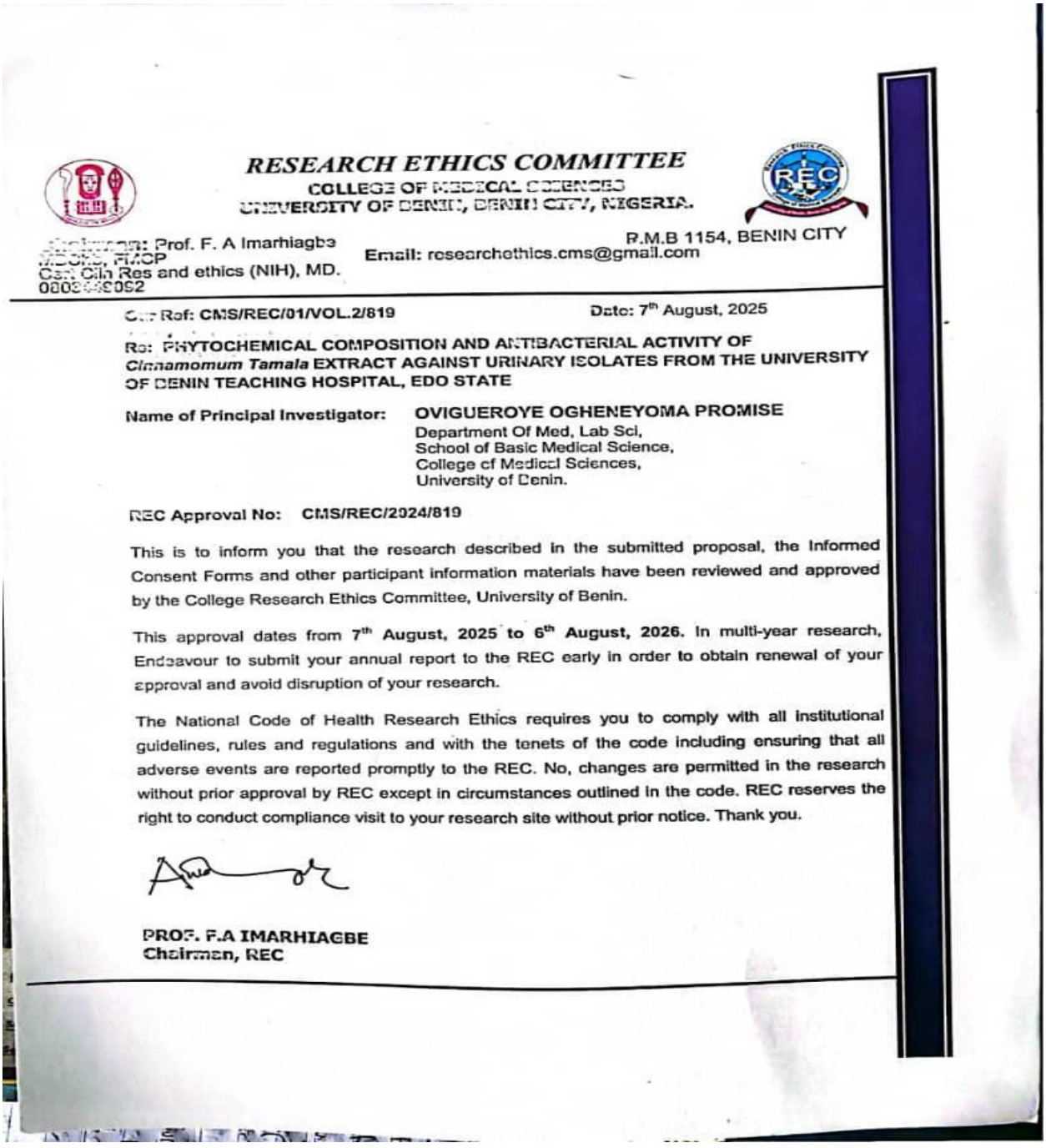
APPENDIX III

ETHICAL APPROVAL CERTIFICATE



RESEARCH ETHICS COMMITTEE
COLLEGE OF MEDICAL SCIENCES
UNIVERSITY OF BENIN, BENIN CITY, NIGERIA.

Chairman: Prof. F. A Imarhiagbe
MBBS, FMCP
Cert. Clin Res and ethics (NIH), MD.
0000-0002-0000-0092

P.M.B 1154, BENIN CITY
Email: researchethics.cms@gmail.com

Our Ref: CMS/REC/01/VOL.2/819 Date: 7th August, 2025

Re: PHYTOCHEMICAL COMPOSITION AND ANTIBACTERIAL ACTIVITY OF *Cinnamomum Tamala* EXTRACT AGAINST URINARY ISOLATES FROM THE UNIVERSITY OF BENIN TEACHING HOSPITAL, EDO STATE

Name of Principal Investigator: OVIGUEROYE OGHENEYOMA PROMISE
Department Of Med, Lab Sci,
School of Basic Medical Science,
College of Medical Sciences,
University of Benin.

REC Approval No: CMS/REC/2024/819

This is to inform you that the research described in the submitted proposal, the Informed Consent Forms and other participant information materials have been reviewed and approved by the College Research Ethics Committee, University of Benin.

This approval dates from 7th August, 2025 to 6th August, 2026. In multi-year research, Endeavour to submit your annual report to the REC early in order to obtain renewal of your approval and avoid disruption of your research.

The National Code of Health Research Ethics requires you to comply with all institutional guidelines, rules and regulations and with the tenets of the code including ensuring that all adverse events are reported promptly to the REC. No, changes are permitted in the research without prior approval by REC except in circumstances outlined in the code. REC reserves the right to conduct compliance visit to your research site without prior notice. Thank you.

PROF. F.A IMARHIAGBE
Chairman, REC

APPENDIX IV

MACERATION OF THE EXTRACT

APPENDIX V