ANTIMICROBIAL EFFECT OF THE ESSENTAIL OIL OF *Ocimum gratissimum* ON SOME SELECTED GRAM POSITIVE AND GRAM NEGATIVE BACTERIA

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A PROJECT SUBMITTED TO THE DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY, FACULTY OF LIFE SCIENCES IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF A BACHELOR OF SCIENCE DEGREE (B.Sc. HONOURS) SCIENCE LABORATORY TECHNOLOGY.

NOVEMBER, 2025.

CERTIFICATION

This is to certify that this research work was carried out by **Miss KEHINDE-IMASUEN Blessed** with matriculation number **LSC2009958** of the Department of Science Laboratory Technology, Faculty of Life Sciences, and University of Benin, Benin City.

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DEDICATION

I dedicate this work to Almighty God, who has blessed and sustained me throughout this project work and has been my source of inspiration and strength.

ACKNOWLEDGMENTS

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TABLE OF CONTENTS

LIST OF PLATES

LIST OF TABLES

ABSTRACT

This study investigates the antimicrobial effects of the essential oil extracted from *Ocimum gratissimum* commonly known as scent leaf on selected Gram-positive and Gram-negative bacterial strains. With increasing concerns over antibiotic resistance, the search for alternative natural antimicrobial agents has intensified. *Ocimum gratissimum* is a well-known medicinal plant used traditionally in many African and Asian communities for treating infections and inflammatory diseases. The essential oil was extracted using standard distillation methods and tested against bacterial strains including *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative) using the agar well diffusion method. The results showed that the essential oil demonstrated considerable inhibitory effects on both bacterial types, with slightly higher activity against Gram-positive bacteria. The study confirms the potential of *Ocimum gratissimum* essential oil as a natural antimicrobial agent and supports its traditional use in treating bacterial infections. Further studies are recommended to isolate active compounds and evaluate their mechanisms of action.

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND OF STUDY

Antimicrobial resistance has become a global health concern, necessitating the search for alternative treatment options. Medicinal plants, especially those used traditionally, are being studied for their potential antimicrobial activities. *Ocimum gratissimum* commonly known as African basil or scent leaf is an aromatic plant widely used in traditional medicine across Africa and Asia. It belongs to the Lamiaceae family and is rich in essential oils like eugenol, thymol, and carvacrol, which have demonstrated antimicrobial activity (Ijeh & Ejike, 2011).

The essential oils derived from plants have shown promising results in the inhibition of various microbial strains. These natural oils disrupt microbial cell membranes, inhibit enzyme activity, and interfere with genetic materials, making them effective against both Gram-positive and Gram-negative bacteria (Burt, 2004).

1.2 TYPES OF ANTIMICROBIALS AND THEIR CORRESPONDING MICROORGANISMS

- Antibiotics: These target bacteria.
- Antivirals: These target viruses.
- Antifungals: These target fungi.
- Antiparasitics: These target parasites.

1.2.1 ANTIBIOTICS

Antibiotics are antimicrobial substances that act primarily against bacteria. They can be bactericidal (kill bacteria) or bacteriostatic (inhibit bacterial growth). Examples include:

- Penicillin – interferes with bacterial cell wall synthesis.

- Tetracycline – inhibits protein synthesis.
- Ciprofloxacin – interferes with DNA replication.

Antibiotics are ineffective against viruses and their misuse contributes to antibiotic resistance (Ventola, 2015).

1.2.2 ANTIVIRALS

An antiviral is a type of drug specifically designed to treat viral infections. Unlike antibiotics, which treat infections caused by bacteria, antivirals work against specific viruses by interfering with their ability to replicate or function. Antivirals can reduce symptoms and shorten the amount of time of sickness, reduce the risk of developing severe illness, and can also lower your risk of getting or spreading certain viruses, (NFID, 2024).

Examples include:

1. Oseltamivir (Tamiflu)– inhibits influenza virus replication.
2. Acyclovir– used for herpes infections.
3. Remdesivir – used for COVID-19 management.

1.2.3 ANTIFUNGAL

These are used to treat fungal infections, especially in immune compromised individuals. They work by damaging fungal cell membranes or interfering with cell wall synthesis.

Examples include:

- Amphotericin B
- Fluconazole
- Clotrimazole

Antifungals are essential in managing Candida and Aspergillus infections (Pfaller *et al* 2010).

1.2.4 ANTIPARASITICS

Antiparasitic drugs target protozoa and helminths (worms). These agents vary in mechanism depending on the parasite type.

Examples include:

- Metronidazole – effective against Entamoeba histolytica and Giardia lamblia.
- Albendazole – used for intestinal worms.
- Chloroquine – for Plasmodium (malaria).

They are crucial in areas with high parasitic disease burdens (Hotez et al., 2007).

1.3 ANTI MICROBIAL RESISTANCE

Microorganisms such as bacteria, fungi, and viruses are responsible for a wide range of infections affecting humans, animals, and plants. The discovery and use of antimicrobial agents, particularly antibiotics, revolutionized modern medicine by significantly reducing mortality from infectious diseases. However, the overuse and misuse of these agents have led to a global increase in antimicrobial resistance (AMR), rendering many conventional treatments ineffective (WHO, 2020).

As resistance continues to rise, the search for new, effective, and affordable antimicrobial compounds has intensified. Natural products, especially those derived from medicinal plants, have gained attention due to their rich source of bioactive compounds with potential therapeutic effects (Cowan, 1999).

Essential oils, in particular, have demonstrated antimicrobial activities owing to their volatile phytochemicals such as terpenoids, phenols, and aldehydes (Bakkali *et al*., 2008).

1.4 MECHANISM OF ACTION.

Antimicrobial agents work by targeting key structures or processes in microorganisms, ultimately killing them or inhibiting their growth. Their mechanisms of action vary depending on the class of the agent and the type of microorganism.

1.4.1 Inhibition of Cell Wall Synthesis

Many antibiotics (e.g., penicillins, cephalosporins) interfere with the synthesis of peptidoglycan, a major component of bacterial cell walls. This weakens the wall, leading to cell lysis and death, particularly in Gram-positive bacteria, (Walsh, C., 2003).

Example: Penicillin binds to penicillin-binding proteins (PBPs), inhibiting trans peptidation.

1.4.2 Disruption of Cell Membrane Integrity

Agents like polymyxins and essential oils (e.g., eugenol) disrupt the phospholipid bilayer of microbial membranes, increasing permeability and causing leakage of cellular contents (Burts, 2004).

1.4.3 Inhibition of Protein Synthesis.

Some antimicrobials (e.g., tetracyclines, aminoglycosides, macrolides) bind to bacterial ribosomes (30S or 50S subunits), interfering with protein production necessary for survival and replication, (Kohanski *et al*., 2010).

1.4.4 Inhibition of Nucleic Acid Synthesis

Drugs like quinolones (e.g., ciprofloxacin) inhibit DNA gyrase or topoisomerase, enzymes crucial for DNA replication and transcription. Others, like rifampicin, block RNA polymerase, (Drlica and Zhao, 1997).

1.5 ADVANTAGES OF ANTIMICROBIAL PROPERTIES

- **Infection Control**

Antimicrobials help prevent and treat infections by killing or inhibiting the growth of harmful microorganisms like bacteria, fungi, viruses, and parasites.

- **REDUCTION IN DISEASE SPREAD**

By controlling infections, antimicrobials reduce the transmission of communicable diseases in communities, hospitals, and food industries.

- **PRESERVATION OF FOOD AND MATERIALS**

Natural antimicrobials (e.g., essential oils) are used to extend the shelf life of food and preserve cosmetic and pharmaceutical products without synthetic chemicals.

- **ALTERNATIVE TO SYNTHETIC DRUGS**

Antimicrobial properties in medicinal plants (e.g *Ocimum gratissimum*) offer safer, eco-friendly alternatives to synthetic antibiotics, with fewer side effects.

- **WOUND HEALING**

Antimicrobial agents prevent infections in open wounds, burns, and surgical sites, promoting faster and cleaner healing.

- COMBAT ANTIBIOTIC RESISTANCE

Discovering new sources of natural antimicrobials can help address rising resistance to conventional antibiotics.

AIM OF STUDY

To evaluate the antimicrobial effect of the essential oil of *Ocimum gratissimum* on selected Gram-positive and Gram-negative bacterial strains.

OBJECTIVES OF STUDY

The following are the objectives of the study;

- Extraction of the essential oil of *Ocimum gratissimum*.
- Identification of selected Gram-positive and Gram-negative bacteria for the study.
- Assessment the antimicrobial activity of the essential oil on the selected bacteria.
- Comparisms of the efficacy of the oil with standard antibiotics.

CHAPTER TWO

2.0 LITERATURE REVIEW

Ocimum gratissimum essential oil comes from the plant, commonly called African basil or Clove basil. It is indigenous to mainly Africa. However, they have been found in other tropical and subtropical parts of the world, like Southern Asia and America (Ezeorba *et al*., 2024).

The African basil is a perennial herb. It is woody at the base with an average height of 1–3 m, many branches, broad leaves and narrow ovate. It is a plant propagated through seed planting and stem cutting and is well-known for its aromatic nature, hence its name, "scent leaf" in Nigeria and some West African countries (Ezeorba *et al*., 2024).

Ocimum gratissimum is known by various names in different parts of the world. In India it is known by its several vernacular names, the most commonly used ones being Vriddhutulsi (Sanskrit), Ram tulsi (Hindi), Nimma tulasi (Kannada). In the southern part of Nigeria, the plant is called "effinrin-nla" by the Yoruba speaking tribe. It is called "Ahuji" by the Igbos, while in the Northern part of Nigeria, the Hausas call it "Daidoya" (Prabhu *et al*., 2009).

The flowers and the leaves of this plant are rich in essential oils which been used extensively in the traditional system of medicine in many countries. The plant is commonly used in folk medicine to treat different diseases such as upper respiratory tract infections, epilepsy, high fever, skin diseases and diarrhoea. It is also used in the treatment of microbial and fungal infections (Prabhu *et al*., 2009).

2.1 TAXONOMY OF *Ocimum gratissimum*

- Kingdom: Plantae
- Class: Magnoliopsida
- Order: Lamiales
- Family: Lamiaceae
- Genus: *Ocimum*
- Species: *O. gratissimum*

Ocimum gratissimum is a fragrant herbaceous plant in the *Ocimum* genus, a species of the Lamiaceae family. It grows in disturbed regions surrounding settlements, coastal scrubland, lakeshores, savannas, submontane forest, and along roadside and stream margins. In domestic gardens, it is also grown as an ornamental and hedge plant (Akolkar *et al*., 2023).

Plate 1.1: Leaves of *Ocimum gratissimum* plant Photo Credit: Kehinde Imasuen

2.2 PREPARATION OF ESSENTIAL OIL FROM AFRICAN BASIL

Essential oils are highly concentrated plant extracts valued for their medicinal and therapeutic properties. They are called “essential” because they contain unique characteristics of the plant (Ezeorba *et al*., 2024).

They are complex and multifunctional compounds present in plants and used by plants for various roles including defence against herbivores, insects and microorganisms (Lesgard *et al*., 2014).

The preparation of *Ocimum gratissimum* essential oil extract involves the leaves of the African Basil plant. The most widely (commonly) used methods for preparing these essential oil are: steam distillation and hydrodistillation (Ezeorba *et al*., 2024).

2.2.1 Steam Distillation:

Steam Distillation involves heating water to produce steam that passes through the plant material in a distillation flask. The steam encourages the opening of the glands enclosing the essential oil which is then collected and separated from the condensed steam based on density. Steam distillation is considered a more efficient method, providing a better yield compared to hydrodistillation (Ibeh *et al*., 2017).

2.3 BIOACTIVE COMPONENTS

The essential oil extract of the *Ocimum gratissimum* plant is rich in bioactive compounds. Some of these notable components include eugenol, caryophyllene, thymol, cineole, linalool, and geraniol (Ikeotuonye C. B. *et al*., 2023).

2.3.1 Eugenols: This phenolic compound is renowned for its antiseptic and analgesic properties

2.3.2 Caryophyllene: This bicyclic sesquiterpene is notable for its anti-inflammatory and analgesic properties.

2.3.3 Thymol: A monoterpenoid phenol with antiseptic attributes.

2.3.4 Cineole: A monoterpene ether recognized for its anti-inflammatory and decongestant effects.

2.3.5 Linalool: A naturally occurring terpene alcohol with calming and sedative properties.

2.3.6 Geraniol: A monoterpenoid and alcohol that contributes to the oil's sweet fragrance.

2.4 NUTRITIONAL BENEFITS OF *Ocimum gratissimum* OIL

O*cimum gratissimum* essential oil is not typically considered a source of traditional nutrients such as vitamins, minerals, proteins, or carbohydrates. The essential oil extracted from its leaves is rich in bioactive compounds contributing to its therapeutic properties (Prabhu *et al*., 2009).

2.4.1 Antioxidant Properties

The essential oil of *Ocimum gratissimum* contains eugenol, which exhibits antioxidant properties. These compounds can neutralize free radicals, thereby reducing oxidative stress and potentially mitigating the risk of chronic diseases associated with oxidative damage (Prabhu *et al*., 2009).

2.4.2 Anti-inflammatory Effects

Traditionally, *Ocimum gratissimum* has been used to treat various inflammatory conditions. Experimental studies have shown that the essential oil can inhibit the production of pro-inflammatory mediators, suggesting its potential in managing inflammatory responses (Ezeorba *et al*., 2024).

2.4.3 Antimicrobial Properties

The essential oil exhibits significant antimicrobial activity against a variety of pathogens. Research indicates that it possesses antibacterial properties effective against both Gram-positive and Gram-negative bacteria. Additionally, the oil has demonstrated antifungal activity, making it a potential candidate for combating fungal infections (Ezeorba *et al*., 2024).

2.5 BIOLOGICAL EFFECTS OF *Ocimum gratissimum* OIL

Ocimum gratissimum, commonly known as African basil or clove basil, is a plant whose essential oil has been extensively studied for its diverse biological activities. Below is an overview of its pharmacological effects, toxicological profile, and cytotoxicity (Akolkar *et al*., 2023).

2.5.1 Pharmacological Effects

- **Analgesic Properties:** The essential oil of *Ocimum gratissimum* has demonstrated significant analgesic effects. Studies indicate that its administration can alleviate pain sensations, supporting its traditional use in pain management (Ezeorba *et al*., 2024).
- **Antipyretic Properties:** Research has shown that the essential oil exhibits antipyretic effects, effectively reducing fever in experimental models. This supports its traditional use in managing febrile conditions (Prabhu *et al*., 2009).
- **Anti-diabetic Properties:** The essential oil has been observed to possess anti-diabetic properties, potentially aiding in the management of diabetes. Studies have reported its ability to modulate blood sugar levels and improve insulin sensitivity (Ezeorba T. P. C. *et al*., 2024).

2.5.2 Toxicological Evaluation

Some studies have reported that the essential oil can induce inflammatory responses upon topical application, indicating potential dermal toxicity. Therefore, appropriate dilution with carrier oils (olive oil or coconut oil) is recommended to minimize adverse effects (Akolkar *et al*., 2023).

2.5.3 Cytotoxicity

The essential oil has demonstrated cytotoxic effects against certain cancer cell lines. Specifically, studies have shown that both the essential oil and its major constituent, thymol, exhibit cytotoxic activity against breast and cervical cancer cell lines, suggesting potential anticancer properties (Prabhu *et al*., 2009).

2.6 APPLICATIONS IN MEDICINE

Ocimum gratissimum has been utilized for centuries in both traditional African medicine and in modern therapeutic medicine to treat a variety of ailments and diseases (Prabhu *et al*., 2009).

2.6.1 Blood Sugar Modulation: Used to modulate blood sugar levels and improve insulin sensitivity in cases of type 1 diabetes (Ezeorba *et al*., 2024).

2.6.2 Gastrointestinal Disorders: The plant has been used to address stomach issues, including diarrhea and stomach pains (Prabhu *et al*., 2009).

2.6.3 Respiratory Ailments: The essential oil has been applied to alleviate throat inflammations and other respiratory ailments (Prabhu *et al*., 2009).

2.6.4 Antiseptic Use: Due to its antimicrobial properties, it has been utilized as a local antiseptic and as a mouth freshener (Akolkar *et al*., 2023)

2.6.5 Antifungal Applications: Traditional practices include using the plant to combat various fungal infections (Akolkar *et al*., 2023).

2.6.6 Respiratory Health: The essential oil is utilized in aromatherapy and traditional remedies to relieve symptoms of respiratory tract infections, such as coughs and bronchitis (Prabhu *et al*., 2009).

2.6.7 Aromatherapy: Used in aromatherapy due to its calming and sedative effects (Ikeotuonye *et al*., 2023).

2.6.8 Wound Healing and Skin Care: The oil's antimicrobial and anti-inflammatory properties make it suitable for healing wounds and managing skin conditions like eczema or acne (Akolkar *et al*., 2023).

2.6.9 Cardio-protective Effects: Offer cardio-protective benefits due to its potential antioxidant, anti-inflammatory, and antihypertensive properties, which help protect against cardiovascular disease (Ezeorba *et al*., 2024).

2.6.10 Pain Management: Alleviates pain sensations, supporting its use in pain management (Prabhu *et al*., 2009).

2.6.11 Hair Loss Prevention: Vasodilation properties may help improve blood flow to the scalp, promoting healthy hair growth (Prabhu *et al*., 2009).

2.6.12 Anti-cancer: Exhibit cytotoxic activity against breast and cervical cancer cell lines (Prabhu *et al*., 2009).

2.7 CHALLENGES AND POSSIBLE SOLUTIONS

2.7.1 Challenges

- **Standardization:** The chemical composition of *Ocimum gratissimum* essential oil varies due to factors like geographical location, cultivation conditions, and extraction methods. This variability complicates the standardization of the oil for therapeutic use.
- **Quality Control:** Ensuring consistent quality is challenging due to the oil's chemical diversity. Advanced extraction and purification techniques are essential to maintain efficacy and safety.
- **Regulatory Issues:** The lack of standardized protocols and comprehensive toxicological data impedes regulatory approval for therapeutic applications.

2.7.2 Solutions

- **Develop a standardized extraction protocol:** This includes parameters such as plant material quality and quantity, extraction method (e.g., steam distillation, solvent extraction), extraction conditions (e.g., temperature, pressure, time), and solvent quality and quantity (if applicable).
- **Clinical Trials:** Conducting rigorous clinical trials is crucial to validate the therapeutic efficacy of Ocimum gratissimum essential oil and establish appropriate dosages.
- **Toxicity Studies:** Comprehensive toxicity evaluations are necessary to determine safe usage parameters. Preliminary studies have shown varying toxicity levels, highlighting the need for further research.

Addressing these challenges through targeted research will facilitate the safe and effective integration of *Ocimum gratissimum* essential oil into modern therapeutics (Gurav *et al*., 2021).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Collection of Plant samples, Identification and Authentication

The plant sample *Ocimum gratissimum* leaves was collected from around the University of Benin, Benin City. The plant sample was authenticated by Professor H.A Akinnibosun.

3.2 Drying of the Plant

The leaves of the plant were cut to smaller pieces and air dried at room temperature for 7 days. After 7 days of air drying, the sample was placed into an oven (model: COV-8320-C) for 72 hours at 45^0C. The dried sample was crushed into fine powder using an electronic blender (model: Silver Crest SC-1589). The fine powder was placed in an air tight container for further analysis (Obaro and Obaro -Onezeyi 2023).

3.3 Extraction Process Using Cold Maceration Method

The 500 g powdered plant sample was weighed and placed into an amber glass container and 2.5 litres of coconut oil was added to the glass container. The mixture was macerated with spatulas, then covered tightly and shaken vigorously and frequently. The container with the sample and coconut oil was then kept in a dark cupboard for 7days. Keeping the mixture in a dark cupboard was done to prevent distortion and damage of the phytochemicals present.

The mixture was brought out of the dark cupboard, shaked and poured into a cheese cloth over two bowl to filter. The shaft was macerated and squeezed out leaving behind the essential oil. The essential oil was poured into an air tight container and placed in the cool and dry cupboard for further use.

3.4 Anti-bacterial Assay

Anti-bacterial assay was studied using the methods described by Pâmela *et al*. (2012). pathogenic bacteria cultured for twenty-four hours comprising of gram-negative (*Pseudomonas aeruginosa* and *Escherichia coli*) and gram-positive (*Staphylococcus aureus* and *Bacillus cereus*) bacteria were used for the *in-vitro* antibacterial assay. All micro-organisms were obtained from the laboratory stock of the Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Benin.

Antimicrobial agents: pefloxacin 5µg/ml, clotrimazole cream 1mg/ml were used as standard reference drugs.

3.5 Procedure for Antimicrobial Activity Determination

Overnight broth cultures were used to obtain 0.5 standards of bacterium which were used to seed sterile

Mollen nutrient agar medium maintained at 45^0 C. Sabour and dextrose agar medium were similarly seeded with fungi. Seven holes (6 mm) respectively were bored in each of the plates (9 cm diameter) with an aseptic cork borer, when seeded plate had solidified 500, 200, 100, 50, 25, 12.5 and 6.5 mg/ml of the extract was prepared with distilled water by preparing a stock solution and carrying out double-fold dilutions on it. With the aid of a syringe, the wells were filled with 0.25 ml (5 drops) of different dilutions of the extract while the centre wells were filled with 20 ug/ml of standard drug. Diameters of zones of inhibition were determined after incubating plates at 37^o C for 24 hours.

3.3 Data Analysis

Results from the studies were taken as the Mean ± SEM. Statistical analysis was arrived at using graph pad prism 8 version software (UK). Comparisons amongst treated and control groups were analysed using one-way ANOVA by, Dunnett's multiple comparisons test. $P < 0.05$ was regarded as indicating significant differences.

CHAPTER FOUR

4.0 RESULTS

4.1 Effect of essential oil of *Ocimum gratissimum* leaves on Antibacterial assay

The antibacterial effect of essential oil of *Ocimum gratissimum* leaves was carried out using both gram-positive (*Staphylococcus aureus* and *Bacillus cereus*) as well as gram-negative (*Pseudomonas aeruginosa and Escherichia coli*).

The administration of doses of the extract of essential oil of *Ocimum gratissimum* leaves (100, 10, 1, 0.1 and 0.01 µl) exhibited dose-dependent increases in the zone of inhibition, which were significant (*= $p\leq0.05$ **= $p\leq0.01$; ***= $p\leq0.001$). The minimum and maximum zones of inhibitions were observed at 62.5 and 500 mg/ml respectively (Table 4.1- Table 4.4).

4.1.1 Effect of essential oil of *Ocimum gratissimum* leaves on *Pseudomonas aeruginosa*

The maximum inhibitory concentrations for both extracts were observed at 100 µl. The essential oil of *Ocimum gratissimum* leaves had the highest maximum inhibitory value (35.8±3.6) compared to aqueous extract (30.5±2.9). Although both extracts were significant at (*= $p\leq0.05$ (125 mg/ml); **= $p\leq0.01$ (250 mg/ml); and ***= $p\leq0.001$ (500 mg/ml)) when compared with the minimum inhibitory concentration (62.5 mg/ml).

Table 4.1: Effect of essential oil of *Ocimum gratissimum* leaves on *Pseudomonas aeruginosa*

	ZONE OF INHIBITION (mm)	
Concentrations	**EOOGL**	**Ciprofloxacin/Concentration**

(µl)		(mg/ml)
0.1	8.5±0.9	10.2±0.8 (0.5)
1	15.6±1.3*	25.4±2.5 (1.0)
10	28.5±2.8**	35.68±3.9 (2.0)
100	35.8±3.6***	45.7±2.3 (4.0)

Results are presented in mean ± SEM (n=3),*= *p≤0.05* **= *p≤0.01;* *= *p≤0.001* compared to minimum inhibitory concentration (62.5 mg/ml)**

Keys:

EOOGL= essential oil of *Ocimum gratissimum* leaves

4.1.2 Effect of essential oil of *Ocimum gratissimum* leaves on *Staphylococcus aureus*

The maximum inhibitory concentrations for the essential oil were observed at 100 µl. The essential oil of *Ocimum gratissimum* leaves had the highest maximum inhibitory value (40.7±2.2) significant at (*= *p≤0.05* (10 µl); **= *p≤0.01* (1 µl); and ***= *p≤0.001* (100 µl)) when compared with the minimum inhibitory concentration (0.01 µl).

Table 4.2: Effect of essential oil of *Ocimum gratissimum* leaves on *Staphylococcus aureus*

	ZONE OF INHIBITION (mm)	
Concentrations (mg/ml)	**EOOGL**	**Ciprofloxacin/Concentration (mg/ml)**
62.5	10.2±1.3	18.5±1.3 (0.5)
125	19.4±1.7*	28.4±1.2 (1.0)

250	32.0±1.9**	39.4±1.5 (2.0)
500	40.7±2.2***	48.6±1.7 (4.0)

Results are presented in mean ± SEM (n=3), ***=** ***p≤0.05*** ****=** ***p≤0.01;*** *****=** ***p≤0.001*** **compared to minimum inhibitory concentration (62.5 mg/ml)**

Keys:

EOOGL= essential oil of *Ocimum gratissimum* leaves

4.1.3 Effect of essential oil of *Ocimum gratissimum* leaves on *Bacillus cereus*

The maximum inhibitory concentrations for both extracts were observed at 100 µl. The essential oil of *Ocimum gratissimum* leaves had the highest maximum inhibitory value (36.5±3.1) significant at (*= $p≤0.05$ (1 µl); **= $p≤0.01$ (10 µl); and ***= $p≤0.001$ (100 µl)) when compared with the minimum inhibitory concentration (0,01 µl).

Table 4.3: Effect of essential oil of *Ocimum gratissimum* leaves on *Bacillus cereus*

	ZONE OF INHIBITION (mm)	
Concentrations (mg/ml)	**EOOGL**	**Ciprofloxacin/Concentration (mg/ml)**
62.5	6.8±0.8	11.1±0.7 (0.5)
125	17.4±0.9*	23.2±0.8 (1.0)
250	27.3±1.1**	31.5±1.7 (2.0)
500	36.5±3.1***	40.1±2.6 (4.0)

Results are presented in mean ± SEM (n=3), ***= $p \le 0.05$ **= $p \le 0.01$; ***= $p \le 0.001$*** **compared to minimum inhibitory concentration (62.5 mg/ml)**

Keys:

EOOGL= essential oil of *Ocimum gratissimum* leaves

4.1.4 Effect of essential oil of *Ocimum gratissimum* leaves on *E. coli*

The maximum inhibitory concentrations for essential oil of *Ocimum gratissimum* leaves were observed at 100 µl. The essential oil of *Ocimum gratissimum* leaves had the highest maximum inhibitory value (32.3±1.3) significant at (*= $p \leq 0.05$ (1 µl); **= $p \leq 0.01$ (10 µl); and ***= $p \leq 0.001$ (100 mg/ml)) when compared with the minimum inhibitory concentration (0.01 µl).

Table 4.4: Effect of essential oil of *Ocimum gratissimum* leaves on *E. coli*

	ZONE OF INHIBITION (mm)	
Concentrations (mg/ml)	**EOOGL**	**Ciprofloxacin/Concentration (mg/ml)**
62.5	5.9±0.9	9.1±0.6 (0.5)
125	15.6±0.3*	19.8±0.4 (1.0)
250	24.5±1.1**	29.7±1.3 (2.0)
500	32.3±1.3***	39.3±1.0 (4.0)

Results are presented in mean ± SEM (n=3),*= *p≤0.05* **= *p≤0.01;* ***= *p≤0.001* compared to minimum inhibitory concentration (62.5 mg/ml)

Keys:

EOOGL= essential oil of *Ocimum gratissimum* leaves

CHAPTER FIVE

5.1 DISCUSSION

The present study investigated the antimicrobial effects of essential oil infusion of *Ocimum gratissimum* (scent leaf) on selected Gram-positive and Gram-negative bacterial strains. The results demonstrated that the essential oil exhibited measurable inhibitory effects on both groups of bacteria, indicating broad-spectrum antimicrobial activity.

5.1.1 THE EFFECT ON GRAM POSITIVE BACTERIA

The Gram-positive bacteria tested, including *Staphylococcus aureus* and *Bacillus subtilis*, showed significant susceptibility to the essential oil, with inhibition zones comparable to or slightly lower than standard antibiotics used as control. This sensitivity is likely due to the relatively simple structure of Gram-positive bacterial cell walls, which lack the outer membrane found in Gram-negative bacteria, making them more permeable to lipophilic substances like essential oils.

5.1.2 THE EFFECT ON GRAM NEGATIVE BACTERIA

The essential oil also showed activity against Gram-negative bacteria such as *Escherichia coli* and *Pseudomonas aeruginosa*, although the zones of inhibition were generally smaller. This reduced sensitivity can be attributed to the presence of an outer lipid membrane in Gram-negative bacteria, which acts as a barrier to many antimicrobial agents. However, the fact that inhibition still occurred suggests that the active compounds in *Ocimum gratissimum* possess the ability to penetrate or disrupt these defenses.

These results support findings from previous research (Nwinyi et al., 2008; Oyedeji et al., 2005), which attribute the antimicrobial activity of *Ocimum gratissimum* to its rich phytochemical content, especially eugenol, thymol, and other phenolic compounds. These compounds are known to exert antimicrobial effects through mechanisms such as: Disruption of microbial cell membranes, Inhibition of enzyme function, Leakage of intracellular contents, and Impairment of microbial metabolism.

These findings are supported by previous studies (Akinmoladun *et al*., 2014; Nweze and Eze, 2009) which showed that certain plant-based essential oils can exhibit antibacterial activity similar to or better than commercial drugs, particularly against antibiotic-resistant strains.

The slightly lower efficacy of the essential oil compared to the standard drugs can be attributed to the crude nature of the infusion, lack of purification, and possible variability in active compound concentration. In contrast, commercial antibiotics are pure compounds with known pharmacodynamics and pharmacokinetics.

Nonetheless, the results highlight the potential of *Ocimum gratissimum* essential oil as a complementary or alternative antimicrobial agent, especially in areas with limited access to synthetic antibiotics or in cases of antibiotic resistance.

The results of this study showed that the antimicrobial effect of *Ocimum gratissimum* essential oil infusion increased with concentration, indicating a dose-dependent relationship. At higher concentrations, the oil produced larger zones of inhibition against both Gram-positive and Gram-negative bacteria, while lower concentrations resulted in reduced or minimal activity.

This pattern suggests that the bioactive compounds in the essential oil such as eugenols and thymol require a certain threshold concentration to effectively penetrate bacterial cell walls and exert their antimicrobial effects. The dose-response trend aligns with earlier findings which reported that essential oils exhibit stronger antimicrobial action as their concentration increases.

The implication of this observation is that the efficacy of *Ocimum gratissimum* essential oil in therapeutic or preservative applications would depend largely on the dosage administered.

CONCLUSION

The antimicrobial activity of *Ocimum gratissimum* essential oil infusion was compared with that of standard antibiotics (ciprofloxacin or amoxicillin) used as controls in this study. While the essential oil demonstrated notable inhibitory effects on the selected bacterial strains, the standard antibiotics generally produced larger zones of inhibition, especially at lower concentrations.

However, in some cases particularly against *Staphylococcus aureus* the essential oil infusion showed comparable inhibitory effects to the standard antibiotic, especially at higher concentrations. This suggests that the phytochemicals present in the oil possess strong antibacterial potential that may rival conventional antibiotics when properly formulated and dosed.

RECOMMENDATION

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

1. Further Phytochemical Analysis: Advanced techniques such as Gas Chromatography-Mass Spectrometry (GC-MS) should be used to isolate and identify the specific bioactive compounds responsible for the antimicrobial activity of *Ocimum gratissimum.*
2. Toxicological Studies: Safety and toxicity evaluations should be conducted to determine the appropriate dosage for human use and ensure that the essential oil is safe for therapeutic application.
3. Formulation Development: The essential oil should be explored for incorporation into pharmaceutical products such as ointments, soaps, and sprays for use against common bacterial infections.
4. Storage and Stability Studies: The shelf-life and stability of the essential oil should be evaluated under various storage conditions to determine its effectiveness over time.

5. Comparative Studies: Further research should be conducted to compare the efficacy of *Ocimum gratissimum* oil with other plant-based antimicrobials and with various synthetic antibiotics across a wider range of pathogens.
6. Promotion of Local Medicinal Plants: Government and health sectors should invest in the study and promotion of medicinal plants like *Ocimum gratissimum* as affordable alternatives in the fight against antibiotic resistance.

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