PROJECT RESEARCH WORK

ON

ANTIBACTERIAL EFFICACY OF BLUE GUM (EUCALYPTUS GLOBULUS) ON METHICILLIN RESISTANT *Staphylococcus aureus* AND E*scherichia coli*

BY

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CERTIFICATION

This is to certify that this Project report was written by ADEMOLA MONSURAT YETUNDE with matric number HND/23/SLT/FT/0465 and submitted to the Department of Science Laboratory Technology (S.L.T), Microbiology Unit, Institute of Applied Sciences (IAS), Kwara State Polytechnic, and has been read and approved as a partial fulfillment for the award of Higher National Diploma (HND) in Science Laboratory Technology.

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DEDICATION

This project work is dedicated to Almighty God and also my parent Mr and Mrs Ademola. Also to my supervisor Mrs Dagba IB for her support and advice through our project write up.

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First and foremost, I express my sincere gratitude to the Almighty God for granting me the strength, wisdom, and perseverance to successfully complete this report.

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To all my lecturers, colleagues, and friends who contributed directly or indirectly to the completion of this work, I say thank you.

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ABSTRACT

This study investigated the antibacterial efficacy of aqueous and ethanol extracts of Eucalyptus globulus leaves against Methicillin-Resistant Staphylococcus aureus (MRSA) and Escherichia coli, two clinically important and drug-resistant bacterial pathogens. The study was carried out at the Microbiology Laboratory Unit of Kwara State Polytechnic, Ilorin, Nigeria. Clinical isolates were obtained from the University of Ilorin Teaching Hospital (UITH) . Fresh Eucalyptus globulus leaves collected locally were processed and extracted using aqueous. Phytochemical screening was performed to identify key bioactive constituents. The antibacterial activity of the extracts was assessed using the agar well diffusion method, and minimum inhibitory concentration (MIC) was determined through the agar dilution technique. Phytochemical analysis revealed the presence of flavonoids, tannins, and phenolic compounds in both extracts. The agar well diffusion test demonstrated that both extracts possessed antibacterial activity, with the ethanol extract showing a significantly higher zone of inhibition compared to the aqueous extract. The zone of inhibition increased with higher volumes of extract applied, indicating a dose-dependent effect. The ethanol extract exhibited maximum inhibitory activity at 300 µl, with mean inhibition zones of 0.80 ± 0.04 mm and 0.80 ± 0.03 mm for MRSA and E. coli respectively. The ethanol and ionized water showed significant inhibition, which confirmed their effect. MIC determination further confirmed the antibacterial potency of the ethanol extract, with complete growth inhibition observed at 0.2 v/v concentration for both bacterial isolates. The results of this study support the potential use of Eucalyptus globulus leaf extracts, particularly ethanol-based extracts, as a source of natural antibacterial agents. The presence of multiple phytochemicals known for antimicrobial activity likely contributed to the observed effects. Further studies are recommended to isolate active compounds, test in vivo effectiveness, and evaluate toxicity to support future applications in pharmaceutical or herbal medicine.

CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

The growing challenge of antibiotic resistance represents one of the most serious threats to public health today. Across the world, bacterial infections are becoming harder to treat because many common antibiotics are losing their effectiveness (World Health Organization, 2020). Two notable examples of antibiotic-resistant pathogens are Methicillin-Resistant *Staphylococcus aureus* (MRSA) and drug-resistant *Escherichia coli* (*E. coli*). MRSA is a major cause of hospital and community-acquired infections, while *E. coli* strains have been linked to serious infections such as urinary tract infections and bloodstream infections (Prestinaci, Pezzotti, & Pantosti, 2019).

Resistance in MRSA and *E. coli* is not only making treatment difficult but is also increasing the costs of healthcare and leading to higher rates of morbidity and mortality. The Centers for Disease Control and Prevention (CDC) in 2022 reported that antibiotic-resistant infections cause over 35,000 deaths annually in the United States alone. The situation is even worse in low and middle-income countries where access to advanced antibiotics is limited (CDC, 2022). As a result, there is a critical need to search for new, effective, and affordable antimicrobial agents.

Plants have always been a valuable source of medicines. Many modern antibiotics and drugs have been derived directly or indirectly from plant sources. In the search for alternatives to synthetic antibiotics, medicinal plants are gaining attention for their antimicrobial activities (Ahmed *et al*., 2021). *Eucalyptus globulus*, commonly known as the Blue Gum tree, is one such plant that had shown promising antimicrobial properties. It belongs to the Myrtaceae family and is widely cultivated for its essential oils, which are rich in bioactive compounds (Silva *et al*., 2020). The essential oils extracted from *Eucalyptus globulus* leaves are known to contain compounds

such as 1,8-cineole (eucalyptol), α-pinene, and limonene. These compounds have demonstrated antibacterial, antiviral, and antifungal activities in several studies (da Silva *et al*., 2020). Notably, 1,8-cineole has been shown to damage bacterial cell walls, disrupt membranes, and inhibit the growth of both Gram-positive and Gram-negative bacteria (Gomes *et al*., 2021). This makes *Eucalyptus globulus* a potential candidate for combating bacterial pathogens like MRSA and *E. coli*.

Recent research indicates that essential oils could be particularly effective against resistant strains of bacteria. Unlike traditional antibiotics that often target a single cellular process, essential oils contain multiple active compounds that can attack bacteria in different ways simultaneously. This multi-target approach reduces the chance of bacteria developing resistance quickly (Aljaafari *et al*., 2022). Moreover, essential oils are generally considered safe, biodegradable, and less toxic compared to synthetic antibiotics when used appropriately (Júnior *et al*., 2020).

Traditionally, *Eucalyptus globulus* has been used in various cultures to treat infections, wounds, and respiratory problems. In modern times, scientific studies have supported some of these uses by demonstrating the antibacterial activity of *Eucalyptus* extracts against a range of pathogens. For example, a study by Salehi *et al*. (2021) found that *Eucalyptus globulus* essential oil exhibited significant inhibitory effects against multidrug-resistant *E. coli* strains isolated from clinical samples. Similarly, Elbehiry *et al*. (2022) reported that *Eucalyptus globulus* oil effectively inhibited the growth of MRSA isolates collected from food and clinical settings.

Despite these findings, there are still gaps in the literature regarding the specific effectiveness of *Eucalyptus globulus* against MRSA and *E. coli*, especially using local strains isolated from different environments. More laboratory-based investigations are needed to confirm its antibacterial potential, explore its mechanism of action, and determine its possible applications in

healthcare settings. In the face of the global antibiotic resistance crisis, exploring natural resources like *Eucalyptus globulus* is not just timely but necessary. Natural products offer a rich and relatively untapped reservoir of potential solutions to one of the most pressing medical challenges of the 21st century.

1.1 Statement of Problem

The rise of antibiotic-resistant bacteria, particularly Methicillin-Resistant *Staphylococcus aureus* (MRSA) and resistant strains of *Escherichia coli,* poses a major public health challenge worldwide. Traditional antibiotics that were once effective are now often failing, leading to persistent infections, increased hospital stays, higher healthcare costs, and greater risk of death (World Health Organization, 2020). Despite ongoing research and the development of new antibiotics, the speed at which bacteria are evolving resistance often outpaces drug discovery efforts (Prestinaci, Pezzotti, & Pantosti, 2019).

Eucalyptus globulus, widely recognized for its traditional medicinal use, has shown potential antibacterial effects against various microorganisms. However, there is limited comprehensive data on its specific efficacy against resistant strains like MRSA and *E. coli* under controlled laboratory conditions (Salehi *et al*., 2021). Most available studies have focused on general antimicrobial properties, but few have directly compared its activity against these two clinically significant bacteria hence this study of its efficacy on *MRSA* and *E. coli.*

1.1 Justification

The growing problem of antibiotic resistance has created an urgent need for alternative antimicrobial agents. This study is significant because it explores the antibacterial potential of *Eucalyptus globulus*, a plant known for its medicinal properties, against two important resistant bacteria: MRSA and *Escherichia coli*. The study could contribute valuable knowledge to the

search for new, plant-based therapies to combat resistant infections. If *Eucalyptus globulus* proves effective, it could offer a safer, cheaper, and more natural alternative to synthetic antibiotics, especially in low- and middle-income countries where access to modern drugs is limited (Salehi *et al*., 2021; Ahmed *et al*., 2021).

The study will also add to the existing body of scientific knowledge by providing updated information on the antibacterial effects of *Eucalyptus globulus* specifically against MRSA and *E. coli*. This could encourage further research, clinical trials, and the development of new natural health products. Finally, the findings could support public health efforts to integrate traditional medicinal plants into modern healthcare practices, promoting more sustainable and holistic approaches to infection management.

1.3 Aim and Objectives of Study

1.3.1 Aim of the Study

The aim of this study is to evaluate the antibacterial efficacy of *Eucalyptus globulus* extracts against Methicillin-Resistant *Staphylococcus aureus* (MRSA) and *Escherichia coli*.

1.3.2 Specific Objectives

The specific objectives of the study are to:

i. extract and prepare an extracts from *Eucalyptus globulus* leaves.
ii. determine the antibacterial activity of *Eucalyptus globulus* extract against MRSA and *Escherichia coli* using standard microbiological methods.
iii. Check the phytochemical properties of *Eucalyptus globulus*
iv. assess the minimum inhibitory concentration (MIC) of *Eucalyptus globulus* extract against the two bacterial strains.

CHAPTER TWO

LITERATURE REVIEW

2.1 Antibacterial Agents

Antibacterial agents are substances that kill or inhibit the growth of bacteria. They are used to treat bacterial infections and can either be synthetic (produced artificially) or natural (derived from plants, animals, or microorganisms) (Ventola, 2019). The increasing resistance of bacteria to synthetic antibiotics has led to growing interest in plant-based antibacterial agents, which often contain a wide range of bioactive compounds with potential therapeutic effects (Gajdács, 2020). Plant extracts, essential oils, and phytochemicals such as flavonoids, tannins, and terpenoids have shown significant antibacterial activities against both Gram-positive and Gram-negative bacteria (Echeverría & Albuquerque, 2020). Their mechanisms of action include disrupting bacterial cell walls, interfering with metabolism, and inhibiting nucleic acid synthesis. Natural products offer advantages such as lower toxicity, multiple modes of action, and the potential to reduce antibiotic resistance when used properly (Borges *et al*., 2020).

Today, antibacterial agents come from various sources, including natural products, chemical synthesis, and biotechnological modifications. They function by targeting essential bacterial structures or processes, such as the cell wall, protein synthesis, nucleic acid replication, and metabolic pathways, ultimately leading to bacterial death or growth inhibition (Echeverría & Albuquerque, 2020). However, despite their critical role, the growing problem of bacterial resistance to existing antibiotics presents a major challenge to global health.

In recent years, attention has increasingly turned toward exploring new antibacterial agents, particularly those derived from natural sources like plants. Studies have shown that certain medicinal plants, such as Eucalyptus globulus, contain essential oils and phytochemicals with

strong antibacterial properties (Salehi *et al*., 2021). These natural compounds offer the advantage of being less likely to induce resistance compared to synthetic antibiotics, and they may provide multiple mechanisms of action against bacterial pathogens (Boukhatem *et al*., 2020). Moreover, the urgency of discovering novel antibacterial agents has been heightened by the emergence of multidrug-resistant organisms such as Methicillin-Resistant Staphylococcus aureus (MRSA) and drug-resistant strains of Escherichia coli (Elbehiry *et al*., 2022). These resistant strains cause infections that are harder and more expensive to treat, and they lead to higher rates of mortality and prolonged hospital stays. As a result, research into plant-based antibacterial agents is gaining significant momentum, offering hope for new, effective treatments that can complement or replace existing antibiotics.

2.1.1 Types of Antibacterial Agents

Antibacterial agents can be broadly classified based on their origin, spectrum of activity, and mechanism of action. These agents have revolutionized the treatment of bacterial infections, enabling healthcare professionals to manage diseases that were once considered fatal. This section explores the different types of antibacterial agents, highlighting their origins, therapeutic roles, and methods of action against pathogenic bacteria.

Origin of Antibacterial Agents

Antibacterial agents can be categorized based on their origin, which determines their composition and the process of their development. The three main types of antibacterial agents based on origin are natural, semi-synthetic, and synthetic agents.

Natural Antibacterial Agents

These agents are derived from naturally occurring sources, such as microorganisms, plants, and other natural substances. One of the most notable examples is penicillin, discovered from the mold

Penicillium . Natural compounds have been a cornerstone of antibiotic development, with many modern antibiotics still based on their natural origins. Plant-based antibacterial agents, such as those derived from Eucalyptus globulus, have also gained attention for their promising antibacterial properties, offering an alternative to synthetic antibiotics (Salehi *et al*., 2021).

Semi-Synthetic Antibacterial Agents

Semi-synthetic antibiotics are chemical derivatives of natural compounds. These agents are modified to enhance their antibacterial activity or to overcome specific bacterial resistances. For example, amoxicillin, derived from penicillin, has been chemically altered to expand its effectiveness against a broader range of bacteria, particularly Escherichia coli and Streptococcus species (Livermore, 2020).

Synthetic Antibacterial Agents

These antibiotics are entirely chemically synthesized in laboratories. Sulfonamides and fluoroquinolones are prime examples of synthetic agents that were developed to target specific bacterial processes (Blair *et al*., 2015). The advantage of synthetic agents lies in their ability to be tailored for specific therapeutic needs, providing a wide range of options for clinicians.

2.1.2 Spectrum of Activity

The spectrum of activity refers to the range of bacterial species that an antibacterial agent can effectively target. Based on their spectrum, antibacterial agents are classified into narrow-spectrum and broad-spectrum agents.

Narrow-Spectrum Antibacterial Agents

These agents target specific types of bacteria. For example, vancomycin is particularly effective against Gram-positive bacteria such as Staphylococcus aureus and Clostridium difficile (Cohen, 2019). Narrow-spectrum agents are typically preferred when the causative bacterial pathogen is

known, as they minimize the disruption to beneficial microbiota and reduce the likelihood of resistance development.

Broad-Spectrum Antibacterial Agents

In contrast, broad-spectrum antibiotics are effective against a wide range of bacterial species, both Gram-positive and Gram-negative. Tetracyclines and fluoroquinolones are prime examples of broad-spectrum antibiotics (Deo *et al*., 2018). These agents are particularly useful when the infecting pathogen is unknown, or when multiple bacteria are involved in an infection. However, overuse of broad-spectrum antibiotics is associated with the development of resistance and disruption of the normal microbiome.

2.1.3 Mechanisms of Action

Antibacterial agents exert their therapeutic effects by targeting specific bacterial structures or processes, thereby interfering with bacterial growth or survival. These mechanisms can be grouped into several categories based on the target within the bacterial cell.

1. Cell Wall Synthesis Inhibitors

Many antibiotics, such as β-lactams (including penicillin and cephalosporins), target bacterial cell wall synthesis. Bacterial cell walls are crucial for maintaining the structural integrity of the cell, and when their synthesis is blocked, the bacteria are unable to survive. This leads to cell lysis and death, particularly in Gram-positive bacteria (Gajdács, 2020).

2. Protein Synthesis Inhibitors

Tetracyclines, macrolides, and aminoglycosides are antibiotics that inhibit bacterial protein synthesis. These agents bind to bacterial ribosomes and prevent the assembly of proteins, essential for bacterial growth and function. Without the ability to synthesize proteins, bacteria are unable to replicate or perform vital functions, leading to their death or inhibition (Boukhatem *et al*., 2020).

3. DNA Synthesis Inhibitors

Fluoroquinolones such as ciprofloxacin and levofloxacin work by inhibiting the enzymes involved in DNA replication, such as DNA gyrase and topoisomerase. These enzymes are necessary for maintaining the integrity of the bacterial genome, and their inhibition prevents bacterial DNA from unwinding, ultimately leading to bacterial cell death (Echeverría & Albuquerque, 2020).

4. Cell Membrane Disruptors

Polymyxins, such as polymyxin B and colistin, interact with the outer membrane of Gram-negative bacteria, increasing membrane permeability. This leads to the leakage of vital cellular contents, causing cell death. Polymyxins are often reserved for multi-drug-resistant Gram-negative infections due to their potency and potential toxicity (Gorib *et al*., 2021).

5. Metabolic Pathway Inhibitors

Sulfonamides and trimethoprim inhibit bacterial folic acid synthesis, a pathway crucial for bacterial survival. By blocking the production of folic acid, which is essential for DNA synthesis and cell division, these antibiotics effectively stop bacterial replication and growth (Blair *et al*., 2015).

2.1.4 Combination Therapy

In the fight against multidrug-resistant bacteria, combination therapies have become an essential tool. These therapies involve the use of two or more antibacterial agents simultaneously, often with complementary mechanisms of action. This approach can enhance the antibacterial effect, prevent the emergence of resistance, and improve treatment outcomes (Paterson & Bonomo, 2020). For example, combining β-lactams with β-lactamase inhibitors can restore the effectiveness

of the former against bacteria that produce β-lactamase, an enzyme that degrades penicillin and related drugs.

2.2 Overview of Eucalyptus globulus

Eucalyptus globulus is commonly known as the blue gum tree and it is a species of evergreen tree native to Australia. It belongs to the Myrtaceae family, which includes a diverse range of trees and shrubs. Eucalyptus globulus is one of the most economically significant species within the genus Eucalyptus, due to its widespread use in the timber, paper, and medicinal industries (Sharma *et al*., 2020). The tree is recognized for its tall stature, with some specimens reaching over 70 meters in height, and its distinctive blue-green leaves that give it the common name "blue gum" (Sharma *et al*., 2020).

Eucalyptus globulus is renowned for its medicinal properties, particularly its antimicrobial, anti-inflammatory, and analgesic activities. The essential oil extracted from the leaves of the tree has long been used in traditional medicine, and its therapeutic potential is now being explored through modern pharmacological research (Mackenzie *et al*., 2019). This essential oil is rich in compounds such as eucalyptol (also known as 1,8-cineole), which is the primary bioactive compound responsible for the plant's antimicrobial properties (Sani *et al*., 2020). The wide application of Eucalyptus globulus in various sectors ranging from the pharmaceutical to the agricultural industries—has fueled interest in its biological activities, particularly its role as a potential source of natural antibacterial agents. Given the rise in antimicrobial resistance, the antibacterial efficacy of Eucalyptus globulus and its essential oil has been the subject of numerous studies, which have demonstrated its effectiveness against a variety of bacterial strains, including Gram-positive and Gram-negative bacteria (Fadeyi *et al*., 2018; Silva *et al*., 2020).

2.3 Methicillin -Resistant Staphylococcus *aureus* (MRSA)

Staphylococcus aureus is a gram-positive bacterium that is a common pathogen in both healthcare and community settings. It is known for causing a wide range of infections, from mild skin infections to severe conditions such as pneumonia, endocarditis, and osteomyelitis (Zong *et al*., 2020). One of the most concerning aspects of S. aureus is its ability to acquire resistance to antibiotics, particularly methicillin, which has led to the emergence of methicillin-resistant Staphylococcus aureus (MRSA).

MRSA refers to strains of S. aureus that have developed resistance to beta-lactam antibiotics, including methicillin, oxacillin, and penicillin. This resistance is due to the acquisition of the mecA gene, which encodes a modified penicillin-binding protein (PBP2a) that has a low affinity for beta-lactam antibiotics (Bouchiat *et al*., 2019). As a result, MRSA is difficult to treat with standard antibiotic therapies, making it a major cause of hospital-acquired infections (HAIs) and a growing concern in community-associated infections (CAIs) (Nash *et al*., 2019).

2.3.1 Mechanisms of Resistance

The resistance of S. aureus to methicillin and other antibiotics is primarily mediated by the mecA gene, which is located on a mobile genetic element known as the staphylococcal cassette chromosome mec (SCCmec). This gene encodes PBP2a, which alters the structure of the bacterial cell wall, rendering it less susceptible to beta-lactams (Zong *et al*., 2020). In addition to the mecA gene, MRSA can also acquire resistance through other mechanisms such as the production of beta-lactamases, which degrade antibiotics, and mutations in other genes involved in cell wall synthesis (Paterson *et al*., 2020).

2.3.2 Epidemiology and Clinical Impact

The prevalence of MRSA has increased significantly over the past few decades, both in hospital and community settings. Hospital-acquired MRSA (HA-MRSA) infections are typically associated with invasive medical procedures such as surgery, catheter insertion, and mechanical ventilation (Zong *et al*., 2020). These infections are often difficult to treat due to the limited number of effective antibiotics available. HA-MRSA infections are associated with higher morbidity, mortality, and healthcare costs (Nash *et al*., 2019).

Community-associated MRSA (CA-MRSA), on the other hand, is a strain of S. aureus that is capable of causing infections in otherwise healthy individuals outside of healthcare settings. These strains tend to be more virulent, often causing skin and soft tissue infections (SSTIs), and have been associated with outbreaks in schools, sports teams, and correctional facilities (Paterson *et al*., 2020). The ability of CA-MRSA to spread in the community is a growing concern, particularly due to the ease of transmission through close contact and shared personal items.

2.4 *Escherichia coli* Infections and Resistance

Escherichia coli (*E. coli*) is a gram-negative bacterium commonly found in the intestines of humans and animals. While many strains of *E. coli* are harmless and even beneficial, certain pathogenic strains can cause a wide variety of infections. These infections range from mild urinary tract infections (UTIs) to severe diseases, including bacteremia, sepsis, and gastroenteritis (Ranjbar *et al*., 2021). In recent years, the increasing resistance of *E. coli* to multiple antibiotics has become a significant public health concern.

2.4.1 Pathogenic Strains of *Escherichia coli*

The majority of *E. coli* infections are caused by specific pathogenic strains. Among the most notable of these are Enterotoxigenic *E. coli* (ETEC), which is commonly associated with traveler's diarrhea, and Enteropathogenic *E. coli* (EPEC), a major cause of infant diarrhea. Another

significant pathogenic strain, Enterohemorrhagic *E. coli* (EHEC), has been linked to outbreaks of foodborne illness, with some strains producing Shiga toxins that can cause hemolytic uremic syndrome (HUS), a potentially fatal condition. Uropathogenic *E. coli* (UPEC) is another key strain, primarily responsible for UTIs, both in community settings and healthcare facilities (Zong *et al*., 2020). These pathogenic strains are equipped with a range of virulence factors, including adhesins, toxins, and the ability to form biofilms, which enable them to adhere to and invade host tissues, evade immune responses, and resist antibiotic treatment (Nataro & Kaper, 2019).

2.4.2 Mechanisms of Antibiotic Resistance in *Escherichia coli*

A significant concern regarding *E. coli* infections is the growing problem of antibiotic resistance. Several mechanisms contribute to the ability of *E. coli* to evade the effects of antibiotics, complicating treatment options and leading to more severe clinical outcomes. One of the most common mechanisms is beta-lactam resistance, which is typically mediated by the production of beta-lactamases. These enzymes degrade beta-lactam antibiotics, such as penicillins and cephalosporins, rendering them ineffective. Extended-spectrum beta-lactamases (ESBLs) are particularly problematic, as they can hydrolyze third-generation cephalosporins and monobactams, which are often used as the first line of treatment in infections caused by *E. coli* (Paterson *et al*., 2020). In addition to beta-lactam resistance, *E. coli* has also developed resistance to other classes of antibiotics, including aminoglycosides and fluoroquinolones. Aminoglycoside resistance is primarily driven by the production of modifying enzymes that inactivate these drugs, while fluoroquinolone resistance is often the result of mutations in the bacterial DNA gyrase and topoisomerase IV, which are the targets of these drugs (Wu *et al*., 2020). Another concerning development is carbapenem resistance. Carbapenems, such as meropenem, are considered last-resort antibiotics for treating multi-drug-resistant (MDR) *E. coli* strains. The emergence of

carbapenemases, such as New Delhi metallo-beta-lactamase (NDM) and Klebsiella pneumoniae carbapenemase (KPC), in *E. coli* poses a serious threat to public health by rendering these antibiotics ineffective and limiting treatment options (Tucker *et al*., 2019). The increasing prevalence of multidrug-resistant *E. coli* strains has significantly impacted clinical outcomes, as these infections are more difficult to treat, often requiring more expensive and toxic drugs. This, in turn, leads to prolonged hospital stays, higher healthcare costs, and increased morbidity and mortality (Guerra *et al*., 2020).

2.4.3 Clinical Impact of *E. coli* Resistance

The rise of antibiotic-resistant *E. coli* strains, particularly those that are multidrug-resistant (MDR), poses significant challenges for healthcare providers. These resistant strains are often more difficult to treat, requiring alternative antibiotics that may not be as effective, or even entirely unavailable in certain settings. In addition to the direct impact on patient outcomes, the spread of resistant *E. coli* strains within healthcare settings can lead to outbreaks, further complicating infection control efforts. For example, resistant strains of *E. coli* are frequently implicated in nosocomial infections, where patients in hospitals or long-term care facilities acquire infections from the healthcare environment itself (Tucker *et al*., 2019).

To combat the increasing prevalence of antibiotic-resistant *E. coli* infections, several strategies have been proposed. Antimicrobial stewardship programs, which aim to optimize antibiotic use in clinical settings, have been shown to be effective in reducing the overuse and misuse of antibiotics, thereby slowing the development of resistance. These programs ensure that patients receive the right antibiotic at the right dose and duration, minimizing unnecessary exposure to broad-spectrum antibiotics (Guerra *et al*., 2020). In addition to stewardship, stringent infection control measures, including hand hygiene, proper sanitation, and isolation of infected patients, are essential for

preventing the transmission of resistant *E. coli* strains in hospitals and other healthcare environments.

Research into alternative therapies is also underway to provide new options for treating resistant *E. coli* infections. One promising approach is bacteriophage therapy, which involves using viruses that specifically target and kill bacteria. This technique has shown promise as an alternative to traditional antibiotics, particularly in cases where conventional treatments have failed (Nataro & Kaper, 2019). Another area of research is the development of vaccines against *E. coli* strains, especially those that cause gastroenteritis and UTIs. Additionally, novel antibiotics targeting new bacterial pathways are being explored, which may provide much-needed solutions for treating resistant *E. coli* infections (Wu *et al*., 2020).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area and Sample Collection

This study was conducted in the Microbiology Laboratory Unit of Kwara State Polytechnic, Ilorin, Nigeria. The clinical isolates of *Methicillin-Resistant Staphylococcus aureus* (MRSA) and *Escherichia coli* (*E. coli*) were obtained from the University of Ilorin Teaching Hospital (UITH), Ilorin, Kwara State. These isolates were selected for their clinical significance and resistance patterns.

3.2 Collection and Preparation of Plant Material

Fresh leaves of *Eucalyptus globulus* were collected from a natural stand in Ilorin, Kwara State, Nigeria. The plant was authenticated by a plant taxonomist at the Department of Plant Biology, University of Ilorin. The leaves were thoroughly washed with clean water to remove dirt, then air-dried at room temperature in a shaded area for 7–10 days. The dried leaves were ground into fine powder using a sterile mortar and pestle.

3.3 Preparation of Plant Extracts

3.3.1 Ethanol Extraction

A total of 30 g portion of the powdered leaves was soaked in 150 ml of absolute ethanol in a sterile conical flask. The mixture was placed in a shaking incubator at 42 °C and 80 rpm for 7 days. After extraction, the solution was filtered using Whatman No.1 filter paper. The filtrate was concentrated by evaporation in a water bath at 40 °C to remove residual ethanol and then stored in sterile bottles at 4 °C for further analysis.

3.4 Phytochemical Screening

Preliminary phytochemical screening was conducted, ethanol extracts of *Eucalyptus globulus* leaves to detect the presence of key secondary metabolites, including alkaloids, flavonoids, tannins, saponins, and phenolic compounds. These tests were performed using standard qualitative methods described by Yakubu *et al*. (2020). Each test is detailed below.

3.4.1 Test for Alkaloids

Two drops of Mayer's reagent were added to 2 ml of each extract . The formation of a cream-colored precipitate indicated the presence of alkaloids in the extract.

3.4.2 Test for Flavonoids

One millilitre of the extract was mixed with a few drops of dilute sodium hydroxide solution. An intense yellow color appeared, which became colorless upon the addition of dilute hydrochloric acid. This color change confirmed the presence of flavonoids.

3.4.3 Test for Tannins

A few drops of 0.1% ferric chloride solution were added to 2ml of the extracts. The formation of a blue-black or greenish-black coloration indicated the presence of tannins.

3.4.4 Test for Saponins

About 5 ml of the extract was mixed with 5 ml of distilled water in a test tube. The mixture was vigorously shaken for 30 seconds and allowed to stand for 10 minutes. The presence of persistent frothing (foam layer) indicated the presence of saponins.

3.4.5 Test for Phenolic Compounds

Two millilitres of the extract were treated with a few drops of 5% ferric chloride solution. The appearance of a deep blue or dark green coloration confirmed the presence of phenolic compounds.

3.5 Standardization of Bacterial Inoculum

The bacterial isolates were standardized by adjusting their turbidity to match that of 0.5 McFarland standard, equivalent to approximately 1.5×10^8 CFU/ml. This standardization ensured uniformity in bacterial concentration during susceptibility testing.

3.6 Antibacterial Susceptibility Testing

3.6.1 Agar Well Diffusion Method

Mueller-Hinton Agar (MHA) plates were prepared and sterilized. The standardized bacterial inoculum was uniformly spread over the surface of the agar plates using a sterile swab. Wells of 6 mm diameter were made using a sterile cork borer. Different volumes (100 µl, 200 µl, and 300 µl) of each extract (aqueous and ethanol) were introduced into the wells. A well containing ethanol alone served as a negative control. The plates were incubated at 37 °C for 24 hours. After incubation, the zones of inhibition were measured in millimeters using a transparent ruler, and the average diameter from three replicates was recorded for each sample.

3.6.2 Minimum Inhibitory Concentration (MIC) Determination

The MIC of the ethanol extract was determined using the agar dilution method. Different concentrations of the extract were incorporated into molten Mueller-Hinton Agar at ratios of 0.15 and 0.2 (v/v). The agar was poured into sterile Petri dishes and allowed to solidify. The standardized bacterial suspensions of MRSA and *E. coli* were then spot-inoculated onto the surface of the agar. The plates were incubated at 37 °C for 24 hours and observed for visible bacterial

growth. The lowest concentration of extract at which no growth was observed was recorded as the MIC.

CHAPTER FOUR

RESULTS

4.1 Phytochemical Screening of Eucalyptus globulus Leaf Extracts

The phytochemical analysis of ethanol extracts of *Eucalyptus globulus* revealed the presence of several bioactive compounds known for their antimicrobial properties. The results are summarized in Table 4.1 below.

Table 4.1: Phytochemical Constituents of Ethanol Extracts of *Eucalyptus globulus*

Phytochemical	Ethanol Extract
Alkaloids	-
Flavonoids	+
Tannins	+
Saponins	-
Phenolic Compounds	+

Key: *(+) = Present*
(-) = Absent

4.2 Antibacterial Activity of Eucalyptus globulus Extracts Using Agar Well Diffusion Method

The antibacterial effects of both aqueous and ethanol extracts of *Eucalyptus globulus* leaves were evaluated against MRSA and *Escherichia coli* using the agar well diffusion method. Different volumes of extracts (100 µl, 200 µl, and 300 µl) were tested, and the zones of inhibition were measured after 24 hours of incubation at 37°C.The ethanol extract showed larger zones of inhibition against both bacterial strains compared to the aqueous extract, indicating higher antibacterial potency.

Table 4.2: Zones of Inhibition (mm) of Eucalyptus globulus Extracts Against MRSA and E. coli Using Agar Well Diffusion

Extract Type	Volume (µl)	Zone of Inhibition (mm) MRSA	Zone of Inhibition (mm) *E. coli*
Aqueous Extract	100	0.30 ± 0.03	0.35 ± 0.04
	200	0.45 ± 0.04	0.50 ± 0.03
	300	0.60 ± 0.05	0.65 ± 0.04
Ethanol Extract	100	0.50 ± 0.04	0.55 ± 0.03
	200	0.65 ± 0.03	0.70 ± 0.02
	300	0.80 ± 0.04	0.80 ± 0.03
Ethanol Control	300	0.00 ± 0.05	0.00 ± 0.04
Aqueous Control (Ionized Water)	300	0.00 ± 0.05	0.00 ± 0.04

4.3 Minimum Inhibitory Concentration (MIC) Determination of Ethanol Extract

The results showed that at the 0.15 concentration, No inhibition of bacterial growth was observed for both organisms. Complete inhibition of growth was achieved at the 0.2 concentration for the *Methicillin-Resistant Staphylococcus aureus (MRSA),* indicating that this represents the minimum inhibitory concentration for MRSA and while no inhibition for *E. coli*.

Table 4.3: MIC of Ethanol Extract of *Eucalyptus globulus* Against MRSA and *E. coli*

Extract Concentration (v/v)	MRSA Growth	*E. coli* Growth
0.15	No Inhibition	No inhibition
0.20	Total Inhibition	No Inhibition

4.5 Discussion

This study was carried out to evaluate the antibacterial efficacy of *Eucalyptus globulus* leaf extracts against two clinically significant bacteria: Methicillin-Resistant *Staphylococcus aureus* (MRSA) alkaloids,and *Escherichia coli*. These organisms were selected due to their public health importance, particularly MRSA, which is resistant to multiple antibiotics, and *E. coli*, which is a common cause of gastrointestinal and urinary tract infections. The confirmation of the bacterial isolates using standard biochemical and microbiological techniques ensured the reliability of the experimental results.

Phytochemical screening of the extracts revealed the presence of several important secondary metabolites, including flavonoids, tannins, and phenolic compounds but alkanoids and saponin were absent. These compounds are known for their various biological activities, especially antimicrobial action. Alkaloids, for instance, interfere with microbial DNA replication; flavonoids disrupt microbial cell membranes; tannins can bind to microbial proteins and enzymes; while phenolics and saponins possess both bacteriostatic and bactericidal effects (Sasidharan *et al*., 2011; Singh *et al*., 2021). The presence of these phytochemicals in *Eucalyptus globulus* may explain the observed antibacterial activity.

The antibacterial assay using the agar well diffusion method showed that both the aqueous and ethanol extracts were effective against MRSA and *E. coli*, but the ethanol extract exhibited greater inhibitory activities. This higher potency of the ethanol extract can be attributed to the better solubility of bioactive compounds in ethanol compared to water. Ethanol, being an organic solvent, is more effective at extracting non-polar or moderately polar compounds like flavonoids, essential oils, and certain alkaloids which possess antimicrobial effects (Alabi *et al*., 2019; Ogunyemi *et al*., 2022). The increase in inhibition zones with higher extract volumes further

demonstrates a dose-dependent relationship. At 300 µl, the ethanol extract produced a zone of inhibition of 0.80 ± 0.04 mm against MRSA and 0.80 ± 0.03 mm against *E. coli*, while the aqueous extract produced zones of 0.60 ± 0.05 mm and 0.65 ± 0.04 mm respectively. These findings align with those reported by Mohamed *et al*. (2020), who found that ethanol extracts of *Eucalyptus globulus* showed significantly higher antibacterial activity than aqueous extracts against drug-resistant strains. The results of the MIC test reinforce the potency of the ethanol extract, as complete inhibition of growth was observed at a relatively low concentration of 0. 2 v/v for Methicillin-Resistant *Staphylococcus aureus* (MRSA). This suggests that even small quantities of the ethanol extract can exert effective antibacterial action. Similar MIC values were reported by Taiwo *et al*. (2021), who tested *Eucalyptus globulus* against multidrug-resistant *Staphylococcus aureus* and other Gram-negative pathogens.

The difference in activity between MRSA (Gram-positive) and *E. coli* (Gram-negative) was minimal in this study, though Gram-negative bacteria are generally considered more resistant due to their outer membrane, which limits the penetration of antibacterial agents. The similar response from both organisms suggests that the phytochemicals in *Eucalyptus globulus* may act through multiple mechanisms that overcome this structural barrier. This supports earlier findings by Akinmoladun *et al*. (2020), who noted that *Eucalyptus globulus* extracts could disrupt both Gram-positive and Gram-negative bacterial membranes. The findings of this study have several practical implications. The growing problem of antibiotic resistance, especially in hospital-acquired infections, necessitates the search for new, effective, and affordable antimicrobial agents. Medicinal plants like *Eucalyptus globulus* offer a promising natural alternative or complementary option, especially in resource-limited settings. The ease of plant collection, low toxicity, and

broad-spectrum activity make them suitable candidates for further development into antimicrobial formulations.

CHAPTER FIVE

SUMMARY, CONCLUSION AND RECOMMENDATION

5.1 Summary

This study evaluated the antibacterial efficacy of aqueous and ethanol extracts of *Eucalyptus globulus* leaves against clinically important bacterial isolates of Methicillin-Resistant *Staphylococcus aureus* (MRSA) and *Escherichia coli*. The bacterial isolates were obtained from the University of Ilorin Teaching Hospital and confirmed using standard microbiological and biochemical methods. Preliminary phytochemical screening revealed no presence of alkaloids and saponins while flavonoids, tannins, and phenolic compounds were present. The antibacterial activity assessed by agar well diffusion showed that ethanol extracts had greater inhibitory effects compared to aqueous extracts, with the zone of inhibition increasing with extract volume. The minimum inhibitory concentration (MIC) determination further confirmed that the ethanol extract inhibited bacterial growth at relatively low concentrations.

5.2 Conclusion

The findings revealed that *Eucalyptus globulus* leaf extracts, especially the ethanol extract, possess significant antibacterial activity against MRSA and *E. coli*. The presence of bioactive phytochemicals (flavonoids, tannins, and phenolic compounds) in the extracts contributes to this effect. These results support the potential use of *Eucalyptus globulus* as a natural source of antibacterial agents, particularly in combating resistant bacterial strains.

5.3 Recommendations

Based on the findings of this study, the following recommendations are made:

1. Further research should be conducted to isolate and characterize the specific bioactive compounds responsible for the antibacterial effects observed.
2. In vivo studies and toxicity evaluations are necessary to assess the safety and efficacy of *Eucalyptus globulus* extracts before clinical applications.
3. Development of formulations using *Eucalyptus globulus* extracts for topical or systemic antibacterial therapy could be explored.
4. Continuous monitoring of resistance patterns in clinical isolates is essential to evaluate the long-term effectiveness of plant-based antibacterial agents.

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APPENDIX

Plate 2: MIC result of *E. coli* at 0.15 concentration

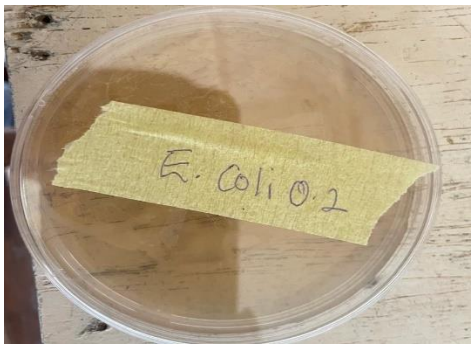

Plate 2: MIC result of *E. coli* at 0.2 concentration

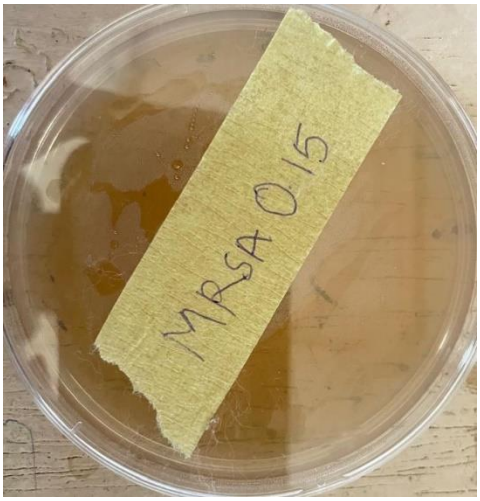

PLATE 3: MIC result of MRSA at 0.15 concentration

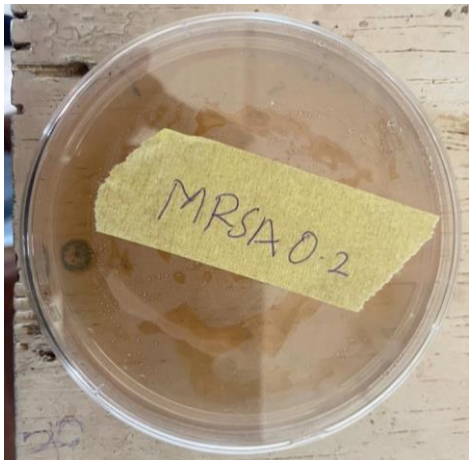

PLATE 3: MIC result of MRSA at 0.2 concentration

UNIVERSITY OF ILORIN, ILORIN, NIGERIA

FACULTY OF LIFE SCIENCES

DEPARTMENT OF PLANT BIOLOGY

HERBARIUM SERVICES FORM

NAME (with Matriculation Number): ADEMOLA MONSURAT Y. HND/23/SLT/FT/0465

INSTITUTION: KWARA STATE POLYTECHNIC

DEPARTMENT: MICROBIOLOGY (SLT)

SERVICES PROVIDED: Eucalyptus globulus

S/No	Services	Descriptions
1	Plant Identification	
2	Collection of Voucher Number	UILH/09/1073/2025
3	Collection of Plant Information	
4	Preparation of Plant Specimen	
5	Collection of Plant materials	
6	Training of personnel	

DATE OF VISIT/SUBMISSION: 26/02/2025

DATE AND TIME OF COLLECTION: 26/02/2025

.....
NAME AND SIGNATURE OF CHAIRMAN

Bdy Ajiji
NAME AND SIGNATURE OF CURATOR