

**ANTIBACTERIAL PROPERTIES OF *Oscuim gratissium* (SCENT LEAF) AGAINST
SELECTED BACTERIA**

BY

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FACULTY OF LIFE SCIENCES

UNIVERSITY OF BENIN

BENIN CITY

NOVEMBER, 2025

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

NOVEMBER, 2025.

CERTIFICATION

This is to certify that this project was carried out by **SAMUEL BORI FAVOUR** with the Matriculation number **LSC12104026** of the Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City, under adequate supervision, in partial fulfillment for the award of a Bachelor of Science degree in Microbiology.

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Date

Prof. E.O IGBINOSA

(Head of Department)

Date

DEDICATION

This project is dedicated to Almighty God, whose wisdom, guidance, and strength have been my constant support throughout this academic journey. His grace made every challenge surmountable and every success possible.

ACKNOWLEDGEMENTS

First and foremost, I give all glory, honor, and praise to Almighty God for His grace, wisdom, and strength throughout the course of this project. His divine guidance, made this achievement.

My heartfelt appreciation also goes to my project supervisor, Prof. (Mrs.) I. S. Obuekwe, for her valuable guidance, patience, and constructive feedback throughout the course of this work day.

My sincere gratitude goes to Mr. Clement Minabowan, who has played a fatherly role in my life. Your support, encouragement, and guidance have meant so much to me. I am deeply thankful to my mother, Mrs. Tina Obaze, for her constant prayers, love, and unwavering support. To my amazing siblings - God's Power, Victor, and Maximilian thank you for always cheering me on, believing in me, and filling my life with love and laughter. I also want to acknowledge my late father, Mr. Samuel Bori, who left this world when I was still a child. Even though you are not here to witness this moment, your memory lives on in my heart. A special thank you to my grandfather, Mr. Obaze Abraham, for your encouragement and for supporting me in my academics.

To my friends, and extended family (Mr Naghede, Simon Ajayi, Blessing Ajayi, Comfort Obaze, Lucky Obaze, Abigail, Esther, Monica and Favour) thank you for always being there for your support, motivation, and companionship during the challenging times. Finally, I would like to acknowledge myself for never giving up, for staying strong through the challenges, and for believing that I could make it. This journey has taught me resilience, patience, and faith.

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ABSTRACT

The growing resistance of pathogenic bacteria to commonly used antibiotics has stimulated renewed global interest in medicinal plants as alternative sources of antimicrobial agents. One such plant, *Ocimum gratissimum* (commonly known as scent leaf), has long been utilized in traditional medicine due to its therapeutic properties. This study investigated the antibacterial activity of scent leaf extracts against selected bacterial pathogens. Fresh leaves of *Ocimum gratissimum* were procured, air-dried, and ground into powder. The powdered material was extracted using ethanol and distilled water to obtain ethanolic and aqueous extracts, respectively. The antibacterial effects of these extracts were evaluated against clinical isolates of *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*, obtained from the University of Benin Teaching Hospital, using the agar well diffusion technique in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines. Phytochemical analysis of both extracts confirmed the presence of alkaloids, flavonoids, tannins, and saponins in varying concentrations, with the ethanolic extract exhibiting a higher abundance of these bioactive compounds. Antibacterial testing revealed a concentration-dependent inhibitory effect for both extracts, with reduced activity observed at lower concentrations. However, the ethanolic extract demonstrated superior antibacterial efficacy, producing inhibition zones of up to 15 mm against *Staphylococcus aureus* and 12 mm against *Pseudomonas* species at a concentration of 100 mg/mL. In contrast, the aqueous extract showed moderate activity, with inhibition zones ranging between 10 mm and 12 mm. The minimum inhibitory concentration (MIC) of the ethanolic extract was determined to be 50 mg/mL for *S. aureus*, *E. coli*, and *Pseudomonas*, while *Klebsiella* species required 100 mg/mL for inhibition. For the aqueous extract, MIC values were only observed at 100 mg/mL for *E. coli* and *Pseudomonas*. Furthermore, the minimum bactericidal concentration (MBC) of the ethanolic extract was 50 mg/mL for *Staphylococcus aureus* and *Pseudomonas*, and 100 mg/mL for *E. coli*, whereas *Klebsiella* species exhibited only bacteriostatic response. The aqueous extract showed bactericidal activity only against *Pseudomonas* at 100 mg/mL. Overall, the results indicate that *Ocimum gratissimum* possesses notable antibacterial properties, particularly when extracted with ethanol. These

findings validate its traditional medicinal use and suggest its potential application in the development of plant-based antimicrobial agents.

CHAPTER ONE

INTRODUCTION

The rapid emergence of resistance among disease-causing microorganisms to commonly used antibiotics has intensified the global search for alternative antimicrobial agents, particularly from plant sources. In this context, *Ocimum gratissimum* L., commonly referred to as scent leaf, has attracted considerable scientific interest due to its notable antibacterial efficacy and rich phytochemical composition. This perennial, aromatic member of the Lamiaceae family is widely distributed across West Africa, especially in Nigeria, where it is deeply embedded in both dietary and traditional healing systems. Locally identified as “Efirin” among the Yoruba, “Nchanwu” by the Igbo, and “Daidoya” in Hausa communities, the plant is valued not only for its characteristic fragrance but also for its medicinal relevance (Ugbogu *et al.*, 2021).

Historically, medicinal plants have played a foundational role in healthcare systems worldwide, with an estimated 80% of the global population depending on traditional medicine for the treatment of various diseases (Chaachouay *et al.*, 2022). Scent leaf, in particular, is commonly cultivated around homes and gardens in West African communities, serving dual purposes in nutrition and therapy. Its aromatic leaves are frequently incorporated into local dishes such as soups, stews, and especially pepper soup, where they enhance both flavor and perceived health benefits (Onoharigho *et al.*, 2022). In recent years, increasing attention has been directed toward its bioactive constituents—including flavonoids, polyphenols, and essential oils—which are believed to contribute to its role in the prevention and management of chronic conditions such as diabetes, cancer, and microbial infections (Eze *et al.*, 2025).

The therapeutic potential of scent leaf is largely linked to its diverse phytochemical makeup. Advanced analytical techniques such as gas chromatography–mass spectrometry (GC-MS) have revealed key compounds like eugenol, thymol, linalool, and β -caryophyllene (Iweala *et al.*, 2023). These constituents are associated with multiple biological activities, including antimicrobial, antifungal, anti-inflammatory, and antioxidant effects. Empirical evidence indicates that ethanolic extracts of *Ocimum gratissimum* exhibit strong inhibitory activity against multidrug-resistant organisms such as *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*, with minimum inhibitory concentrations comparable to those of standard antibiotics (Adeyemi *et al.*, 2023). Moreover, its essential oils have demonstrated the ability to compromise bacterial cell membrane integrity and suppress biofilm formation, mechanisms that are particularly important in addressing antimicrobial resistance (Okoro & Nwankwo, 2024; Chukwuemeka *et al.*, 2024).

Given the increasing global burden of antimicrobial resistance, the exploration of plant-based therapeutics has become more urgent than ever. Scent leaf stands out due to its widespread availability, affordability, and strong ethnomedicinal background, making it a viable candidate for drug development. Beyond its antimicrobial properties, the plant has been reported to exhibit a broad spectrum of pharmacological effects, including antihyperglycemic, antidiarrheal, hepatoprotective, and antihypertensive activities (Akara *et al.*, 2021; Ajayi *et al.*, 2023; Shittu *et al.*, 2024). Ongoing investigations continue to uncover the biochemical pathways responsible for these effects, thereby bridging traditional medicinal knowledge with modern pharmacological science.

Extensive research has validated the antibacterial effectiveness of scent leaf against a variety of pathogenic organisms, including *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Salmonella* species. For example, Adeyemi *et al.* (2023) reported that

ethanolic extracts of the plant significantly inhibited multidrug-resistant strains of *S. aureus* and *E. coli*, with efficacy levels comparable to conventional antimicrobial agents. Similarly, Okoro and Nwankwo (2024) attributed the disruption of bacterial cell membranes by scent leaf essential oils to the presence of potent compounds such as eugenol and thymol.

The plant's phytochemical richness remains central to its therapeutic promise. GC-MS analyses have consistently identified eugenol, linalool, and β -caryophyllene as dominant components, which contribute to its antioxidant, anti-inflammatory, and antimicrobial capabilities (Iweala *et al.*, 2023). These bioactive molecules have been shown to neutralize free radicals and inhibit the formation of bacterial biofilms, a key factor in persistent infections and antibiotic resistance (Chukwuemeka *et al.*, 2024). Furthermore, Eze *et al.* (2025) highlighted the synergistic interactions among these phytochemicals, suggesting that their combined effects may enhance the development of new and more effective therapeutic agents.

Additional findings by Nwosu *et al.* (2024) indicate that the interaction between eugenol and flavonoids in *Ocimum gratissimum* extracts significantly improves antibacterial performance by interfering with multiple metabolic processes within bacterial cells. Beyond medical applications, the plant has also shown promise in food preservation, where its essential oils can inhibit spoilage microorganisms, thereby prolonging shelf life and improving food safety (Okafor *et al.*, 2023). This highlights its potential utility across diverse sectors, including healthcare, agriculture, and the food industry.

Despite these encouraging attributes, certain limitations hinder the full clinical application of scent leaf. One major challenge is the variability in its phytochemical composition, which can be influenced by environmental conditions such as soil quality, climate, and cultivation practices (Eze *et al.*, 2025). Such variations may lead to inconsistencies in therapeutic

outcomes. Consequently, recent research has focused on refining extraction methods and developing standardized formulations to ensure consistent efficacy and safety (Adebayo *et al.*, 2024). As scientific advancements continue, the incorporation of scent leaf into modern healthcare systems appears increasingly feasible, particularly in low-resource settings where access to conventional antibiotics remains limited.

1.2. Aim and Objectives of the study

The aim of this study was to evaluate the antibacterial potential of scent leaf (*Ocimum gratissimum*) on some selected bacterial isolates.

The specific objectives of this research were to:

1. determine the phytochemical constituents present in ethanol and aqueous extract of *Ocimum gratissimum*.
2. evaluate the antibacterial activity of scent leaf extract against some selected bacterial isolates.
3. determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the scent leaf extract against the test bacteria.

CHAPTER TWO

LITERATURE REVIEW

2.1 Botanical Description of *Ocimum gratissimum* (Scent Leaf)

Ocimum gratissimum is a strongly aromatic, herbaceous plant widely recognized by common names such as basil, clove basil, or alfavaca. Taxonomically, it belongs to the family Lamiaceae, under the genus *Ocimum* and species *gratissimum* (Nweze and Eze, 2019). The plant typically grows as an erect, well-branched shrub, characterized by a somewhat woody base and a quadrangular (four-angled) stem structure. Its leaves are arranged oppositely along the stem, appearing slender with slightly serrated or marginal edges, and they emit a distinct fragrance when crushed. This species is perennial in nature and thrives predominantly in tropical and subtropical environments. It is widely distributed across several regions of the world, including countries such as Brazil, India, Vietnam, Rwanda, and Nigeria (Nweze and Eze, 2019), as well as Cameroon, Togo, Côte d'Ivoire, Kenya, Benin (Kpoviessi *et al.*, 2024), and South Africa (Venuprasad *et al.*, 2024). Its adaptability to diverse climatic conditions contributes to its widespread cultivation and use.

Members of the Lamiaceae family are generally known for their aromatic properties and are commonly utilized as culinary herbs, spices, or medicinal plants. This plant family is extensive, consisting of approximately 236 genera and over 7,200 species, which include

herbs, shrubs, vines, and even some tree forms distributed globally. Within this family, the genus *Ocimum* comprises roughly 60 species, many of which are predominantly found in Africa (Tanko *et al.*, 2018).



Figure 2.2. *Ocimum gratissimum* (Sent leaf) (Udochukwu *et al.*, 2015)

2.2 Traditional uses of scent leaf

The basil species *Ocimum gratissimum* is commonly known by several names, including clove basil, African basil, and, in some regions such as Hawaii, wild basil. It originates from parts of southern Asia, Africa, Madagascar, and the Bismarck Archipelago, and has since become naturalized in many regions including Polynesia, Hawaii, Mexico, Panama, the West Indies, Brazil, and Bolivia. The plant is primarily valued for its leaves, which are widely used in food preparation. It belongs to the group of plants categorized as spices. Morphologically, *Ocimum gratissimum* is an erect, small shrub with numerous branches, typically not exceeding one meter in height. It is a member of the Lamiaceae (Labiatae) family and specifically classified under the species *gratissimum*. Although the plant is broadly distributed across tropical and subtropical regions, it exhibits the greatest diversity in tropical

Africa and India. While it is cultivated commercially in countries such as Vietnam, it is more commonly grown as a household garden plant throughout Southeast Asia.

The plant serves multiple purposes, particularly in traditional medicine, where different parts are used to manage ailments such as headaches, fever, asthma, and other conditions (Taur and Patil, 2011). In many parts of the world, scent leaf is also incorporated into a variety of dishes, including salads, soups, pasta, vinegars, and jellies. It is especially popular in Thai cuisine, where it is used as a flavoring agent (Amit, Uddin *et al.*, 2017). Beyond its culinary relevance, scent leaf extracts have long been employed in traditional healing practices for the treatment of fever, cough, body pain, and similar conditions (Ghaleb, 2022). The essential oil derived from its leaves contains notable compounds such as eugenol, camphor, pinene, and limonene, which are largely responsible for its therapeutic properties (Akpogheli *et al.*, 2022).

2.3 Health Benefits of Scent Leaf

Research indicates that chewing the stem of *Ocimum gratissimum* can help combat bad breath and strengthen teeth, largely due to its activity against oral microorganisms that contribute to tooth decay (Mbegbu, Nwajinka and Amaefule, 2021). The plant offers numerous health benefits, partly because it contains essential minerals like calcium and magnesium, which contribute to the regulation of blood sugar levels (Okoduwa, Umar, James and Inuwa, 2017). It also plays a protective role for the pancreatic islets responsible for insulin production. In addition, scent leaf supports cardiovascular health by aiding in the removal of harmful substances and excess cholesterol from the body (Ademiluyi *et al.*, 2023).

Furthermore, *Ocimum gratissimum* promotes proper digestion by facilitating bowel movement, relaxing the gastrointestinal tract, and enhancing the breakdown of food (Oppong *et al.*, 2021). Its ability to lower blood glucose levels and protect pancreatic function further

underscores its relevance in metabolic health (Ademiluyi *et al.*, 2023). Recent findings have also suggested its potential use as a supportive therapy for individuals living with HIV/AIDS (Ugbogu *et al.*, 2021).

Scent leaf has also been traditionally used to manage gastrointestinal disorders such as stomach ache, dysentery, diarrhea, and vomiting, while simultaneously improving digestion (Ademiluyi *et al.*, 2023). Regular consumption is associated with the normalization of blood sugar levels, highlighting one of its key health advantages. Additionally, it has been suggested that scent leaf may help mitigate some of the harmful effects of smoking and nicotine exposure (Agholor *et al.*, 2018). The plant has long been recognized in traditional medicine for its wide range of therapeutic applications (Akinyemi *et al.*, 2005).

Although diarrhea is often perceived as a minor and temporary condition, it remains a significant public health concern, particularly in children under five years of age, where it ranks as the second leading cause of mortality globally (Mokomane *et al.*, 2018). Scent leaf has been identified as one of several natural remedies for managing diarrhea. Its effectiveness is largely attributed to eugenol, an active compound with antibacterial and antispasmodic properties (Das *et al.*, 2020). This compound helps eliminate pathogens responsible for diarrhea while also relieving associated symptoms such as abdominal cramps and bloating (Ukoroiye *et al.*, 2018).

In addition, extracts of *Ocimum gratissimum* have been traditionally used in the treatment of ulcers due to their anti-inflammatory, analgesic, and anti-ulcer activities (Agholor *et al.*, 2018). The compounds eugenol and carvacrol, both phenolic in nature, are believed to play key roles in this effect (Leyva-López *et al.*, 2017). Eugenol has been shown to possess antioxidant and anti-inflammatory properties and can inhibit cyclooxygenase-2 (COX-2), an enzyme involved in ulcer formation (da Fonsêca *et al.*, 2019; Prasad and Tyagi, 2015).

Carvacrol, on the other hand, exhibits antimicrobial, antioxidant, and anti-inflammatory activities and contributes to gastric protection by enhancing mucus production and reducing acid secretion (Maćzka *et al.*, 2023; Mbegbu *et al.*, 2021). The combined action of these compounds helps protect the stomach lining and supports the healing of ulcers (Hassan *et al.*, 2021).

Scent leaf is often referred to as the “fever leaf” due to the antiseptic, antifungal, and antibacterial properties of its essential oil (Irshad *et al.*, 2020). It is also widely used in the treatment of skin conditions, as its antimicrobial properties make it effective against infections such as ringworm. When prepared as a paste and applied topically, it helps in managing various skin diseases (Ademiluyi *et al.*, 2023).

2.4 Phytochemistry of Scent Leaf

2.4.1 Flavonoids

Ocimum gratissimum contains numerous phenolic compounds such as rosmarinic acid, caffeic acid, catechins, and flavonoids including rutin, apigenin, kaempferol, and quercetin (Dharsono *et al.*, 2022). Flavonoids are the largest group of plant phenolics and occur either freely or as glycosides (Kumar and Pandey, 2013). Structurally, they possess a benzopyrone nucleus and are known for strong antioxidant activity (Nwosu *et al.*, 2022).

These compounds are widely distributed in plants and play important roles in medicine due to their ability to neutralize free radicals, chelate metals, and inhibit oxidative enzymes (Pizzino *et al.*, 2017). They also exhibit antimicrobial, anti-inflammatory, anti-ulcer, and anticancer properties (Yakubu *et al.*, 2019). For example, quercetin found in scent leaf has been linked to cancer prevention through mechanisms such as apoptosis induction and inhibition of tumor growth (Onyibe *et al.*, 2021).

Additionally, flavonoids improve vascular function and are useful in managing conditions like hemorrhoids (Ali *et al.*, 2021). Their antiviral potential has also been widely reported, making them important bioactive compounds in medicinal plants (Jing *et al.*, 2016).

2.4.2 Glucosinolates

Glucosinolates are sulfur- and nitrogen-containing compounds derived from glucose and are responsible for the characteristic flavor of plants in the Brassicaceae family (Forman *et al.*, 2009; Fleck *et al.*, 2019). Although inactive in their original form, they become biologically active when hydrolyzed by the enzyme myrosinase, producing compounds such as isothiocyanates (Angelino *et al.*, 2015).

These hydrolysis products possess antimicrobial, antifungal, and insecticidal properties, contributing to plant defense (Nguyen *et al.*, 2020). They also exhibit health benefits in humans, including antioxidant and chemoprotective effects against diseases like cancer and cardiovascular disorders (Kamal *et al.*, 2022).

However, excessive intake may lead to adverse effects such as digestive discomfort and reduced growth in animals (Škubník *et al.*, 2021). Despite this, glucosinolates remain valuable due to their roles in plant protection and potential pharmaceutical applications (Barba *et al.*, 2016).

2.4.3 Terpenes

Terpenes are abundant plant secondary metabolites found in *Ocimum gratissimum* and are known for diverse biological activities (Dharsono *et al.*, 2022). They exhibit anti-inflammatory effects by inhibiting pro-inflammatory pathways and are beneficial in conditions like bronchitis and arthritis (Del Prado-Audelo *et al.*, 2021).

Terpenes also show anticancer potential by interfering with processes such as cell proliferation, angiogenesis, and metastasis (Kamran *et al.*, 2022). In addition, they possess neuroprotective properties, supporting brain health, with compounds like borneol playing key roles (Ameade *et al.*, 2018).

Overall, terpenes contribute to plant defense against environmental stress and have promising applications in medicine due to their wide range of therapeutic effects (Ninkuu *et al.*, 2021).

2.4.4 Alkaloids

Alkaloids are bioactive compounds known for their ability to reduce blood pressure and stabilize the nervous system, especially in neurological disorders. They also exhibit antimalarial and antibacterial properties, making them useful in traditional medicine (Othman *et al.*, 2019). These nitrogen-containing plant metabolites, such as caffeine and morphine, have significant physiological effects and are widely used in pharmaceuticals. Their presence in plants highlights the importance of phytochemicals as natural therapeutic agents (Kurek, 2019).

2.4.5 Sterols

Sterols are secondary metabolites derived from cholesterol with diverse biological and chemical functions. They share a common structural backbone but vary due to different functional group attachments (Atanasov *et al.*, 2021). Some plant sterols, such as ecdysteroids found in *Ocimum gratissimum*, exhibit pharmacological effects including anti-inflammatory, analgesic, antidiabetic, and anthelmintic activities, making them valuable in drug development.

2.5 Biological Activities of Scent Leaf

2.5.1 Antioxidant

Scent leaf possesses strong antioxidant properties due to vitamins like tocopherol and ascorbic acid (Idris *et al.*, 2019). Its phenols and flavonoids help protect cells from oxidative damage by neutralizing free radicals and inhibiting oxidative enzymes (Hussain *et al.*, 2019; Nimse and Pal, 2015).

Studies show that extracts of *Ocimum gratissimum* contain high levels of polyphenols and flavonoids, contributing to strong free radical scavenging activity and protection of DNA, proteins, and lipids from damage (Ayele *et al.*, 2022). The plant is also widely used in traditional medicine to treat various conditions such as infections, fever, and digestive disorders (Ojewumi *et al.*, 2021).

2.5.2 Anti-Convulsant

Scent leaf extracts have demonstrated anticonvulsant activity by delaying seizure onset and protecting against seizure-related mortality in experimental models (Loshali *et al.*, 2021). These effects are linked to interactions with the GABA system in the brain. Additionally, the plant shows anxiolytic properties by influencing behavioral responses associated with stress and anxiety (da Fonsêca *et al.*, 2019).

2.5.3 Antimicrobial

Antimicrobial activity refers to the ability to inhibit or destroy microorganisms. *Ocimum gratissimum* has been widely reported to possess strong antimicrobial effects due to its phytochemical content (Chaudhary *et al.*, 2018). Its essential oils act as natural preservatives and can inhibit bacteria, fungi, and toxin-producing organisms, making them useful in food preservation (Maurya *et al.*, 2021).

Studies also indicate that the plant exhibits high antimicrobial activity even at low concentrations, with minimal toxicity (Melo *et al.*, 2019).

2.5.4 Anti-inflammatory and Antiviral

Scent leaf contains compounds such as eugenol, thymol, and rosmarinic acid, which help reduce inflammation by inhibiting pro-inflammatory mediators and oxidative stress (Abd Rani *et al.*, 2018).

It also shows antiviral potential, as these compounds can inhibit the replication of viruses like influenza, herpes simplex virus, and HIV (Zakaryan *et al.*, 2017). Additionally, it may enhance immune function, contributing to broader antiviral effects (Lin, Hsu and Lin, 2014).

2.6 Test Organisms

2.6.1 *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is a Gram-negative opportunistic pathogen responsible for infections such as pneumonia and bloodstream infections (Qin *et al.*, 2022). Its virulence is

linked to factors like toxins, biofilm formation, and secretion systems that enhance survival and resistance (Sultan *et al.*, 2021).

It exhibits strong antibiotic resistance through mechanisms such as efflux pumps and enzyme production, making it a critical priority pathogen globally (Sendra *et al.*, 2024).

2.6.2 Escherichia coli

Escherichia coli is a Gram-negative bacterium that ranges from harmless to highly pathogenic strains causing infections like diarrhea and urinary tract infections (Kaper *et al.*, 2023). Its virulence depends on adhesins, toxins, and biofilm formation.

Antibiotic resistance, especially in ESBL-producing strains, is a major concern, as it increases disease severity and treatment difficulty (Murray *et al.*, 2023).

2.6.3 Staphylococcus aureus

Staphylococcus aureus is a Gram-positive pathogen responsible for skin infections and systemic diseases. Its virulence is due to toxins, adhesins, and immune evasion mechanisms (Cheung *et al.*, 2023).

Methicillin-resistant *S. aureus* (MRSA) is a major global health concern due to its resistance to multiple antibiotics and its ability to maintain high virulence (Turner *et al.*, 2023).

2.6.4 *Klebsiella pneumoniae*

Klebsiella pneumoniae is a Gram-negative bacterium associated with hospital-acquired infections such as pneumonia and urinary tract infections (Paczosa and Meccas, 2023). Its pathogenicity is linked to its capsule, biofilm formation, and iron-acquiring systems.

Carbapenem-resistant strains pose serious treatment challenges due to high resistance and enhanced virulence (Murray *et al.*, 2023).

2.10 Antibacterial Potential of Scent Leaf (*Ocimum gratissimum*)

The increasing problem of antimicrobial resistance has led to interest in plant-based antimicrobials like *Ocimum gratissimum*. The plant is widely used in traditional medicine and cuisine, especially in West Africa (Ugbogu *et al.*, 2021).

Its antibacterial activity is attributed to compounds such as eugenol, thymol, and flavonoids, which disrupt bacterial membranes, inhibit enzymes, and prevent biofilm formation (Iweala *et al.*, 2023; Okoro and Nwankwo, 2024).

Studies show that scent leaf extracts are effective against multidrug-resistant bacteria like *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*, often with effects comparable to conventional antibiotics (Adeyemi *et al.*, 2023).

Additionally, these compounds act synergistically and may enhance the effectiveness of standard antibiotics, making scent leaf a promising candidate for developing alternative antimicrobial therapies (Nwosu *et al.*, 2024).

2.11 Challenges and Future Directions

Although scent leaf shows strong antibacterial potential, several limitations restrict its clinical use. Variations in phytochemical composition—caused by differences in soil, climate, and harvesting conditions—can lead to inconsistent therapeutic effects (Eze *et al.*, 2025). Therefore, standardizing extraction methods and developing stable formulations are essential for safety and reproducibility. Recent studies have explored improved techniques such as ultrasound-assisted extraction to enhance the yield of active compounds (Adebayo *et al.*, 2024). In addition, toxicity evaluations are necessary, as high levels of compounds like eugenol may be harmful to mammalian cells (Onoharigho *et al.*, 2022).

Future studies should focus on clinical trials to confirm the safety and effectiveness of scent leaf in humans. Advanced approaches such as genomic and proteomic analyses can help clarify how its bioactive compounds interact with microbial targets. Furthermore, innovative delivery systems like nanoencapsulation may improve the stability and bioavailability of the extracts, enhancing their therapeutic value (Nwosu *et al.*, 2024). Overall, incorporating scent leaf into modern medicine holds great promise, particularly in combating antimicrobial resistance and supporting sustainable healthcare.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials and Apparatus

Laboratory materials including Petri dishes, pipettes, conical flasks, round-bottom flasks, and bottles were properly washed, dried, wrapped in aluminum foil, and sterilized in a hot-air oven at 160°C for one hour. After cooling, they were used under aseptic conditions

maintained with a Bunsen burner flame, while work surfaces were disinfected with alcohol. Culture media were prepared according to manufacturer instructions and sterilized at 121°C (15 psi) for 15 minutes unless otherwise specified.

3.2 Sample Preparation and Collection

Ocimum gratissimum leaves were obtained from a local market in Benin City, Edo State, Nigeria. The leaves were air-dried, ground into powder using an electric blender, and stored in airtight containers for further analysis. Bacterial isolates were collected from the University of Benin Teaching Hospital.

3.3 Extraction

Five hundred grams (500 g) of the powdered sample were soaked separately in ethanol and water for extraction. The mixtures were filtered using Whatman No. 1 filter paper and then concentrated by evaporation to obtain crude extracts (Abdul-Hammed et al., 2021).

3.4 Determination of Phytochemicals

3.4.1 Total Flavonoids

Flavonoid content was determined using a modified method of Pandey et al. (2020). The extract was mixed with distilled water, sodium nitrite, aluminum chloride, and sodium hydroxide. After incubation, absorbance was measured at 510 nm. Results were calculated using a quercetin standard curve and expressed as mg quercetin equivalent per gram (QE/g) (Bouret et al., 2015).

3.4.2 Test for Saponins

The extract was diluted with water and shaken vigorously. Persistent frothing indicated the presence of saponins.

3.4.3 Tannins

Tannin content was determined using Folin-Denis reagent. After reaction and incubation, absorbance was read at 760 nm, and results were expressed as tannic acid equivalents (AOAC, 2005; Bouret et al., 2015).

3.4.4 Test for Alkaloids

The extract was treated with dilute hydrochloric acid, filtered, and tested with Dragendorff's, Mayer's, and Wagner's reagents. Formation of precipitates confirmed the presence of alkaloids.

3.4.5 Test for Glycosides

The extract was hydrolyzed with sulfuric acid, neutralized, and treated with Fehling's solution. A brick-red precipitate indicated the presence of glycosides.

3.5 Bacterial Isolates

Bacterial isolates were obtained from the Microbiology Laboratory of the University of Benin Teaching Hospital and transported using nutrient slants. They were further identified through morphological and biochemical tests before use.

3.6 Culture Media Preparation

3.6.1 Nutrient Agar

Twenty-eight grams of nutrient agar were dissolved in 1000 mL of distilled water, sterilized at 121°C for 15 minutes, and cooled before use.

3.6.2 Mueller-Hinton Agar

Thirty-eight grams of Mueller-Hinton agar were dissolved in distilled water, sterilized, cooled to about 40°C, and poured into sterile Petri dishes.

3.7 Confirmatory Tests

3.7.1 Gram Staining

Bacterial smears were heat-fixed, stained with crystal violet, iodine, decolorized with alcohol, and counterstained with safranin. Slides were examined under oil immersion ($\times 100$).

3.8 Biochemical Identification

3.8.1 Oxidase Test

Addition of oxidase reagent producing a purple color within 10 seconds indicated a positive result.

3.8.2 Urease Test A color change from yellow to pink in urease agar after incubation confirmed urease activity.

3.8.3 Indole Test

After incubation in peptone broth, addition of Kovac's reagent producing a red ring indicated a positive result.

3.8.4 Citrate Test

A color change from green to blue in Simon's citrate agar indicated citrate utilization.

3.8.5 Catalase Test

Formation of bubbles after adding hydrogen peroxide confirmed catalase activity.

3.8.6 Sugar Fermentation (TSI Test)

Changes in color, gas production, or blackening of the medium indicated sugar fermentation and hydrogen sulfide production.

3.9 Antimicrobial Sensitivity

3.9.1 Agar Well Diffusion

Mueller-Hinton agar plates were prepared, wells created, and extracts of varying concentrations added. Plates were inoculated with bacteria and incubated at 37°C for 24 hours. Zones of inhibition were measured.

3.9.2 Minimum Inhibitory Concentration (MIC)

Using broth dilution, extracts were prepared in different concentrations and inoculated with standardized bacterial suspensions. After incubation, the lowest concentration without visible growth was recorded as MIC.

3.9.3 Minimum Bactericidal Concentration (MBC)

Samples from clear MIC tubes were cultured on agar plates. The lowest concentration with no bacterial growth was recorded as MBC.

3.10 Data Analysis

Data were analyzed using SPSS version 21.0. Results were expressed as mean \pm standard deviation of triplicates, and differences between means were determined using Duncan's Multiple Range Test (SPSS, 2010).

CHAPTER FIVE

DISCUSSION

The antibacterial assessment of *Ocimum gratissimum* (scent leaf) extracts revealed that both ethanolic and aqueous extracts exhibited inhibitory effects against the tested organisms—*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas* spp., and *Klebsiella pneumoniae*. However, the degree of activity varied, largely due to differences in the concentration and availability of bioactive constituents (Hamma *et al.*, 2020).

Phytochemical screening confirmed the presence of key secondary metabolites, including alkaloids, flavonoids, glycosides, tannins, and saponins, though their concentrations differed between extracts. The ethanolic extract contained higher levels of flavonoids and tannins, which likely contributed to its stronger antibacterial effect. These compounds act by disrupting bacterial membranes, inhibiting nucleic acid synthesis, and interfering with

metabolic processes. Additionally, saponins and alkaloids may enhance antimicrobial activity through synergistic effects, such as increasing membrane permeability and inhibiting essential enzymes (Olawale and Olusola-Makinde, 2021).

At a concentration of 100 mg/mL, the ethanolic extract produced inhibition zones between 11–15 mm, while the aqueous extract showed smaller zones of 3–10 mm. *Staphylococcus aureus* was the most susceptible organism, showing the highest inhibition values. These results align with previous findings that reported stronger activity of ethanolic extracts against similar bacteria (Lawrence and Olusola-Makinde, 2023).

The Minimum Inhibitory Concentration (MIC) results indicated that the ethanolic extract inhibited *S. aureus*, *E. coli*, and *Pseudomonas* spp. at 50 mg/mL, while *K. pneumoniae* required 100 mg/mL. In contrast, the aqueous extract showed weaker activity, requiring higher concentrations and failing to inhibit some organisms. Similarly, the Minimum Bactericidal Concentration (MBC) findings showed that the ethanolic extract was bactericidal against most isolates, whereas the aqueous extract was mainly bacteriostatic. These observations agree with earlier studies showing superior bactericidal effects of ethanol extracts (Hamma *et al.*, 2020).

The enhanced activity of the ethanolic extract can be attributed to ethanol's ability to extract both polar and non-polar compounds, resulting in a richer phytochemical profile. It also improves penetration into bacterial cells, increasing the effectiveness of active compounds. In contrast, water extracts fewer bioactive substances, leading to reduced antibacterial potency. This is supported by studies showing higher concentrations of active compounds like eugenol in ethanolic extracts (Lawrence and Olusola-Makinde, 2023).

Overall, these findings support previous reports that *Ocimum gratissimum* possesses significant antibacterial activity against both Gram-positive and Gram-negative bacteria, reinforcing its potential as a natural antimicrobial agent (Olawale and Olusola-Makinde, 2021).

5.2 Recommendations

Based on this study, it is recommended that ethanolic extracts of *Ocimum gratissimum* be prioritized for antibacterial applications due to their higher efficacy. The plant should be further explored for use in traditional and primary healthcare, especially in areas with limited access to antibiotics. Advanced analytical methods such as HPLC and GC-MS should be employed to identify specific active compounds.

Further studies should include toxicity testing and in vivo evaluations to ensure safety. Research into combining scent leaf extracts with conventional antibiotics is also encouraged to enhance treatment outcomes and combat resistance. Additionally, the development of plant-based antimicrobial products—such as creams, soaps, and preservatives—should be considered. Cultivation and conservation of the plant should also be promoted to ensure sustainable availability.

5.3 Contribution to Knowledge

This study highlights the superiority of ethanol as an extraction solvent for obtaining potent antibacterial compounds from *Ocimum gratissimum*. It provides updated data on its activity against clinically important pathogens and establishes baseline MIC and MBC values for future research.

The findings also strengthen the link between phytochemicals such as flavonoids, tannins, and alkaloids and antimicrobial activity. Importantly, the study offers scientific evidence supporting the traditional use of scent leaf in treating infections and lays a foundation for the development of plant-based antimicrobial agents.

5.4 Conclusion

In conclusion, *Ocimum gratissimum* contains a wide range of bioactive compounds—including flavonoids, tannins, alkaloids, glycosides, and saponins—that contribute to its strong antibacterial activity against both Gram-positive and Gram-negative bacteria such as *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas* spp., and *Klebsiella pneumoniae*. These compounds exert their effects through mechanisms like membrane disruption, enzyme inhibition, and interference with microbial metabolism.

The ethanolic extract demonstrated significantly higher antibacterial activity than the aqueous extract, confirming ethanol as a more effective solvent for extracting active compounds. Overall, the study supports the potential of scent leaf as a valuable natural source for antimicrobial development.

