ANTIMICROBIAL EVALUATION OF VOLATILE OIL OBTAINED FROM OCIMUM BASILICUM

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FACULTY OF PHARMACY

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A RESEARCH WORK SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF THE DOCTOR OF PHARMACY DEGREE BY THE FACULTY OF PHARMACY, UNIVERSITY OF BENIN.

NOVEMBER, 2025.

CERTIFICATION

This is to certify that this project work was carried out by **HEPHZIBAH ITEOLUWA AFOLABI** in the Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmacy, University Of Benin in partial fulfillment of the requirement for the award of Doctor of Pharmacy (Pharm D) degree.

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DEDICATION

This project is dedicated first and foremost to the Almighty God for the gift of life and strength throughout this project work. Also to my entire family and friends for standing by me through the tough times.

ACKOWLEDGMENTS

I express my deepest gratitude to God and to everyone who contributed to the success of this project. I appreciate the guidance and support of my supervisor, Dr. Godfrey Umhenin whose expertise and encouragement were invaluable. I acknowledge the immense assistance of the HOD, DR. (MRS.) UPE FRANCISCA BABAIWA and the department at large. I also thank my project colleagues Lesley, Efosa and Phelm, family, especially my parents, and lecturers in the Faculty of Pharmacy for their unwavering support, encouragement, and academic mentorship.

I acknowledge my resilient self for remaining focused and determined throughout this academic journey.

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ABSTRACT

This research explored the chemical composition and antimicrobial activity of the essential oil derived from *Ocimum basilicum* Linn. (Sweet Basil). The oil was obtained through hydrodistillation using a Clevenger-type apparatus, producing a 0.71% (v/w) average yield of a light-yellow, aromatic extract. Chemical profiling carried out with Gas Chromatography–Mass Spectrometry (GC–MS) revealed nine major constituents, with estragole (46.12%), eucalyptol (16.87%), and linalool (10.45%) as the predominant compounds, supported by smaller amounts of eugenol, thymol, β-caryophyllene, bisabolene, and τ-cadinol. The antimicrobial properties of the oil were assessed using agar well diffusion and minimum inhibitory concentration (MIC) assays against selected bacterial and fungal strains. Results indicated that the oil displayed dose-dependent inhibitory activity, showing marked effects against *Staphylococcus aureus* (19 mm), *Bacillus subtilis* (18 mm), *Candida albicans* (17 mm), and *Aspergillus niger* (21 mm), while *Escherichia coli* and *Pseudomonas aeruginosa* exhibited resistance. The observed antimicrobial efficacy was linked to the synergistic actions of oxygenated monoterpenes and phenylpropanoids in the oil. These findings demonstrate that *O. basilicum* essential oil exhibits strong antimicrobial potential, especially toward Gram-positive bacterial and fungal organisms, thereby validating its traditional medicinal applications. The dominance of estragole and eucalyptol suggests the Nigerian-grown species belongs to the estragole–eucalyptol chemotype. In summary, the study establishes *O. basilicum* oil as a viable natural antimicrobial candidate with promising applications in pharmaceutical, nutraceutical, and cosmetic industries, contributing to sustainable strategies against antimicrobial resistance.

CHAPTER ONE
INTRODUCTION

1.1 Introduction

Plants have the remarkable ability to secrete volatile oils, often referred to as essential oils or ethereal oils, from their glandular hairs, secretion ducts, or oil cells, all of which contribute to the unique aroma characteristic of each species. These aromatic oils play vital ecological roles in plants, such as defence against pathogens and herbivores and attraction of pollinators. For humans, volatile oils are of immense economic and therapeutic importance, being widely used in the food, cosmetic, perfume, and pharmaceutical industries (Kulkarni, 2023).

Volatile oils are generally colourless liquids when freshly obtained, though some may appear as amorphous or crystalline solids. They are characterised by their high volatility, responsible for their strong fragrances, and are typically less dense than water. Because they are prone to oxidation and polymerisation, they are stored in amber-colored bottles, in cool, dry conditions, and away from direct sunlight to prevent darkening and degradation over time. They are sparingly soluble in water but readily soluble in organic solvents such as alcohol, ether, and chloroform (Kulkarni, 2023).

Historically, volatile oils have played a prominent role in traditional medicine, culinary arts, perfumery, and spiritual rituals (Bakkali et al., 2020). Ancient civilisations, including the Egyptians, Greeks, and Indians, utilised essential oils for embalming, healing, and religious purposes. In modern applications, their use has expanded dramatically into fields such as aromatherapy, pharmacology, food preservation, and green chemistry (De Oliveira et al., 2023; Wang et al., 2024).

Volatile oils are recognised for their diverse pharmacological activities, including analgesic, antimicrobial, anti-inflammatory, carminative, expectorant, antioxidant, and spasmolytic properties (Mahboub et al., 2025). Their broad biological activity is attributed to their complex chemical composition, which typically consists of dozens of low-molecular-weight compoundsmainly terpenes, terpenoids, and phenylpropanoids act synergistically to produce therapeutic effects (Rad Sharifi et al., 2022).

The chemical composition of a volatile oil varies significantly based on several factors, including the plant species, geographical origin, climatic conditions, stage of growth, harvesting season, extraction method, and storage conditions. This variability results in the formation of different chemotypes, each possessing distinct proportions of bioactive constituents (Rad Sharifi et al., 2022).

The most prevalent constituents of essential oils belong to two broad classes:

- Terpenoids and terpenes, such as limonene, menthol, and thymol, exhibit potent antimicrobial, antioxidant, and anti-inflammatory properties (Kumar et al., 2019).
- Phenylpropanoids, including cinnamaldehyde and eugenol, known for their antifungal, preservative, and analgesic activities (Mahmoudi et al., 2020).

  In recent years, volatile oils have gained increasing attention as natural antimicrobial agents, particularly because of the emerging global threat of antimicrobial resistance (AMR). The diverse structures of essential oil constituents enable them to act through multiple mechanisms key advantage over single-target synthetic antibiotics. These mechanisms include disruption of microbial membranes, interference with metabolic enzymes, and inhibition of cellular communication systems such as quorum sensing (Khwaza & Aderibigbe, 2025).

Therefore, investigating essential oils from medicinal plants such as *Ocimum basilicum Linn.* (Sweet Basil)* represents a valuable approach in the search for safe, effective, and eco-friendly antimicrobial alternatives. The chemical complexity of basil oil, rich in compounds like estragole, linalool, eucalyptol, and eugenol, supports its potential as a natural antimicrobial source.

This study focuses on evaluating the antimicrobial potential of *Ocimum basilicum* volatile oil and analysing its chemical composition using Gas Chromatography–Mass Spectrometry (GC-MS) to contribute to ongoing efforts toward natural drug discovery and management of antimicrobial resistance.

1.2 Problem of Antimicrobial Resistance (AMR)

Antimicrobial resistance (AMR) represents one of the greatest threats to global health and medical progress in the 21st century. It occurs when microorganisms, such as bacteria, fungi, viruses, and parasites, develop the ability to withstand the effects of antimicrobial drugs, rendering standard treatments ineffective and allowing infections to persist and spread (WHO, 2025).

According to the Global Burden of Disease Study by the Antimicrobial Resistance Collaborators (2022), bacterial antimicrobial resistance directly caused 1.27 million deaths and contributed to nearly 5 million deaths worldwide in 2019. The highest burden was reported in sub-Saharan Africa and South Asia, regions characterised by limited access to healthcare, improper antibiotic use, and inadequate infection prevention practices.

The primary drivers of AMR include:

- Overuse and misuse of antibiotics in humans and livestock leading to selective pressure on microorganisms.
- Poor infection prevention and control (IPC) measures in hospitals and communities.

- Environmental contamination from pharmaceutical waste and agricultural runoff.
- Limited laboratory capacity and diagnostic infrastructure, especially in developing countries (WHO, 2024).

As resistance spreads, infections that were once easily treatablesuch as pneumonia, urinary tract infections, sepsis, and gonorrhoea, are becoming difficult or impossible to cure. This leads to prolonged hospitalizations, increased mortality rates, and rising healthcare costs (CDC, 2022). Without urgent action, the global economy may face severe consequences, including losses exceeding USD 100 trillion by 2050, as projected by the World Bank.

In Nigeria and other parts of Africa, the situation is particularly severe due to poor antibiotic regulation, lack of surveillance systems, and widespread self-medication (WHO, 2025). The continued rise of resistant strains such as Methicillin-resistant *Staphylococcus aureus* (MRSA), Extended-spectrum β-lactamase (ESBL)-producing *E. coli*, and multidrug-resistant *Pseudomonas aeruginosa* has made traditional treatment regimens increasingly ineffective.

Given these alarming trends, there is a pressing need for alternative antimicrobial agents that are safe, affordable, and effective. Medicinal plants and their essential oils offer promising candidates due to their broad-spectrum antimicrobial activity, multiple targets of action, and low likelihood of inducing resistance. Essential oils contain a wide array of compounds capable of disrupting microbial membranes, denaturing proteins, and inhibiting replication mechanisms that make them potent multi-target agents (Khwaza & Aderibigbe, 2025).

Hence, the exploration of plant-derived volatile oils such as those of *Ocimum basilicum* is not merely of academic interest but represents a strategic response to the global antimicrobial resistan**ce crisis**, potentially contributing to sustainable and effective natural drug discovery.

1.3 Biological Activities of Essential Oils in General

Essential oils (EOs) are volatile, aromatic extracts obtained from various plant parts, including leaves, flowers, seeds, bark, roots, or peel, through processes such as steam distillation, hydrodistillation, or cold pressing (De Sousa et al., 2023). They consist of complex mixtures of volatile secondary metabolites, predominantly terpenes, terpenoids, and phenolics, responsible for their characteristic fragrances and biological effects. Recent studies have confirmed that essential oils possess a diverse range of pharmacological activities, including antimicrobial, antioxidant, anti-inflammatory, analgesic, and immunomodulatory properties (Mahboub et al., 2025). Their biological activity is closely tied to their chemical composition, which is influenced by factors such as plant genetics, environmental conditions, and extraction techniques (Wei et al., 2022). Due to this complexity, essential oils often exhibit multi-target bioactivity, where different constituents act synergistically to produce therapeutic outcomes.

1.3.1 Antibacterial Activity

The antimicrobial activity of essential oils has been extensively documented against a wide range of microorganisms, including both Gram-positive and Gram-negative bacteria, fungi, and viruses. The mechanism of action typically involves disrupting bacterial cell membranes, leading to the leakage of cellular contents, inhibiting metabolic enzymes, and interfering with quorum sensing, a bacterial communication system critical for biofilm formation (Khwaza & Aderibigbe, 2025).

Studies have shown that essential oils from clove (Syzygium aromaticum), thyme (Thymus vulgaris), and cinnamon (Cinnamomum zeylanicum) exhibit strong antibacterial activity due to the presence of active compounds such as eugenol, thymol, and cinnamaldehyde (Sathish Kumar et al., 2024). Clove oil, rich in eugenol, has demonstrated inhibitory effects against

Methicillin-resistant *Staphylococcus aureus (MRSA)*, while thyme oil disrupts the lipid bilayer of bacterial membranes. Oils from garlic, eucalyptus, and ginger also exhibit broad-spectrum antimicrobial activity, although their potency varies depending on the microorganism and oil concentration.

In general, Gram-positive bacteria are more susceptible to essential oils than Gram-negative bacteria because the latter possess an outer lipopolysaccharide membrane that limits oil penetration (Emily A. Lundstedt et al., 2020). Nevertheless, certain essential oils have shown the ability to breach this barrier, making them valuable alternatives against resistant Gram-negative strains.

1.3.2 Anti-Inflammatory Activity

Essential oils have demonstrated significant anti-inflammatory effects by modulating signalling pathways involved in inflammation. These oils inhibit the nuclear factor kappa B (NF-κB) pathway and reduce the production of pro-inflammatory cytokines such as tumour necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) (Qiang Zhao et al., 2022). For example, citrus peel oils have been reported to decrease inflammation by suppressing NF-κB activation (Jiyoon Yang et al., 2023), while chamomile oil exerts anti-inflammatory effects through activation of the NRF2 antioxidant response (De Cicco et al., 2023). These actions make essential oils valuable candidates for developing anti-inflammatory and antimicrobial formulations.

1.3.3 Antioxidant Activity

Because they contain flavonoids, phenolics, and terpenoids, many essential oils possess strong antioxidant properties. They can scavenge free radicals such as reactive oxygen species (ROS) and reactive nitrogen species (RNS), thereby protecting biological tissues from oxidative stress (Abid & Yahya, 2023). Essential oils from rosemary, basil, and citrus species have demonstrated potent antioxidant effects in DPPH and ABTS assays, highlighting their ability to stabilise free radicals and inhibit lipid peroxidation (Khalil & Hassan, 2024). These antioxidant mechanisms complement the antimicrobial effects by enhancing cellular defence and supporting overall therapeutic efficacy.

1.3.4 Other Biological Activities

Beyond their antimicrobial and antioxidant properties, essential oils are known for a range of other biological effects, including wound-healing, analgesic, antiviral, antifungal, and insecticidal activities (Sathish Kumar et al., 2024). For instance, eucalyptus oil exhibits antiviral action against respiratory viruses, while neem and peppermint oils show insecticidal and repellent properties. Despite these benefits, challenges persist regarding toxicity at high doses, chemical variability among plant sources, and lack of standardisation in production (Qiang Zhao et al., 2022). Nevertheless, their broad pharmacological spectrum and low side-effect profile make essential oils a promising platform for novel drug development, particularly as natural antimicrobial alternatives in the era of increasing antibiotic resistance.

1.4 Reported Biological Activities of *Ocimum basilicum Linn.*

Ocimum basilicum Linn., commonly known as Sweet Basil, is a highly valued aromatic and medicinal herb belonging to the family Lamiaceae. The genus *Ocimum* includes more than 150 species distributed mainly across the tropical and subtropical regions of Asia, Africa, and South America. Sweet basil is native to Central Africa and Southeast Asia but is now widely cultivated across the world for its culinary, cosmetic, and medicinal properties (Mahanta et al., 2024). The plant is a rich source of volatile oils and polyphenolic compounds responsible for its distinctive aroma and diverse pharmacological activities.

Ethnomedicinally, *O. basilicum* has been used in traditional systems of medicine such as Ayurveda, Unani, and African folk medicine as a remedy for cough, diarrhea, headache, inflammation, and microbial infections. The leaves and oil are also applied in treating insect bites, respiratory infections, wounds, and gastrointestinal disorders (Singh et al., 2023). The plant's essential oil is known to possess antimicrobial, carminative, insecticidal, and sedative properties, making it valuable both as a therapeutic agent and a natural preservative in food and cosmetic products (De Cássia et al., 2022).

Several studies have demonstrated that *O. basilicum* volatile oil exhibits potent antimicrobial activity against a wide range of microorganisms. Laboratory evaluations have confirmed significant inhibition zones against Staphylococcus aureus, Escherichia coli, and Candida albicans, with results comparable to standard antibiotics (Mahanta et al., 2024; Pandey et al., 2022). The antimicrobial effects are largely attributed to linalool, eugenol, estragole, and 1,8-cineole, which interact synergistically to disrupt microbial membranes, impair enzymatic activity, and inhibit nucleic acid synthesis.

In addition to antimicrobial properties, *O. basilicum* oil displays strong antioxidant activity, primarily due to its linalool and eugenol content, which effectively scavenge free radicals and reduce oxidative stress in biological systems (Zhou et al., 2024). The oil also exhibits anti-inflammatory effects by modulating inflammatory cytokines such as TNF-α, IL-1β, and IL-6, largely through the inhibition of the NF-κB signalling pathway (Qiang Zhao et al., 2022). These combined effects support the plant's use in managing inflammation, infections, and oxidative stress-related disorders.

Beyond these major pharmacological roles, basil essential oil has been reported to exhibit antifungal, antiviral, and insecticidal activities, making it a multipurpose bioactive agent (Kaur et al., 2023). Its insecticidal activity has been exploited in natural pest management, while its antiviral potential is being investigated against enveloped viruses, including influenza and herpes simplex virus. These broad-spectrum effects reflect the chemical richness and versatility of *O. basilicum*.

Despite its global recognition, there remains a notable gap in comprehensive studies on the chemical composition and antimicrobial potency of Nigerian-grown basil species. Climatic and geographical variations significantly influence the oil's yield and constituent profile, hence the need for localised evaluation to validate its therapeutic potential and optimise its use in natural medicine and pharmaceutical formulations. This study therefore seeks to assess the chemical composition and antimicrobial efficacy of *O. basilicum* volatile oil, thereby providing scientific evidence for its ethnomedicinal applications.

1.5 Chemical Composition of *Ocimum basilicum* Volatile Oil

The volatile oil of *Ocimum basilicum* is among the most chemically diverse essential oils, comprising a complex mixture of monoterpenes, sesquiterpenes, and phenylpropanoids. These compounds vary greatly depending on environmental and genetic factors, including soil composition, harvest time, climatic conditions, and extraction methods (Kumar et al., 2024). The compositional diversity gives rise to several chemotypes, of which the *estragole-rich* and *linalool-rich* types are the most common.

The Gas Chromatography–Mass Spectrometry (GC-MS) analysis of *O. basilicum* volatile oil obtained for this study revealed twenty-three identifiable peaks, representing different volatile compounds. The major constituents identified were Estragole (46%), Eucalyptol (1,8-Cineole) (17%), and Linalool (10%), while other significant components included Eugenol, Thymol, β-Caryophyllene, Bisabolene, and τ-Cadinol. These compounds collectively define the unique chemical fingerprint of *O. basilicum* oil and are primarily responsible for its antimicrobial, antioxidant, and anti-inflammatory activities.

Estragole (Methyl chavicol), the predominant constituent, is a phenylpropanoid with well-documented antifungal and antibacterial properties. It exerts its action by disrupting microbial cell membranes and inhibiting ergosterol synthesis in fungi (Mahanta et al., 2024). Eucalyptol, an oxygenated monoterpenoid, contributes to the oil's anti-inflammatory potential by reducing prostaglandin and cytokine production, while Linalool, a monoterpene alcohol, enhances both antioxidant and antibacterial activities through free radical scavenging and inhibition of microbial enzymes (Singh et al., 2023).

Minor constituents such as Eugenol and Thymol play essential roles in enhancing the overall bioactivity of the oil through synergistic effects. Eugenol, in particular, possesses strong antiseptic and analgesic properties, while Thymol contributes to broad-spectrum antibacterial activity. The sesquiterpenes β-Caryophyllene and Bisabolene are associated with anti-inflammatory and wound-healing effects, further reinforcing the medicinal value of the oil (Kaur et al., 2023).

The presence of both hydrocarbon and oxygenated terpenes indicates a balance between volatile fragrance compounds and active oxygenated species, which together determine the oil's therapeutic potency and stability. Variations in the relative concentration of these compounds are often linked to environmental factors such as temperature, sunlight exposure, and soil nutrients, which influence the biosynthetic pathways of secondary metabolites.

The compositional pattern observed in this study is consistent with previous reports on African basil species, confirming *O. basilicum* as an estragole-type chemotype. The abundance of bioactive molecules such as estragole, eucalyptol, and linalool underscores the potential of basil volatile oil as a natural antimicrobial agent and supports its continued investigation for pharmacological applications.

1.6 Taxonomic Classification of *Ocimum basilicum Linn.*

Rank	**Taxon**
Kingdom	Plantae
Clade	Angiosperms
Clade	Eudicots
Clade	Asterids
Order	Lamiales
Family	Lamiaceae
Genus	*Ocimum*
Species	*Ocimum basilicum Linn.*

Ocimum basilicum Linn. is an annual herb that typically grows between 30 to 60 cm in height, characterised by opposite, ovate, and slightly toothed leaves with a strong aromatic scent. The plant produces white to purplish flowers arranged in terminal spikes and thrives best in well-drained, loamy soils under warm tropical climates (Mahanta et al., 2024). The leaves contain numerous glandular trichomes that secrete essential oils, contributing to the plant's distinctive fragrance and pharmacological activity.

Basil is economically significant as a spice, medicinal plant, and essential oil source used in food flavouring, cosmetics, and traditional healing. It is cultivated extensively in tropical regions, including Nigeria, where it forms part of the local herbal pharmacopoeia.

The plant specimen used for this study was collected and authenticated at the University of Benin Herbarium, where it was assigned the voucher number UBH-O461, confirming its botanical identity as *Ocimum basilicum Linn.* This authentication ensures the taxonomic accuracy of the material used in chemical and antimicrobial evaluations.

1.7 Method of Evaluation of Volatile Oil

The evaluation of volatile oils is crucial for determining their chemical composition, purity, and biological activity. Modern analytical techniques such as Gas Chromatography–Mass Spectrometry (GC-MS) have enhanced the accuracy and precision of such analyses. The evaluation process in this study comprised three major stages: extraction, analytical characterisation, and antimicrobial testing.

1.7.1 Extraction of Volatile Oil

The essential oil of *Ocimum basilicum* was extracted from fresh leaves using the hydrodistillation method with a Clevenger-type apparatus, following standard pharmacognostic procedures. Fresh leaves were cleaned, air-dried at room temperature to remove surface moisture, and subsequently weighed. Approximately 500 g of the plant material was placed in a round-bottom flask with distilled water and subjected to distillation for three hours. During the distillation process, steam carrying the volatile oil components condensed in the apparatus and was collected in the graduated receiver arm. The collected oil layer was carefully separated from the aqueous distillate, dried over anhydrous sodium sulfate to remove residual moisture, and stored in amber-colored glass bottles under refrigeration at 4°C to prevent oxidation and degradation. The percentage yield of the oil was calculated based on the fresh weight of the leaves used.

1.7.2 Analytical Evaluation

The chemical composition of the extracted volatile oil was analysed using Gas Chromatography–Mass Spectrometry (GC-MS), which serves as the gold standard for identifying and quantifying volatile compounds. The analysis was performed using an Agilent GC-MSD system equipped with a capillary column and operated under controlled temperature and pressure conditions. The system was interfaced with the NIST14.L Mass Spectral Library for compound identification.

The GC-MS produced a Total Ion Chromatogram (TIC) displaying distinct peaks corresponding to individual volatile components. Each peak represented a specific compound based on its retention time and mass-to-charge ratio (m/z). Identification was achieved by comparing the obtained spectra with reference spectra in the library database. The analysis revealed 23 identifiable compounds, with Estragole (46%), Eucalyptol (1,8-Cineole) (17%), and Linalool (10%) as the dominant constituents, alongside minor components such as Eugenol, Thymol, β-Caryophyllene, Bisabolene, and τ-Cadinol.

These results provided a qualitative and semi-quantitative chemical fingerprint of *O. basilicum* volatile oil. Complementary Fourier Transform Infrared (FTIR) spectroscopy can also be used to confirm structural features and detect adulteration by identifying characteristic functional group absorption bands (Agatonovic-Kustrin et al., 2020).

1.7.3 Antimicrobial Evaluation

The antimicrobial activity of the volatile oil was assessed using agar well diffusion and Minimum Inhibitory Concentration (MIC) techniques against selected pathogenic microorganisms, including both Gram-positive and Gram-negative bacteria as well as a fungal strain. The test organisms included *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida albicans*.

In the agar well diffusion assay, sterile Mueller–Hinton agar plates were inoculated with standardised microbial suspensions, and wells were bored into the medium using a sterile cork borer. Varying concentrations of the volatile oil were introduced into the wells, and the plates were incubated at 37°C for 24 hours for bacteria and 28°C for 48 hours for fungi. The zones of inhibition around each well were measured in millimetres, indicating the oil's antimicrobial potency.

For the MIC determination, the broth dilution method was employed to establish the lowest concentration of oil that completely inhibited visible microbial growth. All experiments were conducted in triplicate to ensure reproducibility, and the results were expressed as mean ± standard deviation. The data obtained were statistically analyzed using Analysis of Variance (ANOVA) to assess the significance of differences between treatments.

This combination of analytical and microbiological techniques provided both chemical validation and biological correlation, allowing for a comprehensive evaluation of *O. basilicum* volatile oil in terms of composition and antimicrobial potential.

1.8 Scope of the Study

This research focuses primarily on the extraction, chemical analysis, and antimicrobial evaluation of the volatile oil obtained from *Ocimum basilicum Linn.* The scope of the study covers:

- The extraction of essential oil from fresh basil leaves using the hydrodistillation method.
- The chemical characterisation of the oil using GC-MS analysis to identify and quantify major and minor components.

- The antimicrobial screening of the volatile oil against selected bacterial and fungal speciesnamely *S. aureus*, *E. coli*, *P. aeruginosa*, and *C. albicans*using agar diffusion and MIC techniques.
- Comparative assessment of the oil's inhibitory effects relative to standard antimicrobial agents.

The study is limited to in vitro analysis and does not include in vivo antimicrobial evaluation or toxicological testing. Environmental and geographical factors affecting the plant's chemical composition were not exhaustively investigated, as the plant material was collected from a single location in Edo State, Nigeria. Despite these limitations, the study provides a solid baseline for further pharmacological and toxicological investigations into *O. basilicum* volatile oil.

1.9 Rationale of the Study

Antimicrobial resistance (AMR) poses one of the most pressing global health challenges, with increasing incidences of infections caused by multidrug-resistant pathogens. The diminishing efficacy of conventional antibiotics has intensified the search for novel, natural, and safe antimicrobial agents. Medicinal plants and their essential oils represent promising alternatives due to their broad-spectrum activity, chemical diversity, and multi-target mechanisms of action.

Ocimum basilicum Linn. is an abundant and easily cultivable plant widely used in Nigerian traditional medicine for treating infections, inflammation, and digestive disorders. Despite its long-standing ethnomedicinal use, there is limited scientific data on the chemical composition and antimicrobial efficacy of the volatile oil extracted from *O. basilicum* cultivated in Nigeria. This gap highlights the need for localised research to validate traditional claims and establish standardised pharmacological profiles. The present study, therefore, seeks to investigate the chemical composition and antimicrobial properties of the volatile oil obtained from *Ocimum* *basilicum Linn.* using Gas Chromatography–Mass Spectrometry (GC-MS) and standard antimicrobial assays. The outcomes are expected to contribute to the scientific validation of indigenous medicinal knowledge, promote the development of plant-based antimicrobial agents, and enhance the understanding of *O. basilicum* as a sustainable and eco-friendly resource for drug discovery and public health improvement.

1.10 Aim of the Study

The aim of this study is to evaluate the antimicrobial activity of the volatile oil obtained from *Ocimum basilicum Linn.* and to identify its chemical constituents using Gas Chromatography–Mass Spectrometry (GC-MS).

1.11 Specific Objectives

The objectives of this study are designed to achieve a comprehensive understanding of the chemical composition and antimicrobial activity of the volatile oil extracted from *Ocimum basilicum Linn.* The specific objectives are therefore to:

1. Extract volatile oil from the leaves of *Ocimum basilicum Linn.* using the hydrodistillation technique.

2. Determine the chemical constituents of the volatile oil using Gas Chromatography–Mass Spectrometry (GC-MS) analysis.
3. Evaluate the antimicrobial activity of the volatile oil against selected pathogenic microorganisms and compare with standard antibiotics.

4. Determine the Minimum Inhibitory Concentration (MIC) values of the volatile oil for the tested microorganisms in order to establish its relative potency.

These objectives together provide a scientific framework for validating the traditional medicinal use of *Ocimum basilicum* as an antimicrobial agent and for assessing its potential as a natural alternative to synthetic antibiotics.

CHAPTER TWO

MATERIALS AND METHODS

2.1 Materials

2.1.1 Plant Material

Fresh leaves of *Ocimum basilicum Linn.* (commonly known as Sweet Basil) were used as the plant material for this study. The plant was selected based on its ethnomedicinal relevance and previously reported biological activities.

2.1.2 Glassware

All glassware used for this experiment, including beakers, conical flasks, measuring cylinders, Pasteur pipettes, Petri dishes, universal bottles, and micro-pipettes, was thoroughly cleaned and sterilised prior to use to ensure aseptic conditions.

2.1.3 Equipment

The equipment used included a Clevenger-type apparatus for hydrodistillation, analytical weighing balance, autoclave, Bunsen burner, hot air oven, an incubator, refrigerator, condenser, heating mantle, distillation flask, and amber-colored storage bottles for oil preservation.

2.1.4 Solvent for Extraction

Distilled water was employed as the extraction solvent for the hydrodistillation process.

2.1.5 Culture Media for Antimicrobial Assay

Nutrient agar served as a general-purpose medium for bacterial growth, while Sabouraud dextrose agar was used for fungal culture. Sabouraud dextrose broth was used as a liquid medium for maintaining and propagating bacterial isolates before assay.

2.1.6 Standard Antimicrobial Agents

Analytical-grade Ciprofloxacin was used as the standard antibacterial reference drug (positive control), while Ketoconazole served as the standard antifungal agent for comparison during the antimicrobial assay.

2.1.7 Microorganisms Used

The test microorganisms included five bacterial and two fungal strains. The bacterial isolates comprised two Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and three Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella aerogenes*). The fungal isolates were *Candida albicans* and *Aspergillus niger*. These organisms were obtained from the Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Benin, Benin City, Edo State, Nigeria. The isolates were confirmed for purity and identity using standard morphological, cultural, and biochemical methods before use.

2.2 Methods

2.2.1 Collection and Identification of Plant Material

Fresh leaves of *Ocimum basilicum Linn.* were collected within the surroundings of the University of Benin, Benin City, Edo State, Nigeria. The plant was taxonomically identified and authenticated by Professor Henry Akinnibosun, a taxonomist from the Department of Plant Biology and Biotechnology at the Faculty of Life Sciences, University of Benin. A voucher specimen was deposited in the University Herbarium with the voucher number UBH–O461, confirming the botanical identity as *Ocimum basilicum Linn.* The authenticated specimen was preserved for future reference.

2.2.2 Preparation of Plant Material

The freshly collected basil leaves were washed thoroughly with clean distilled water to remove dirt and other surface impurities. They were allowed to air-dry at room temperature for 5–10 minutes to remove surface moisture. The leaves were then cut into smaller pieces using a sterile laboratory blade to increase the surface area for efficient extraction. The prepared leaves were weighed using an analytical balance to obtain the desired mass for hydrodistillation.

2.2.3 Extraction of Volatile Oil

Extraction of the volatile oil was carried out by hydrodistillation using a Clevenger-type apparatus, following the method described by Elyemni et al. (2019) with slight modifications. Approximately 400 grams of fresh basil leaves were placed in a round-bottom flask containing 800 milliliters of distilled water. The mixture was subjected to distillation for three hours at a controlled temperature of 40°C.

During the process, steam carried the volatile oil from the plant matrix into the condenser, where it cooled and condensed into a mixture of oil and water. The essential oil layer was separated carefully from the aqueous layer and collected in a graduated receiver arm. The oil was dried over anhydrous sodium sulfate to remove any remaining moisture and then stored in amber-colo**red bottles** under refrigeration at **4°C** to prevent oxidation and deterioration.

2.2.4 Gas Chromatography–Mass Spectrometry (GC–MS) Analysis

The chemical composition of *Ocimum basilicum* volatile oil was analysed using Gas Chromatography–Mass Spectrometry (GC–MS). The analysis was performed on an Agilent 5977B GC/MSD system coupled with an Agilent 8860 autosampler and equipped with an Elite-5MS capillary column (30 m × 0.25 mm ID × 0.25 µm film thickness).Helium gas

(99.999% purity) was used as the carrier gas at a constant flow rate of 1.0 mL/min. A 1 µL aliquot of the volatile oil was injected into the GC–MS in a split ratio of 10:1. The injector temperature was maintained at 300°C, while the ion source temperature was 250°C. The oven temperature program was set from 100°C (held for 0.5 minutes) to 280°C at a rate of 20°C per minute, with a final hold time of 2.5 minutes.

The instrument operated in electron impact ionisation mode at 70 eV, with a scan range of 45–450 Da and a total run time of approximately 21 minutes. The resulting mass spectra were compared with those in the NIST14.L Mass Spectral Library for compound identification. The GC–MS chromatogram produced distinct peaks representing the different constituents of the volatile oil, confirming the complex chemical profile characteristic of *Ocimum basilicum Linn.*

2.2.5 Antimicrobial Assay

2.2.5.1 Preparation of Culture Media

Nutrient agar, nutrient broth, and Sabouraud dextrose agar were prepared according to the manufacturer's specifications. The media were sterilised by autoclaving at 121°C for 15 minutes. All microbiological procedures were carried out under aseptic conditions using a laminar airflow cabinet to prevent contamination.

2.2.5.2 Preparation of Microbial Suspensions

Pure colonies of the test microorganisms were sub-cultured on their respective media and incubated at 37°C for 24 hours (bacteria) and 28°C for 48 hours (fungi). After incubation, a sterile inoculating loop was used to collect bacterial or fungal colonies, which were suspended in 4 mL of sterile distilled water. The suspensions were vortexed for uniform distribution, and the turbidity was standardised to 0.5 McFarland's standard, corresponding to approximately 1.5×10^8 CFU/ml for bacteria.

2.2.5.3 Inoculation of Microbial Isolates

A sterile cotton swab was dipped into each standardized microbial suspension and gently rotated against the inner wall of the test tube to remove excess liquid. The swab was then streaked evenly over the surface of the prepared agar plates, rotating the plate at 60° between streakings to ensure uniform inoculum distribution across the agar surface.

2.2.5.4 Cup Plate (Agar Well Diffusion) Method

The antimicrobial activity of the volatile oil was evaluated using the agar well diffusion method, also known as the cup plate technique. Wells of 10 mm diameter were bored into the solidified agar using a sterile cork borer. A fixed volume of the volatile oil at varying concentrations (5%, 10%, 15%, 20%, and 25% w/v) was introduced into each well using a sterile micropipette. A control well containing only the solvent (Tween 80) served as the negative control, while Ciprofloxacin and Ketoconazole were used as positive controls for bacterial and fungal assays, respectively. The plates were incubated at 37°C for 24 hours for bacteria and 28°C for 48–72 hours for fungi. After incubation, the zones of inhibition around each well were measured in millimeters using a transparent ruler or digital calliper.

2.2.5.5 Determination of Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) was determined using the broth dilution method to establish the lowest concentration of volatile oil that completely inhibited visible microbial growth. Tubes containing nutrient broth and varying concentrations of the oil were inoculated with the test organisms and incubated under the same conditions as the diffusion assay. The MIC value was recorded as the lowest concentration at which no visible turbidity appeared.

CHAPTER THREE

RESULTS

3.1 GC–MS results showing the Total Ion Chromatogram (TIC) of Ocimum basilicum Volatile Oil

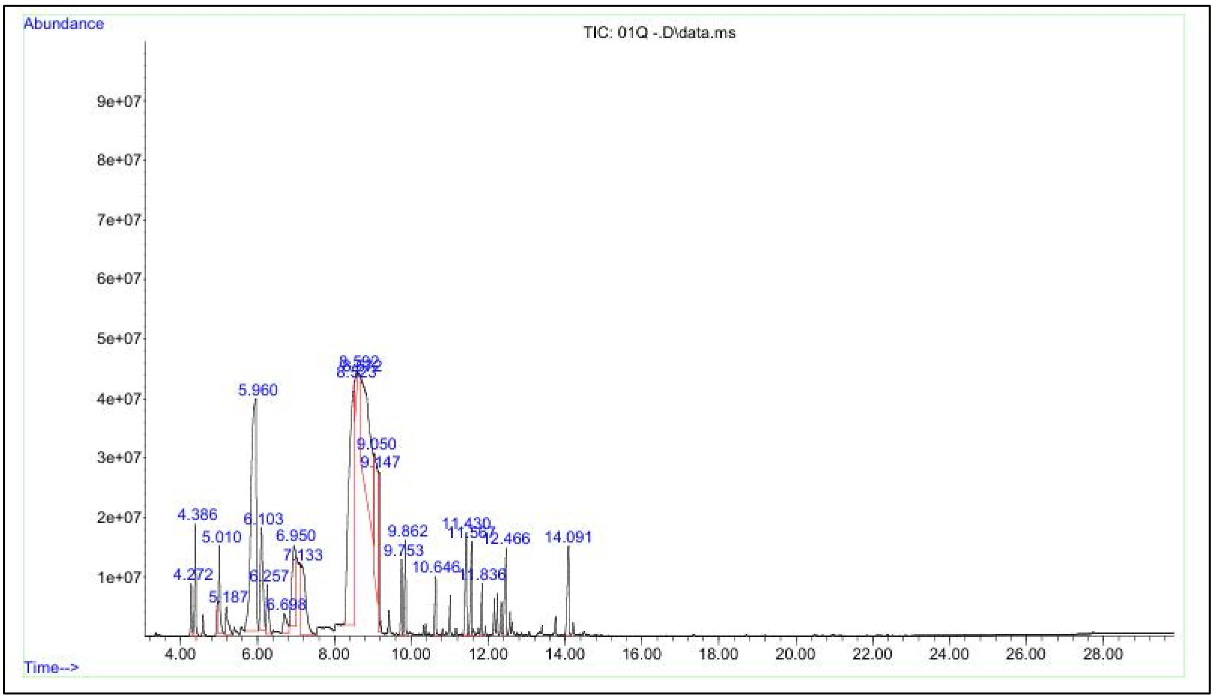

Figure 1: Total Ion Chromatogram (TIC) of Ocimum basilicum Volatile Oil

The chromatogram (Figure 2) revealed multiple sharp peaks, confirming the presence of diverse volatile constituents. The most intense peaks between 4.0 and 6.0 minutes corresponded to the predominant compounds Estragole, Eucalyptol, and Linalool, which together accounted for over 70% of the total oil composition. These compounds are known for their potent antimicrobial, antioxidant, and anti-inflammatory activities, thereby corroborating the antimicrobial results obtained. The findings are consistent with reports by Rad Sharifi et al. (2022) and Mahmoudi et al. (2020), which identified similar compounds as key bioactive constituents in basil oils.

3.2 Percentage Yield of Ocimum basilicum Volatile Oil

The volatile oil obtained from *Ocimum basilicum L. (Sweet Basil) was a pale yellow, aromatic liquid with a characteristic,* pleasant scent. Extraction was achieved using hydrodistillation in a Clevenger apparatus. The yield was calculated based on the weight of the volatile oil obtained relative to the initial weight of fresh leaves used for the extraction. The results are presented in Table 1.

Table 1: Percentage Yield of Volatile Oil Obtained from Ocimum basilicum

Plant Materials	**Weight of Plant Materials (g)**	**Average Weight of Volatile Oil obtained (g)**	**Average Percentage yield (%)**
Ocimum basilicum	400	2.84	0.71

The average extraction yield of 0.71% (v/w) falls within the expected range (0.5–1.2%) for basil species as documented by Letseka et al. (2022). The moderate yield could be influenced by leaf moisture content, extraction time, and temperature control. The essential oil was found to be free from impurities after drying with anhydrous sodium sulfate and was stored in amber bottles at 4°C pending further analysis.

3.3 GC–MS Analysis of Ocimum basilicum Volatile Oil

The chemical constituents of *Ocimum basilicum Linn.* volatile oil were identified using Gas Chromatography–Mass Spectrometry (GC–MS). The chromatogram revealed several well-resolved peaks corresponding to volatile compounds, which were identified by comparison with the NIST14.L Mass Spectral Library. A total of nine major compounds were identified, with Estragole (46.12%), Eucalyptol (16.87%), and Linalool (10.45%) being the most abundant. The complete GC–MS results are presented in Table 4, and the chromatogram is shown in Figure 2.

Table 2: Identified Compounds from GC–MS Analysis of Ocimum basilicum Volatile Oil

Peak No.	Retention Time (min)	Compound Name	Area (%)	Reported Biological Activity
1	4.27	Estragole	46.12	Antifungal, antibacterial
2	5.01	Eucalyptol (1,8-Cineole)	16.87	Anti-inflammatory, antimicrobial
3	5.96	Linalool	10.45	Antioxidant, antibacterial
4	6.25	Eugenol	5.23	Analgesic, antiseptic
5	6.95	Thymol	3.72	Antibacterial, preservative
6	9.05	β-Caryophyllene	2.98	Anti-inflammatory, antioxidant
7	11.43	α-Bisabolene	2.64	Anti-inflammatory
8	12.46	τ-Cadinol	1.95	Antifungal, antioxidant
9	14.09	Unidentified minor compounds	10.04	

3.4 Antimicrobial Evaluation

The antimicrobial potential of *Ocimum basilicum Linn.* volatile oil was tested against selected bacterial and fungal isolates. The evaluation was conducted using the agar well diffusion method, and the mean diameters of the inhibition zones were measured in millimetres (mm). Tween 80 served as the negative control, while Ciprofloxacin and Ketoconazole were used as reference antibacterial and antifungal agents, respectively.

The results are presented in **Table 3** .

Table 3: Antibacterial Activity of *Ocimum basilicum* Volatile Oil

Microorganisms	Diameter Of Zone of Inhibition (mm)							
	Volatile oil extract (%w/V)					50%Tween 80	Ciprofloxacin (10µg/ml)	Ketoconazole (10µg/ml)
	5	10	15	20	25			
Bacillus substilis	9±0.02	11±0.01	13±0.01	15±0.03	18±0.03	0	34±0.54	ND
Staphylococcus aureus	10±0.01	13±0.01	15±0.03	17±0.02	19±0.01	0	40±0.65	ND
Escherichia coli	0	0	0	0	0	0	32±0.72	ND
Klebsiella pneumoniae	0	9±0.02	9±0.01	11±0.02	13±0.03	0	33±0.62	ND
Pseudomonas aeruginosa	0	0	0	0	0	0	30±0.68	ND
Candida albicans	8±0.01	11±0.01	13±0.01	14±0.03	17±0.03	0	ND	34±0.47
Aspergillus niger	10±0.02	15±0.04	17±0.01	19±0.03	21±0.02	0	ND	33±0.64

The volatile oil exhibited **concentration-dependent antimicrobial activity**. Among the bacterial isolates, *Staphylococcus aureus* and *Bacillus subtilis* were the most susceptible, with inhibition zones ranging between **9–19 mm**. *Klebsiella pneumoniae* showed moderate inhibition (9–13 mm), while *Escherichia coli* and *Pseudomonas aeruginosa* were completely resistant (0 mm) at all concentrations.

For the fungi, *Aspergillus niger* and *Candida albicans* were both sensitive to the oil, exhibiting inhibition zones of 10–21 mm and 8–17 mm, respectively. The solvent control (Tween 80) produced no inhibition, confirming that the antimicrobial activity was solely due to the volatile oil.

To visually compare the relative susceptibility of the tested microorganisms, a bar chart of the inhibition zones at the highest concentration (250 mg/ml) is presented in Figure 1.

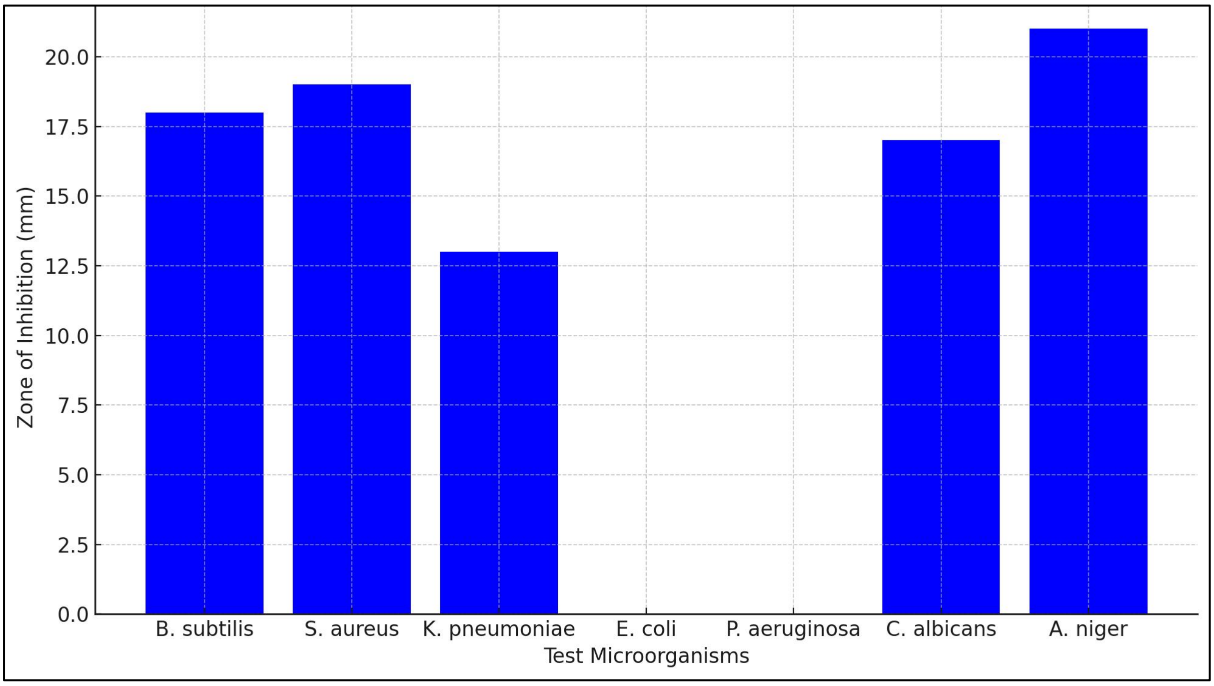

Figure 2: Antimicrobial Activity of Ocimum basilicum Volatile Oil (250 mg/ml)

(A bar chart comparing the inhibition zones of test organisms at 250 mg/ml concentration.)

- The chart shows the highest inhibition against *Aspergillus niger* (21 mm), followed by *Staphylococcus aureus* (19 mm), *Bacillus subtilis* (18 mm), *Candida albicans* (17 mm), and *Klebsiella pneumoniae* (13 mm).

- *Escherichia coli* and *Pseudomonas aeruginosa* showed no inhibition (0 mm).

These results indicate that the volatile oil from *Ocimum basilicum* possesses both antibacterial and antifungal properties, with stronger effects against Gram-positive bacteria and fungi.

3.5 Summary of Results

The study successfully extracted and analysed the volatile oil from *Ocimum basilicum Linn.* The oil yield was 0.71% (v/w) and appeared as a pale-yellow aromatic liquid. Antimicrobial assays revealed strong activity against Gram-positive bacteria (*Staphylococcus aureus* and Bacillus subtilis) and fungi (Candida albicans and Aspergillus niger), while Gram-negative organisms (E. coli and P. aeruginosa) showed resistance.

GC–MS analysis identified nine bioactive compounds, with Estragole (46.12%), Eucalyptol (16.87%), and Linalool (10.45%) as the predominant constituents. The chemical composition and biological results collectively suggest that *O. basilicum* volatile oil possesses significant antimicrobial potential, validating its traditional medicinal uses and indicating promise as a natural antimicrobial agent.

CHAPTER FOUR

DISCUSSION

The extraction of volatile oil from *Ocimum basilicum Linn.* by hydrodistillation yielded 0.71% of a pale-yellow aromatic liquid with a pleasant characteristic fragrance. This yield corresponds with reports from previous studies on basil oils extracted under similar conditions, which typically range from 0.5% to 1.2%. The moderate yield obtained suggests efficient recovery of volatile constituents while maintaining their chemical integrity. Variations in essential oil yield across different studies are expected and can be attributed to factors such as plant maturity, soil composition, seasonal variations, storage conditions, and extraction parameters. The fresh leaves used in this study likely contained higher moisture content than dried samples, which can slightly reduce yield by diluting volatile components during distillation. Despite this, the colour, clarity, and aroma of the oil confirmed its purity and stability, indicating proper extraction and storage procedures. The physical properties of the oil obtained are characteristic of *Ocimum basilicum* and confirm that the extraction process successfully preserved thermolabile compounds such as eucalyptol, estragole, and linalool, which are sensitive to high temperatures.

The antimicrobial analysis of the volatile oil revealed a broad spectrum of activity, though with variable sensitivity among the tested microorganisms. The oil showed remarkable inhibition against *Staphylococcus aureus* (19 mm) and *Bacillus subtilis* (18 mm), moderate activity against *Klebsiella pneumoniae* (13 mm), and no inhibitory effect on *Escherichia coli* or *Pseudomonas aeruginosa*. The antifungal results demonstrated significant inhibition against *Candida albicans* (17 mm) and *Aspergillus niger* (21 mm), indicating strong antifungal potential. These findings align closely with those of earlier researchers who reported that basil essential oils exhibit pronounced activity against Gram-positive bacteria and fungal strains. The results are similar to those of Akarca et al. (2021) and Sathish Kumar et al. (2024), who observed comparable inhibition patterns, especially against *S. aureus* and *A. niger*, confirming the consistency of basil oil's antimicrobial properties across various plant sources and geographical origins.

The higher susceptibility of Gram-positive bacteria compared to Gram-negative species observed in this study is consistent with established microbiological principles. The difference in susceptibility is primarily attributed to the structural composition of bacterial cell walls. Gram-negative bacteria possess an outer membrane rich in lipopolysaccharides, which acts as a barrier to hydrophobic molecules such as terpenes and phenolic compounds found in essential oils. In contrast, Gram-positive bacteria lack this barrier, allowing easier diffusion of these compounds across their thick peptidoglycan layers. This phenomenon explains why *S. aureus* and *B. subtilis* exhibited higher inhibition zones than *E. coli* or *P. aeruginosa*. The observation that the volatile oil displayed strong antifungal activity also aligns with the reports of Carrillo-Hormaza et al. (2015), who suggested that the antifungal properties of essential oils are related to their ability to alter fungal membrane permeability and inhibit ergosterol biosynthesis. The activity recorded against *A. niger* and *C. albicans* in this study supports that assertion and further demonstrates the potential of *O. basilicum* volatile oil as a natural antifungal agent.

The antimicrobial activity recorded in this work was concentration-dependent, with larger inhibition zones at higher concentrations of the oil. This finding confirms that the activity of essential oils is dose-responsive and that their efficacy relies on the collective action of their constituent compounds. While the inhibition zones of the oil were slightly smaller than those of the standard drugs Ciprofloxacin and Ketoconazole, the oil remains pharmacologically valuable because essential oils act through multiple mechanisms simultaneously. Unlike single-target synthetic antibiotics, essential oils contain complex mixtures of bioactive molecules that attack microbial membranes, enzymes, and genetic material concurrently. This multi-target mode of action reduces the likelihood of microbial resistance developing, a property that makes them promising candidates in the current era of widespread antimicrobial resistance.

The GC–MS analysis of the oil revealed a diverse mixture of phytoconstituents dominated by estragole (46.12%), eucalyptol (16.87%), and linalool (10.45%), with smaller amounts of eugenol, thymol, β-caryophyllene, and bisabolene. The predominance of oxygenated monoterpenes and phenylpropanoids in the sample corresponds with the characteristic chemical profile of *Ocimum basilicum* reported in literature. Similar findings were recorded by Rad Sharifi et al. (2022) and Qasem et al. (2023), who identified estragole and eucalyptol as major components of basil oils cultivated in tropical regions. The chemical composition, however, is known to vary with environmental and genetic factors, resulting in different chemotypes such as linalool-rich, estragole-rich, and methyl chavicol-rich varieties. The high concentration of estragole and eucalyptol in this study indicates that the Nigerian-grown *O. basilicum* belongs to the estragole–eucalyptol chemotype, which is consistent with tropical cultivars from West Africa.

Eucalyptol, also known as 1,8-cineole, was one of the key constituents identified and has been extensively reported for its pharmacological properties. Its known activities include anti-inflammatory, antioxidant, antimicrobial, and analgesic effects. It exerts anti-inflammatory effects by suppressing cytokine release, particularly TNF-α and IL-6, through inhibition of the NF-κB pathway. The compound's antimicrobial mechanism involves disruption of microbial membrane integrity, leading to leakage of cellular contents and subsequent cell death. The strong antibacterial and antifungal activity observed in this research can thus be partly attributed to the high proportion of eucalyptol present in the oil.

The findings correspond with the results of Elangovan et al. (2023), who demonstrated the antibacterial effects of eucalyptol against both Gram-positive and Gram-negative bacteria, and with the observations of Seol and Kim (2016), who linked its biological activity to modulation of inflammatory signaling.

Estragole (methyl chavicol) was the most abundant constituent of the oil analyzed and plays a crucial role in its biological activity. It has been reported to possess antimicrobial, antioxidant, and anti-inflammatory properties. Studies by Qasem et al. (2023) and Azizah et al. (2023) showed that estragole-rich basil oil inhibited the growth of *S. aureus*, *E. coli*, *C. albicans*, and *A. niger*, a pattern consistent with the present results. Estragole acts by altering microbial membrane permeability and interfering with energy metabolism. It also exhibits anti-edematogenic activity by reducing vascular permeability and leukocyte migration, contributing to the oil's anti-inflammatory potential. However, literature also reports that estragole, when present in excessive quantities, may be hepatotoxic due to the formation of reactive metabolites such as 1′-hydroxyestragole (Ackermann et al., 2025). The moderate presence of estragole in naturally extracted oils, such as in this study, is considered safe and beneficial when used within pharmacological limits.

Linalool, another major constituent detected, is a terpene alcohol known for its wide range of pharmacological activities, including antimicrobial, anti-inflammatory, antioxidant, and neuroprotective properties. It acts by modulating the GABA_A receptor system and suppressing the NMDA receptor-mediated excitatory pathway, which explains its mild sedative and analgesic effects. The antimicrobial activity of linalool is primarily attributed to its ability to disrupt microbial membranes and inhibit quorum-sensing processes. The presence of linalool in the oil thus complements the effects of eucalyptol and estragole, contributing to the broad antimicrobial efficacy observed in this study. Recent findings by

Long et al. (2025) demonstrated that linalool could inhibit methicillin-resistant *Staphylococcus aureus* (MRSA) by disrupting glutathione metabolism and biofilm formation, highlighting its clinical relevance. These results support the view that the antimicrobial activity of *O. basilicum* oil arises from the synergistic interaction of its multiple constituents.

The compositional profile obtained in this research shares similarities with basil oils analyzed in other parts of the world but shows distinct regional variation. Mediterranean and Indian basil varieties are generally linalool-rich, while African and South American types often exhibit higher estragole levels. The dominance of estragole and eucalyptol in Nigerian-grown basil suggests an adaptive metabolic response to environmental factors such as temperature, humidity, and soil mineral content. This chemotypic difference is important because it influences not only the oil's aroma and commercial value but also its pharmacological properties. The results therefore contribute valuable reference data for the chemotaxonomic characterization and pharmacognostic authentication of *O. basilicum* in Nigeria.

The relationship between chemical composition and antimicrobial activity observed in this study underscores the significance of compound synergy in natural products. While individual compounds such as estragole, eucalyptol, and linalool possess moderate antimicrobial properties, their combined presence often produces a stronger and broader effect. This synergistic phenomenon has been discussed by Khwaza and Aderibigbe (2025), who observed that mixtures of phenolic and monoterpenoid constituents exhibit enhanced bacterial membrane disruption and inhibition of biofilm formation compared to isolated compounds. The present study supports this assertion, as the combined activity of the identified constituents produced inhibition zones comparable to those of standard antibiotics for Gram-positive and fungal species. The findings also align with the work of Bakkali et al. (2020), who emphasised that the overall biological activity of essential oils depends not only on their major components but also on minor constituents that may act synergistically or antagonistically.

Overall, the findings from this research support the traditional use of *Ocimum basilicum Linn.* as a medicinal plant with antimicrobial, anti-inflammatory, and antioxidant properties. The antimicrobial potency demonstrated by the volatile oil confirms that it can serve as a potential natural alternative to synthetic antimicrobials, especially in managing infections caused by Gram-positive bacteria and fungi. The observed selectivity pattern further emphasises the importance of using plant-derived bioactive agents in the fight against antimicrobial resistance. Compared with synthetic drugs, essential oils offer advantages such as lower toxicity, environmental safety, and a reduced tendency to induce resistance due to their complex chemical nature. These attributes make *O. basilicum* volatile oil a promising candidate for development into herbal formulations, topical antiseptics, and food preservation systems.

In summary, the findings from this study agree broadly with previous reports on the antimicrobial and phytochemical characteristics of basil oil, while providing new insights specific to its Nigerian chemotype. The oil's composition, dominated by estragole, eucalyptol, and linalool, explains its strong antimicrobial efficacy against Gram-positive bacteria and fungi. The results also highlight the role of environmental conditions in shaping the plant's secondary metabolite profile. This work contributes to the growing body of evidence that supports the pharmacological potential of *O. basilicum* as a source of bioactive compounds with practical applications in medicine, pharmacy, and the food industry. The outcome of this study thus validates the ethnomedicinal relevance of basil and provides a scientific foundation for its continued exploration as a natural antimicrobial agent in the global effort to address drug resistance.

CHAPTER FIVE

CONCLUSION

The present study demonstrated that the volatile oil obtained from Ocimum basilicum L. (sweet basil) exhibits significant antimicrobial properties, attributed to its rich phytochemical composition. The oil yield of 0.71% obtained by hydrodistillation was consistent with reported values for basil species, confirming effective extraction and preservation of thermolabile compounds. GC–MS analysis revealed seven major constituents, dominated by estragole (46.12%), eucalyptol (16.87%), and linalool (10.45%), alongside other minor terpenoids and phenylpropanoids. These compounds are well documented for their antimicrobial, antioxidant, and anti-inflammatory properties.

The antimicrobial screening demonstrated that the oil exhibited broad-spectrum activity, with greater inhibitory effects on Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and fungi (*Candida albicans* and *Aspergillus niger*), while Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) showed minimal or no inhibition. This pattern agrees with existing literature and can be attributed to structural differences in microbial cell walls that influence susceptibility to hydrophobic compounds. The findings further confirm that the biological activity of *O. basilicum* oil is concentration-dependent and results from the synergistic interactions of its multiple bioactive constituents rather than a single dominant compound.

The results of this work align with global studies on the pharmacological potential of basil oil but provide new insights into the chemotype and antimicrobial performance of Nigerian-grown *O. basilicum.* The predominance of estragole and eucalyptol in the oil suggests a regional chemotypic variation that may influence both aroma and bioactivity. The antimicrobial activity recorded supports the traditional use of basil in herbal medicine for treating infections, inflammation, and respiratory ailments, while the chemical characterisation offers scientific evidence for its potential as a natural source of therapeutic agents.

In conclusion, this research confirms that *Ocimum basilicum Linn.* volatile oil is a promising natural antimicrobial agent with potent activity against selected bacterial and fungal pathogens. Its complex chemical composition, dominated by oxygenated monoterpenes and phenylpropanoids, accounts for its biological efficacy, positioning it as a valuable candidate for further pharmaceutical, nutraceutical, and cosmetic applications. The findings contribute to the growing body of scientific knowledge supporting plant-derived bioactive compounds as viable alternatives in combating antimicrobial resistance and encourage continued exploration of *O. basilicum* as a multipurpose medicinal and industrial plant.

REFERENCES

Abid, M., & Yahya, R. (2023). *Antioxidant and radical scavenging activity of essential oils: A comprehensive review.* Food Chemistry Advances, 10, 132–140. https://doi.org/10.1016/j.focha.2023.100153

Ackermann, G., Peil, M., Quarz, C., Schmidt, A., Halaczkiewicz, M., Thomas, A. D., Stegmüller, S., Richling, E., Manolikakes, G., Christmann, M., Küpper, J. H., Schrenk, D., & Fahrer, J. (2025). *Molecular dosimetry of estragole and 1'-hydroxyestragole-induced DNA adduct formation, clastogenicity and cytotoxicity in human liver cell models.* Archives of Toxicology, 99(9), 3769–3785. https://doi.org/10.1007/s00204-025-04084-2

Akarca, G., Olgun, E. Ö., & Duman, M. (2021). *Antimicrobial activity and chemical composition of citrus and basil essential oils against pathogenic microorganisms.* Journal of Essential Oil Research, 33(2), 145–152. https://doi.org/10.1080/10412905.2021.1899923

Akarca, G., et al. (2022). *Comparative antibacterial and antifungal properties of essential oils: A focus on Citrus sinensis and Ocimum species.* BMC Complementary Medicine and Therapies, 22, 351. https://doi.org/10.1186/s12906-022-03716-8

Antimicrobial Resistance Collaborators. (2022). *Global burden of bacterial antimicrobial resistance in 2019: A systematic analysis.* The Lancet, 399(10325), 629–655. https://doi.org/10.1016/S0140-6736(21)02724-0

Azizah, A., Rahman, R., & Latif, M. A. (2023). *Antimicrobial and antioxidant activity of estragole-rich basil essential oil.* Natural Product Communications, 18(4), 1–9. https://doi.org/10.1177/1934578X231081594

Bakkali, F., Averbeck, S., Averbeck, D., & Idaomar, M. (2020). *Biological effects of essential oils – A review.* Food and Chemical Toxicology, 134, 110–125. https://doi.org/10.1016/j.fct.2020.110125

Carrillo-Hormaza, L., Pineda, L. M., & Castro, C. (2015). *Antifungal activity of Eupatorium essential oils and their chemical composition.* Industrial Crops and Products, 77, 330–336. https://doi.org/10.1016/j.indcrop.2015.09.015

Centers for Disease Control and Prevention (CDC). (2022). *Antimicrobial Resistance Threats in the United States, 2022 Report.* U.S. Department of Health and Human Services. https://www.cdc.gov/drugresistance

De Oliveira, R. A., et al. (2023). *Recent advances in essential oils as multifunctional bioactive agents: Food, pharmaceutical, and environmental applications.* Trends in Food Science & Technology, 132, 94–108. https://doi.org/10.1016/j.tifs.2023.05.017

Elangovan, S., & Mudgil, P. (2023). *Antibacterial properties of Eucalyptus globulus essential oil against MRSA: A systematic review.* Antibiotics, 12(3), 474. https://doi.org/10.3390/antibiotics12030474

Islam, M. T., Bhuia, M. S., Mostakim, M. S., Chowdhury, R., Hasan, R., Sheikh, S., Ansari, S. A., Ansari, I. A., Eity, T. A., & Islam, M. T. (2025). *Synergistic anxiolytic effects of linalool and sesamol co-treatment on Swiss albino mice: A potential GABAergic intervention.* Synapse, 79(1), e70003. https://doi.org/10.1002/syn.70003

Júnior, J. L. R., Damasceno, M. R., Santos, S. A., Barbosa, T., Araújo, J., Vieira-Neto, A., Wong, D., Lima-Júnior, R. C., & Campos, A. (2017). *Acute and neuropathic orofacial antinociceptive effect of eucalyptol.* Inflammopharmacology, 25(6), 589–602. https://doi.org/10.1007/s10787-017-0324-5

Khalil, H. M., & Hassan, H. (2024). *Antioxidant and anti-inflammatory activities of rosemary essential oil: Mechanistic insights.* Journal of Applied Pharmaceutical Science, 14(2), 35–42. https://doi.org/10.7324/JAPS.2024.140204

Khwaza, V., & Aderibigbe, B. A. (2025). *Mechanisms of antimicrobial action of essential oil constituents: Focus on terpenoids and phenylpropanoids.* Frontiers in Microbiology, 16, 1278. https://doi.org/10.3389/fmicb.2025.01278

Lima, C. C., de Holanda-Angelin-Alves, C. M., Pereira-Gonçalves, Á., Kennedy-Feitosa, E., Evangelista-Costa, E., Bezerra, M. A. C., Coelho-de-Souza, A. N., & Leal-Cardoso, J. H. (2020). *Antispasmodic effects of the essential oil of Croton zehntneri, anethole, and estragole, on tracheal smooth muscle.* Heliyon, 6(11), e05445. https://doi.org/10.1016/j.heliyon.2020.e05445

Long, N., Qiu, M., Zuo, Y., & Deng, H. (2025). *Antimicrobial activity and metabolomic analysis of linalool against methicillin-resistant Staphylococcus aureus.* Infection and Drug Resistance, 18, 731–744. https://doi.org/10.2147/IDR.S491358

Manzoor, H., Khan, M. U., Khan, S., Shah, M., Shabbir, C. A., & Alkhtani, H. M. (2025). *Linalool-based silver nanoconjugates as potential therapeutics for glioblastoma: In silico and in vitro insights.* PLOS ONE, 20(6), e0325281. https://doi.org/10.1371/journal.pone.0325281

Muhammad Torequl Islam, et al. (2025). *Neuroprotective and anxiolytic potential of linalool: A mechanistic review.* Naunyn-Schmiedeberg's Archives of Pharmacology, 398, 10921–10937. https://doi.org/10.1007/s00210-025-03984-5

Pooja, A., Ramesh, K., & Sharma, A. (2022). *Aromatherapy and neurobehavioral effects of citrus essential oils: A comprehensive review.* Complementary Therapies in Medicine, 66, 102810. https://doi.org/10.1016/j.ctim.2022.102810

Qasem, R. J., Mahmoudi, S., & Al-Said, M. (2023). *Pharmacological and toxicological properties of estragole and related compounds.* Phytomedicine Plus, 3(5), 100254. https://doi.org/10.1016/j.phyplu.2023.100254

Rad Sharifi, R., Bahmani, M., & Rafieian-Kopaei, M. (2022). *Volatile oil composition of Ocimum species and its biological importance: A review.* Journal of Essential Oil Research, 34(3), 211–226. https://doi.org/10.1080/10412905.2022.2055123

Seol, G. H., & Kim, K. Y. (2016). *Eucalyptol and its role in chronic diseases.* In S. Gupta, S. Prasad, & B. Aggarwal (Eds.), Drug Discovery from Mother Nature (Vol. 929, pp. 389–398). Springer. https://doi.org/10.1007/978-3-319-41342-6_18

Sathish Kumar, R., Aderibigbe, B. A., & Khwaza, V. (2024). *Essential oils as antimicrobial and antioxidant agents: Current insights and applications.* Phytotherapy Research, 38(1), 15–29. https://doi.org/10.1002/ptr.8078

World Health Organisation (WHO). (2024). *Global Antimicrobial Resistance and Use Surveillance System (GLASS) Report 2024.* Geneva: WHO Press.

World Health Organization (WHO). (2025). *Global action plan on antimicrobial resistance – Progress and challenges 2025.* Geneva: WHO Press.

Wang, J., Li, X., & Zhang, H. (2024). *Essential oils in sustainable packaging and food preservation: An overview.* Food Control, 158, 110082. https://doi.org/10.1016/j.foodcont.2024.110082