

**COMPARATIVE BIOACTIVE COMPOUND AND QUANTIFICATION OF *Ocimum  
gratissimum* OILS FROM THREE LOCAL GOVERNMENTS IN EDO STATE**



**BY**

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**MICROBIOLOGY TECHNIQUE**

**DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY**

**FACULTY OF LIFE SCIENCES**

**UNIVERSITY OF BENIN**

**BENIN CITY**

**NOVEMBER, 2025**

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**AN UNDERGRADUATE PROJECT WORK SUBMITTED TO THE DEPARTMENT OF  
SCIENCE LABORATORY TECHNOLOGY, FACULTY OF LIFE SCIENCES,  
UNIVERSITY OF BENIN, BENIN CITY, EDO STATE, NIGERIA; IN PARTIAL  
FULFILMENT OF THE REQUIREMENTS FOR AWARD OF BACHELOR OF  
SCIENCE (B.SC.) DEGREE IN SCIENCE LABORATORY TECHNOLOGY**

**NOVEMBER, 2025.**

## CERTIFICATION

This is to certify that this research titled “**COMPARATIVE BIOACTIVE COMPOUND AND QUANTIFICATION OF Ocimum gratissimum OILS FROM THREE LOCAL GOVERNMENTS IN EDO STATE**” was carried out by “**Victoria Ifeoma ANI (Miss)**” with matriculation number “**LSC2010043**” and presented to the Department of Science Laboratory Technology, Faculty of Life Sciences, University of Benin, Benin City; in partial fulfillment of the requirements for the award of Bachelor of Science (B.Sc.) in Science Laboratory Technology. It was conducted under suitable conditions, was carefully supervised and subsequently approved as having met the requirements for the award of Bachelor of Science degree in Science Laboratory Technology.

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## DECLARATION

I “Victoria Ifeoma ANI (Miss)” declare that “COMPARATIVE BIOACTIVE COMPOUND AND QUANTIFICATION OF Ocimum gratissimum OILS FROM THREE LOCAL GOVERNMENTS IN EDO STATE” is my own work and that all sources that I have used or quoted have been acknowledged by means of complete references and that this work has not been submitted before for any other degree at any other University.

.....

Victoria Ifeoma ANI (Miss)

.....

DATE

## **DEDICATION**

This project work is dedicated to the Almighty God for his grace and mercies and to my family for their support and love throughout my period of study.

## **ACKNOWLEDGEMENT**

All thanks to almighty God, the source of strength for making this work a success. I am forever grateful to God for his grace and faithfulness. I am profoundly indebted to my project supervisor Prof E.O. Oshomoh whose invaluable insights and corrections contributed to the success of this project work. Thanks to all my lecturers for their great impact of knowledge. My generous gratitude to my parents, MR. and MRS. JOHN NKECHI ANI, I am so grateful for your unwavering love, guidance and belief in me that has brought me to this point in my academic journey. And I want to thank myself for my resilience and consistency.. Also, I want to appreciate my siblings for their encouragement during the course of this work. My sincere appreciation goes to Mr Agho Timothy whose assistance and support were instrumental to the completion of this work, I am grateful for his expertise and encouragement all through this journey. My joy knows no bounds in thanking my wonderful friend Kelly and others for their love and cooperation while in school. I appreciate all the insignia coursemates of mine for being part of the wonderful experience I had during my stay at the University of Benin.

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## ABSTRACT

This study investigated the chemical composition of *Ocimum gratissimum* (scent leaf) essential oils collected from Oredo, Ikpoba-Okha, and Ovia-North East Local Government Areas of Edo State, Nigeria. The aim was to comparatively evaluate the bioactive constituents and determine variations in phytochemical profiles that influence the plant's medicinal and industrial applications. Essential oils were extracted using Soxhlet extraction with n-hexane and analyzed by Gas Chromatography – Mass Spectrometry (GC – MS) and Fourier Transform Infrared Spectroscopy (FTIR). The GC–MS results revealed a complex mixture of aromatic hydrocarbons, monoterpenoids, phenolics, long-chain alkanes, fatty acids, esters, and sterols. Major bioactive compounds identified included thymol, caryophyllene oxide, o-cymene, neophytadiene, phytol, and squalene, with thymol emerging as the dominant bioactive constituent. Among the samples, Oredo extract showed higher concentrations of oxygenated terpenes and hydrocarbons, indicating stronger antimicrobial and antioxidant potential, while Ikpoba-Okha and Ovia-North samples exhibited broader chemical diversity. FTIR analysis confirmed the presence of functional groups such as hydroxyl (–OH), carbonyl (C = O), and carbon – carbon double bonds (C = C), characteristic of phenolics, terpenoids, and aromatic compounds. These findings validate the therapeutic and aromatic relevance of *O. gratissimum*, highlighting its potential applications in medicine, cosmetics, and food industries.

The study concludes that environmental and geographical factors significantly influence the chemical variability and quality of *O. gratissimum* essential oils.

# CHAPTER ONE

## INTRODUCTION

### 1.0 BACKGROUND OF THE STUDY

Medicinal plants have served as one of the oldest and most dependable sources of therapeutic agents for humanity. Long before the advent of modern pharmaceuticals, natural herbs were used to prevent and treat diseases in nearly every culture. They contain bioactive constituents that form the foundation for many synthetic and semi-synthetic drugs used today (Adebayo *et al.*, 2021; Nguyen *et al.*, 2021). According to the World Health Organization (WHO, 2021), nearly 80% of the global population relies on herbal medicine as part of their primary healthcare system, particularly in developing nations where access to modern drugs may be limited. The pharmacological potential of medicinal plants is largely derived from their phytochemical constituents such as alkaloids, flavonoids, tannins, terpenoids, saponins, and phenolic compounds which interact with biological systems to exert therapeutic effects (Oke *et al.*, 2023; Ajayi *et al.*, 2022).

The renewed global interest in medicinal plants stems from their perceived safety, accessibility, and lower cost compared with synthetic medicines, as well as growing concerns about antimicrobial resistance and the side effects of conventional drugs (Owolabi *et al.*, 2020). Researchers have continued to isolate, characterize, and evaluate plant-derived compounds for their antimicrobial, antioxidant, anti-inflammatory, and anticancer potentials (Adeyemi *et al.*, 2022). Among the numerous medicinal plants widely studied for their ethnomedicinal and pharmacological significance is *Ocimum gratissimum L.*, a member of the Lamiaceae family.

*Ocimum gratissimum* L., commonly referred to as clove basil, African basil, or scent leaf, is a perennial aromatic herb that plays a dual role as both a culinary spice and a medicinal plant (Ugbogu *et al.*, 2021). It typically grows as an erect, branching shrub with ovate leaves that are rich in essential oil glands, which emit a characteristic clove-like fragrance when crushed (Ademiluyi *et al.*, 2023). The plant is indigenous to tropical Africa and parts of Asia but has become naturalized across many tropical and subtropical regions because of its adaptability and usefulness (Ugbogu *et al.*, 2021).

Ethnobotanically, *O. gratissimum* holds a central place in traditional medicine across West and Central Africa, India, and Southeast Asia. The leaves, stems, and essential oils are employed in the treatment of a wide range of ailments such as malaria, fever, cough, diarrhoea, respiratory infections, and skin diseases (Edo, 2023; Ugbogu *et al.*, 2021). The leaves are often used as infusions or decoctions for internal ailments and as poultices for wounds and skin infections. Additionally, steam inhalation or fumigation with the leaves is traditionally used to relieve cold, catarrh, and throat infections (Edo, 2023). These ethnomedicinal uses have motivated modern pharmacological studies aimed at validating the traditional claims associated with the plant.

Phytochemical analyses of *O. gratissimum* have revealed a rich composition of both volatile and non-volatile constituents responsible for its biological activity. Its essential oils are primarily composed of eugenol, thymol,  $\beta$ -caryophyllene, and other terpenoids compounds known for antimicrobial and antioxidant properties (Ikeotuonye *et al.*, 2023; Granados *et al.*, 2024). Non-volatile constituents such as flavonoids, alkaloids, tannins, and phenolic acids contribute further to its pharmacological functions (Ikokide *et al.*, 2025). However, the phytochemical composition can vary depending on factors such as geographical origin, harvest season, soil type, and

extraction technique, leading to differences in chemical profiles and biological potency among samples (Ikeotuonye *et al.*, 2023).

Experimental pharmacology has substantiated many traditional applications of *O. gratissimum*. Studies have demonstrated antimicrobial, antifungal, antioxidant, anti-inflammatory, antidiarrhoeal, and hepatoprotective activities in both in vitro and in vivo systems (Xie *et al.*, 2023; Ugbogu *et al.*, 2021). Essential oil extracts have exhibited inhibitory effects on Gram-positive and Gram-negative bacteria, including *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* (Silva *et al.*, 2022). The plant's bioactive compounds have also been shown to interfere with microbial biofilm formation and potentiate the effects of conventional antibiotics (Xie *et al.*, 2023). Furthermore, animal studies suggest low acute toxicity and a wide margin of safety, supporting its traditional use for prolonged periods (Ugbogu *et al.*, 2021).

Beyond its medicinal significance, *O. gratissimum* possesses economic and ecological value. It is cultivated for the production of fresh leaves, dried herbs, and essential oils used in food flavoring, cosmetics, and herbal pharmaceuticals (Meneses *et al.*, 2018). The plant's insect-repellent and antimicrobial properties have prompted research into its use as a natural pesticide and food preservative, offering an eco-friendly alternative to synthetic chemicals (Granados *et al.*, 2024). As a result, *O. gratissimum* contributes not only to public health but also to rural economic development and environmental sustainability (Edo, 2023).

Despite extensive research, several knowledge gaps persist regarding *O. gratissimum*. The diversity of its chemical constituents across different ecotypes is yet to be fully characterized, and standardization of extracts for clinical use remains a challenge (Ikeotuonye *et al.*, 2023). Moreover, while laboratory evidence supports its pharmacological efficacy, comprehensive

clinical trials and pharmacokinetic studies are still limited (Ugbogu *et al.*, 2021; Xie *et al.*, 2023). Additional studies focusing on dosage standardization, toxicity profiles, and formulation technologies are therefore essential to fully harness its therapeutic and economic potential (Granados *et al.*, 2024).

In conclusion, *Ocimumgratissimum* stands as a quintessential example of a multipurpose medicinal plant, bridging traditional healing, modern pharmacology, and sustainable economic use. Continued scientific investigation into its phytochemistry, bioactivity, and safe therapeutic application is necessary to transform this traditional resource into a standardized and globally accepted herbal medicine (Ugbogu *et al.*, 2021; Granados *et al.*, 2024).

## 1.1 AIM OF STUDY

The aim of this study is to comparatively quantify and evaluate the bioactive compounds present in the essential oils of *Ocimum gratissimum* collected from Oredo, Ikpoba-Okha, and Ovia North-East Local Government Areas of Edo State, Nigeria, in order to determine variations in chemical composition and potential implications for medicinal and industrial applications.

## 1.2 OBJECTIVES OF STUDY

The specific objectives of the study are:

- To extract essential oils from *Ocimum gratissimum* leaves collected from Oredo, Ikpoba-Okha, and Ovia North-East Local Government Areas using standard extraction methods (e.g., hydrodistillation).
- To characterize and quantify the bioactive compounds present in the oils using analytical techniques such as Gas Chromatography–Mass Spectrometry (GC–MS).
- To compare the qualitative and quantitative differences in the bioactive compound profiles among the three geographical locations.
- To identify major chemical constituents that contributes to the pharmacological and aromatic properties of *O. gratissimum* oils.
- To provide insights into the potential applications of the oils in medicine, cosmetics, and food industries based on their chemical composition and bioactive compound diversity.

## CHAPTER TWO

### LITERATURE REVIEW

#### 2.1 OVERVIEW OF OCIMUM

The genus *Ocimum*, commonly referred to as basil, is one of the most diverse and economically valuable genera in the family Lamiaceae, encompassing over 150 species distributed mainly in tropical and subtropical regions (Nguyen *et al.*, 2021). Members of this genus are renowned for their aromatic properties, attributed to a high concentration of essential oils and secondary metabolites that contribute to their medicinal, culinary, and industrial uses. Among the various species, *Ocimum gratissimum*, *O. basilicum*, and *O. tenuiflorum* (syn. *O. sanctum*) are the most widely studied due to their distinctive chemical compositions and pharmacological properties (Ajayi *et al.*, 2022).

*Ocimum gratissimum* commonly known as “African basil” or “scent leaf” holds exceptional ethnobotanical significance in Africa, Asia, and South America. It is extensively cultivated in home gardens and used as a spice, herbal remedy, and insect repellent. Its leaves and essential oil possess high concentrations of eugenol, thymol, and ocimene, which impart strong antimicrobial, antioxidant, and flavoring qualities (Akinmoladun *et al.*, 2021). The plant serves as an integral part of traditional medicine systems, being used in the management of cough, fever, diarrhea, and microbial infections. Recent advances in chemical profiling have established that the variations in the composition of *O. gratissimum* oils are influenced by environmental factors, soil type, genotype, and post-harvest processing (Singh *et al.*, 2020).

Phytochemical and metabolomic studies of *Ocimum* species reveal a rich diversity of bioactive compounds, including phenolics, flavonoids, alkaloids, tannins, terpenoids, and glycosides, each contributing to the plant's pharmacological spectrum. Comparative analyses across *Ocimum* species demonstrate that *O. gratissimum* tends to exhibit a higher proportion of eugenol and thymol than *O. basilicum* or *O. tenuiflorum*, which accounts for its more intense clove-like aroma and stronger antimicrobial efficacy (Ademiluyi *et al.*, 2023).

## 2.2 TAXONOMY OF OCIMUM

The genus *Ocimum* belongs to the family Lamiaceae, which is characterized by aromatic herbs and shrubs with glandular trichomes that secrete essential oils. The taxonomic classification of *Ocimum* is as follows (Ogundele *et al.*, 2022):

- Kingdom: Plantae
- Division: Magnoliophyta
- Class: Magnoliopsida
- Order: Lamiales
- Family: Lamiaceae
- Genus: *Ocimum*
- Species: *Ocimum gratissimum* Linn

Taxonomically, *O. gratissimum* is distinguished by its quadrangular stem, opposite leaves, and bilabiate flowers typical of Lamiaceae members. The essential oils of the genus serve as chemotaxonomic markers, as chemical polymorphism within *Ocimum* species often correlates

with genetic variation. For instance, chemotypes rich in eugenol, thymol, or citral are used to differentiate varieties within *O. gratissimum* populations (Singh *et al.*, 2020).

Recent molecular phylogenetic analyses using chloroplast DNA barcoding and nuclear ITS sequences have provided deeper insights into *Ocimum* taxonomy. These studies indicate that *O. gratissimum* forms a distinct clade closely related to *O. americanum* and *O. basilicum*, although significant intraspecific variation exists due to domestication and hybridization (Nguyen *et al.*, 2021; Oke *et al.*, 2023).



Plate 1: *Ocimum gratissimum*

**Photocredit:** (Ani Victoria).

### **2.3 BOTANICAL DESCRIPTION OF *Ocimum gratissimum***

*Ocimum gratissimum* is a perennial herb or sub shrub that can grow between 1 – 3 meters in height, depending on environmental conditions. It exhibits a quadrangular stem, opposite leaves, and terminal inflorescences bearing white to purplish flowers. The leaves are simple, ovate, and typically 5 – 10 cm long with serrated margins and a characteristic pungent aroma due to the presence of oil glands (Ogundele *et al.*, 2022).

The plant reproduces both by seeds and vegetative propagation. Its essential oil is localized in glandular trichomes present on the leaf surface, especially on the abaxial (lower) side. The oil content varies with leaf age, harvest time, and climatic factors. The plant's adaptability allows it to thrive in a range of ecological conditions, from semi-arid to humid environments, although it grows best in well-drained loamy soils under full sunlight (Ademiluyi *et al.*, 2023).

Botanical features such as leaf morphology, glandular density, and flower pigmentation are significant in distinguishing regional ecotypes. In tropical Africa, *O. gratissimum* populations tend to exhibit darker green leaves with higher oil yield compared to those cultivated in Asian regions, which may have lighter pigmentation and a more citrus-like aroma (Adeyemi *et al.*, 2022).

### **2.4 DISTRIBUTION AND ECOLOGY**

*Ocimum gratissimum* is native to tropical Africa but has become naturalized in several parts of Asia, South America, and the Caribbean. It grows abundantly in West Africa, particularly in Nigeria, Ghana, and Cameroon, where it is commonly found in home gardens, roadsides, and

farmlands (Adebayo *et al.*, 2021). The plant's wide distribution is attributed to its resilience and ability to thrive under diverse climatic and edaphic conditions.

Ecologically, *O. gratissimum* plays an important role in maintaining pollinator diversity, as its flowers attract bees and butterflies. The plant also demonstrates allelopathic potential by releasing volatile organic compounds that inhibit the growth of competing weeds and soil pathogens (Eze *et al.*, 2021).

Geographical variations significantly influence the phytochemical profile of *O. gratissimum*. For example, plants grown in coastal regions exhibit higher eugenol content, while those from upland or semi-arid zones have elevated concentrations of thymol and camphor (Okafor *et al.*, 2020). This chemotypic diversity forms the basis for comparative studies in bioactive compound quantification.

## **2.5 ETHNOMEDICINAL IMPORTANCE OF *Ocimum gratissimum***

For centuries, *O. gratissimum* has been an integral part of African, Asian, and Latin American ethnomedicine. In Nigeria, it is popularly known as “Efirin” (Yoruba), “Nchuanwu” (Igbo), and “Daidoya” (Hausa). The plant's leaves and oil are used in managing a wide range of ailments such as cough, fever, diarrhea, convulsions, headache, and microbial infections (Ademiluyi *et al.*, 2023).

The essential oil serves as a natural antiseptic, carminative, and insect repellent. Decoctions from the leaves are used for the treatment of malaria, respiratory tract infections, and gastrointestinal disorders. Additionally, *O. gratissimum* is employed in post-partum care, wound healing, and as an aphrodisiac (Eze *et al.*, 2021; Ezenwa and Onwuama, 2022).

Pharmacologically, the ethnomedicinal use of *O. gratissimum* aligns with its chemical composition. The dominance of eugenol in the oil contributes to its antimicrobial, antioxidant, and analgesic properties, while thymol and linalool enhance its antifungal and anti-inflammatory efficacy (Adebayo *et al.*, 2021).

## **2.6 PHYTOCHEMICAL AND BIOACTIVE COMPOUNDS IN *Ocimum gratissimum***

Phytochemical investigations over the last decade have consistently demonstrated that *Ocimum gratissimum* is a chemically rich medicinal plant containing numerous bioactive secondary metabolites. These compounds include alkaloids, flavonoids, saponins, tannins, phenolic acids, glycosides, steroids, terpenoids, and essential oils, all of which collectively contribute to the plant's pharmacological potency and therapeutic diversity (Oke *et al.*, 2023; Adebayo *et al.*, 2021). The presence, concentration, and relative proportions of these compounds can vary widely depending on the plant's genetic makeup, geographical location, cultivation practices, harvest time, and extraction method (Ademiluyi *et al.*, 2023).

Phytochemicals in *O. gratissimum* perform dual biological roles. In plants, they serve as natural defense mechanisms against pathogens, herbivores, and environmental stressors. In humans and animals, these same compounds act as antioxidants, antimicrobial, anti-inflammatory, and cytoprotective agents (Nguyen *et al.*, 2021; Ibrahim and Hassan, 2022). The major bioactive constituents are described below in greater detail.

### **2.6.1 PHENOLIC COMPOUNDS**

Phenolic compounds represent one of the most abundant and biologically significant classes of secondary metabolites in *O. gratissimum*. They include simple phenols, phenolic acids, and

polyphenols such as eugenol, rosmarinic acid, caffeic acid, gallic acid, and ferulic acid. Among these, eugenol is the dominant phenolic compound and the major constituent of the plant's essential oil, often accounting for 35–70% of the total volatile fraction (Ajayi *et al.*, 2022; Adefegha and Oladipo, 2021).

#### **2.6.1.1 EUGENOL**

Eugenol (4-allyl-2-methoxyphenol) is primarily responsible for the plant's characteristic clove-like aroma and numerous pharmacological activities. It exhibits potent antimicrobial, antioxidant, analgesic, and anti-inflammatory properties through its ability to scavenge free radicals, inhibit lipid peroxidation, and modulate enzymatic activity (Ademiluyi *et al.*, 2023). Studies have shown that eugenol disrupts bacterial cell membranes and denatures proteins, leading to cell death in both Gram-positive and Gram-negative microorganisms (Okolie *et al.*, 2021).

#### **2.6.1.2 ROSMARINIC ACID AND CAFFEIC**

Rosmarinic acid and caffeic acid are other key phenolic compounds found in *O. gratissimum*. These phenolic acids are potent natural antioxidants that enhance the plant's defensive capacity against oxidative stress. They exert protective effects on biological membranes and enzymes by neutralizing reactive oxygen species (ROS) and upregulating endogenous antioxidant enzymes such as catalase and superoxide dismutase (Nguyen *et al.*, 2021). Rosmarinic acid also contributes to the plant's anti-inflammatory, neuroprotective, and hepatoprotective effects (Oke *et al.*, 2023).

The high phenolic content of *O. gratissimum* underlines its potential as a functional food ingredient and natural preservative, particularly in food and cosmetic industries seeking safer alternatives to synthetic antioxidants.

## **2.6.2 FLAVONOIDS**

Flavonoids are polyphenolic compounds with diverse biological roles, and they are abundantly present in the leaves of *O. gratissimum*. Major flavonoids identified include quercetin, kaempferol, luteolin, and apigenin (Nguyen *et al.*, 2021; Oke *et al.*, 2023). These compounds are known for their free radical scavenging, anti-inflammatory, antiallergic, and cardioprotective effects.

### **2.6.2.1 QUERCETIN**

Quercetin acts as a powerful antioxidant by donating hydrogen atoms to neutralize free radicals and inhibiting lipid peroxidation in cell membranes (Adebayo *et al.*, 2021). It also exhibits anti-inflammatory properties through the downregulation of NF- $\kappa$ B and suppression of pro-inflammatory cytokines such as TNF- $\alpha$  and IL-6 (Ademiluyi *et al.*, 2023).

### **2.6.2.2 KAEMPFEROL AND APIGENIN**

Kaempferol has been reported to modulate vascular function, reduce oxidative stress, and protect against cardiovascular diseases. Similarly, apigenin enhances antioxidant defenses and exerts neuroprotective and anticarcinogenic effects (Ajayi *et al.*, 2022).

Recent studies have suggested that the synergistic interaction between flavonoids and phenolic acids in *O. gratissimum* enhances the plant's overall therapeutic effect, as these compounds act on complementary biochemical pathways to reinforce antioxidant and immune-regulating activities (Akinmoladun *et al.*, 2021).

## **2.6.3 ALKALOIDS**

Although present in lower concentrations compared to phenolics and flavonoids, alkaloids contribute significantly to the pharmacological profile of *O. gratissimum*. These nitrogen-

containing compounds exhibit broad bioactivities, including antimicrobial, analgesic, antimalarial, and cytotoxic effects (Oke *et al.*, 2023). In small quantities, alkaloids enhance immune function and neurotransmission, but at high doses, they can be toxic due to their interference with enzyme systems and membrane potential regulation (Eze *et al.*, 2021).

The alkaloid fraction of *O. gratissimum* is thought to include indole and pyrrolidine derivatives, which have been linked to the plant's observed antimalarial and sedative properties. Some of these compounds have also demonstrated antiproliferative activity against cancer cells by inducing apoptosis and disrupting cell cycle progression (Nguyen *et al.*, 2021).

#### **2.6.4 SAPONINS AND TANNINS**

Saponins and tannins are non-volatile secondary metabolites that contribute to the plant's astringency, foam-forming ability, and immune-modulatory potential. Saponins, which are glycosides of triterpenoids or steroids exhibit antifungal, hypocholesterolemic, and anti-inflammatory activities (Ademiluyi *et al.*, 2023). They work by interacting with cholesterol molecules in cell membranes, increasing membrane permeability and leading to lysis of pathogenic microorganisms. However, at high concentrations, saponins may cause hemolysis of red blood cells, indicating that dosage regulation is essential for safe use (Eze *et al.*, 2021).

Tannins, on the other hand, are polyphenolic compounds known for their antimicrobial, antioxidant, and anti-diarrheal properties. They exert their effects through protein precipitation, which inhibits microbial growth and prevents oxidative degradation of biomolecules (Ajayi *et al.*, 2022). Tannins also contribute to wound healing by promoting tissue contraction and forming protective layers over damaged epithelium.

### 2.6.5 TERPENOIDS AND VOLATILE COMPOUNDS

Terpenoids constitute one of the largest groups of bioactive compounds in *O. gratissimum*, responsible for the plant's aroma and many of its pharmacological effects. They include monoterpenes such as thymol,  $\alpha$ -pinene, camphene, and  $\gamma$ -terpinene, and sesquiterpenes like  $\beta$ -caryophyllene and germacrene D (Nguyen *et al.*, 2021; Adebayo *et al.*, 2021; Oladipo and Balogun, 2023).

Monoterpenes are mainly responsible for the fragrance and antimicrobial activity, while sesquiterpenes contribute to the oil's stability and antioxidant potential. For example,  $\beta$ -caryophyllene exhibits anti-inflammatory and analgesic activities by selectively binding to cannabinoid receptor type 2 (CB2), modulating immune responses and reducing pain sensation (Oke *et al.*, 2023; Ali and Khan, 2021). Thymol, another key monoterpene, disrupts microbial cell membranes and enhances the efficacy of antibiotics by increasing cell permeability (Ademiluyi *et al.*, 2023).

Environmental and developmental factors influence the relative concentrations of these terpenes. Plants cultivated in warmer climates or exposed to mild drought stress tend to accumulate higher levels of oxygenated terpenoids such as eugenol and thymol, which serve as adaptive defense mechanisms (Eze *et al.*, 2021).

### 2.6.6 GLYCOSIDES AND STEROIDS

*O. gratissimum* also contains glycosides and phytosterols that play physiological roles in plant metabolism and human health. Cardiac glycosides, though present in small amounts, have been implicated in cardiotonic and anti-hypertensive effects. Phytosterols, including stigmasterol and

$\beta$ -sitosterol, have been reported to reduce cholesterol absorption and exhibit anti-inflammatory and anti-ulcer properties (Ajayi *et al.*, 2022).

These compounds, together with phenolics and flavonoids, contribute synergistically to the plant's diverse pharmacological profile, reinforcing its value as a multi-component natural therapeutic resource.

### **2.6.7 QUANTITATIVE ANALYSIS OF PHYTOCHEMICALS**

Quantitative analyses using gas chromatography–mass spectrometry (GC–MS), high-performance liquid chromatography (HPLC), and Fourier transform infrared spectroscopy (FTIR) have revealed significant variation in the concentration of major compounds among *O. gratissimum* samples from different regions (Adebayo *et al.*, 2021; Ajayi *et al.*, 2022).

In eugenol-type chemotypes, eugenol concentration ranges from 35–70%, while thymol and p-cymene predominate in thymol-type oils, comprising 20–40% of total volatiles (Okafor *et al.*, 2020). Minor compounds such as  $\alpha$ -terpinene, linalool, and myrcene occur in trace amounts but contribute to aroma complexity and potential therapeutic synergy.

Furthermore, the ratio of phenolic to terpenoid compounds influences not only the bioactivity but also the organoleptic properties of the oil plants richer in eugenol tend to have a stronger clove-like aroma, while thymol-dominant samples possess a more pungent, herbal scent (Nguyen *et al.*, 2021). This correlation between phytochemical content and sensory characteristics forms the basis for quality control and chemotypic classification in *O. gratissimum* research.

## **2.7 ESSENTIAL OILS OF *OCIMUM GRATISSIMUM***

Essential oils represent the most significant and pharmacologically active constituents of *Ocimum gratissimum*, conferring the plant its distinctive aroma and wide range of therapeutic

applications. These oils are volatile, aromatic mixtures composed primarily of terpenoids, phenylpropanoids, and other low molecular weight secondary metabolites that are synthesized and stored in glandular trichomes on the leaf surface (Adebayo *et al.*, 2021). The essential oil of *O. gratissimum* is typically pale yellow to golden in color, with a strong clove-like or camphoraceous odor that varies according to the dominant chemical constituents and environmental conditions (Nguyen *et al.*, 2021).

### **2.7.1 CHEMICAL COMPOSITION OF THE ESSENTIAL OIL**

The chemical profile of *O. gratissimum* essential oil has been extensively studied and found to be highly variable, with eugenol, thymol,  $\gamma$ -terpinene,  $\alpha$ -pinene,  $\beta$ -caryophyllene, camphene, and ocimene commonly reported as the principal constituents (Okafor *et al.*, 2020; Ajayi *et al.*, 2022). Among these, eugenol (4-allyl-2-methoxyphenol) is often the dominant compound, sometimes accounting for up to 70% of the total oil content in West African populations (Adebayo *et al.*, 2021). Thymol, another major component, is more prevalent in Asian varieties, contributing to a sharper, herbaceous aroma and enhanced antifungal activity.

Other important compounds include  $\beta$ -caryophyllene, a sesquiterpene with analgesic and anti-inflammatory properties,  $\gamma$ -terpinene and  $\alpha$ -pinene, which contribute to the antioxidant and antimicrobial profile, and ocimene, which enhances fragrance and exhibits insect-repelling potential (Eze *et al.*, 2021). Trace constituents such as linalool, camphor, and myrcene are also present and are thought to contribute synergistically to both therapeutic and sensory qualities.

The essential oils of *O. gratissimum* are complex mixtures whose activity is not due to any single compound but to the synergistic interactions among their components. This synergy enhances

bioactivity, leading to potent antioxidant, antimicrobial, and anti-inflammatory effects greater than those of isolated compounds (Ademiluyi *et al.*, 2023).

### **2.7.2 CHEMOTYPES AND GEOGRAPHICAL VARIATION**

Comparative phytochemical analyses have shown that *O. gratissimum* exhibits considerable chemotypic diversity, resulting in the identification of at least two primary chemotypes the eugenol-type and the thymol-type each characterized by distinct organoleptic and bioactive profiles (Okafor *et al.*, 2020; Nguyen *et al.*, 2021).

The Eugenol-type chemotype is predominant in West and Central Africa, particularly in Nigeria, Ghana, and Cameroon. This type contains high concentrations of eugenol (40–70%) and  $\beta$ -caryophyllene, giving it a rich, sweet-spicy aroma similar to clove oil. The eugenol-type oil demonstrates strong antibacterial, antioxidant, and analgesic activities, making it highly valued for medicinal and pharmaceutical purposes (Ademiluyi *et al.*, 2023).

The Thymol-type chemotype, in contrast, is more common in India, Thailand, and Southeast Asia. It contains high levels of thymol (30–50%), p-cymene, and  $\gamma$ -terpinene, producing a more herbal, camphor-like scent. This type exhibits superior antifungal and insecticidal activities, explaining its frequent use in pest control and aromatherapy formulations (Ajayi *et al.*, 2022).

In addition to these major chemotypes, intermediate forms and mixed chemotypes have been reported in tropical regions, reflecting intraspecific chemical plasticity influenced by genetics and environmental adaptation (Nguyen *et al.*, 2021; Oke *et al.*, 2023).

The existence of distinct chemotypes underscores the importance of regional and ecological factors in determining oil composition. For example, *O. gratissimum* grown in the humid coastal zones of Nigeria exhibits a higher concentration of eugenol and sesquiterpenes, while samples

from semi-arid zones show elevated thymol and monoterpene levels (Eze et al., 2021). These differences are of both scientific and economic interest, as they affect the plant's therapeutic efficacy, fragrance profile, and commercial value.

### **2.7.3 BIOSYNTHESIS AND LOCALIZATION OF ESSENTIAL OILS**

Essential oil biosynthesis in *O. gratissimum* occurs primarily through the mevalonate (MVA) and methylerythritol phosphate (MEP) pathways, which are responsible for the formation of terpenoid precursors such as isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP) (Nguyen *et al.*, 2021). These precursors serve as building blocks for monoterpenes and sesquiterpenes, which constitute the majority of the volatile components.

The oil glands (glandular trichomes) located on the leaf surface, petioles, and floral parts serve as the main storage sites for essential oils. Microscopic studies reveal that the density of glandular trichomes correlates positively with essential oil yield and aroma intensity (Ademiluyi *et al.*, 2023). Young leaves generally contain higher concentrations of essential oil than mature or senescent leaves due to greater metabolic activity.

Factors such as light exposure, soil nutrients, water availability, and temperature strongly influence essential oil biosynthesis. For example, exposure to sunlight enhances monoterpene synthesis through increased photosynthetic energy, while moderate water stress has been shown to induce the production of more oxygenated terpenes such as thymol and eugenol as part of the plant's defense mechanism (Eze *et al.*, 2021).

#### **2.7.4 INFLUENCE OF ENVIRONMENTAL AND AGRONOMIC FACTORS**

Environmental and agronomic variables play crucial roles in determining the quantity and quality of essential oils in *O. gratissimum*. These include soil composition, altitude, rainfall, humidity, temperature, and cultivation practices.

Plants grown in nitrogen-rich soils tend to produce higher total biomass but lower oil concentration, whereas plants grown in slightly nutrient-deficient or sandy soils exhibit reduced growth but enhanced oil content and aromatic intensity (Oke *et al.*, 2023; Aina *et al.*, 2022). Similarly, altitude and temperature significantly affect the metabolic pathways involved in volatile synthesis. For instance, high-altitude populations have been reported to accumulate more sesquiterpenes, whereas lowland populations tend to be richer in monoterpenes (Adebayo *et al.*, 2021).

Post-harvest handling and extraction techniques also influence oil yield and composition. Steam distillation, hydrodistillation, and solvent extraction are commonly used, but hydrodistillation is preferred for maintaining the integrity of volatile compounds. The duration of distillation and particle size of plant material affect the recovery of oxygenated terpenoids such as eugenol and thymol (Ajayi *et al.*, 2022). Improper drying or prolonged storage, on the other hand, can lead to oxidation and degradation of key components, resulting in diminished aroma and bioactivity.

#### **2.7.5 PHARMACOLOGICAL AND INDUSTRIAL RELEVANCE OF THE ESSENTIAL OIL**

The essential oil of *O. gratissimum* is a multifunctional natural product with applications in medicine, food, cosmetics, and agriculture. Its broad-spectrum antimicrobial properties make it

an effective natural preservative in food processing and storage (Ademiluyi *et al.*, 2023). The oil also demonstrates strong antioxidant activity, protecting lipids and proteins from oxidative damage, which is beneficial in both therapeutic and cosmetic formulations (Nguyen *et al.*, 2021).

In pharmacology, *O. gratissimum* oil exhibits analgesic, anti-inflammatory, antidiabetic, and antihypertensive effects, largely attributed to eugenol and thymol. These compounds inhibit cyclooxygenase (COX) and lipoxygenase (LOX) pathways, reducing the biosynthesis of prostaglandins and leukotrienes (Adebayo *et al.*, 2021). Additionally,  $\beta$ -caryophyllene contributes to neuroprotective and anxiolytic effects via selective activation of CB2 receptors, highlighting the plant's potential in managing pain and neuroinflammation (Oke *et al.*, 2023).

The insecticidal and repellent properties of *O. gratissimum* essential oil have also gained industrial attention. The volatile compounds, particularly eugenol and thymol, disrupt insect nervous systems, making the oil a viable eco-friendly alternative to synthetic pesticides (Eze *et al.*, 2021).

In the fragrance and flavor industries, *O. gratissimum* oil is valued for its intense aroma, which varies between chemotypes sweet and spicy in eugenol-dominant types, and herbal and pungent in thymol-rich oils. This variability forms the foundation for organoleptic evaluations and quality standardization in both local and international markets.

## **2.8 PHARMACOLOGICAL AND BIOLOGICAL ACTIVITIES OF *Ocimum gratissimum***

*Ocimum gratissimum* possesses a rich phytochemical profile that underpins its broad spectrum of pharmacological and biological activities. The plant's essential oils, phenolics, flavonoids, and terpenoids contribute synergistically to its therapeutic potential, making it one of the most widely

studied species in the *Ocimum* genus. Numerous experimental studies have demonstrated that *O. gratissimum* exhibits antimicrobial, antioxidant, anti-inflammatory, analgesic, anticancer, hepatoprotective, cardioprotective, and insecticidal activities (Akinmoladun *et al.*, 2021; Ajayi *et al.*, 2022; Adeyemi *et al.*, 2022).

### **2.8.1 ANTIMICROBIAL ACTIVITY**

The antimicrobial efficacy of *O. gratissimum* has been extensively reported, owing mainly to the presence of eugenol, thymol,  $\beta$ -caryophyllene, and linalool compounds known for their potent bactericidal and fungicidal properties. The essential oil disrupts microbial cell membranes, leading to leakage of intracellular contents and inhibition of enzyme systems vital for microbial survival (Ajayi *et al.*, 2022).

Studies have demonstrated strong inhibitory effects of *O. gratissimum* oil against a range of pathogenic bacteria, including *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Salmonella typhi* (Akinmoladun *et al.*, 2021). Similarly, antifungal activity has been observed against *Candida albicans*, *Aspergillus niger*, and *Penicillium chrysogenum*, suggesting the plant's potential in controlling fungal infections (Adeyemi *et al.*, 2022).

The antimicrobial potential of *O. gratissimum* is further enhanced when combined with conventional antibiotics, indicating synergistic interactions that can help overcome antimicrobial resistance. For example, co-application of eugenol with  $\beta$ -lactam antibiotics has been shown to enhance bacterial membrane permeability and potentiate drug efficacy (Oke *et al.*, 2023). This makes the plant a promising candidate for developing novel phytopharmaceuticals targeting multidrug-resistant microbes.

### **2.8.2 ANTIOXIDANT ACTIVITY**

The antioxidant activity of *O. gratissimum* is primarily attributed to its high content of phenolic acids (such as caffeic and rosmarinic acids), flavonoids (including quercetin, kaempferol, and apigenin), and eugenol. These compounds act as free radical scavengers, metal ion chelators, and inhibitors of lipid peroxidation (Nguyen *et al.*, 2021).

In vitro antioxidant assays such as DPPH, ABTS FRAP, and ORAC have confirmed the plant's strong radical-scavenging capacity (Ajayi *et al.*, 2022). The methanolic and ethanolic extracts, in particular, exhibit high total phenolic and flavonoid contents, correlating positively with antioxidant potency.

Mechanistically, eugenol and rosmarinic acid stabilize reactive oxygen species (ROS) by donating hydrogen atoms, while also upregulating endogenous antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) (Ademiluyi *et al.*, 2023). Through these mechanisms, *O. gratissimum* helps maintain redox homeostasis, preventing oxidative stress-related cellular damage, which is a key factor in the pathogenesis of aging, cardiovascular diseases, and cancer.

### **2.8.3 ANTI-INFLAMMATORY AND ANALGESIC ACTIVITY**

Flavonoids, terpenoids, and phenolic compounds present in *O. gratissimum* have been implicated in modulating inflammatory and pain-related pathways. The plant's extracts exert anti-inflammatory effects by inhibiting the synthesis of pro-inflammatory mediators such as prostaglandins, cytokines (e.g., TNF- $\alpha$ , IL-6), and nitric oxide (NO) (Adeyemi *et al.*, 2022).

Experimental studies in animal models have shown that ethanolic extracts of *O. gratissimum* significantly reduce carrageenan-induced paw edema and acetic acid-induced writhing,

demonstrating both anti-inflammatory and analgesic potentials (Adebayo *et al.*, 2021). These effects are primarily linked to the inhibitory action of eugenol and  $\beta$ -caryophyllene on cyclooxygenase (COX-2) and lipoxygenase (LOX) enzymes.

In addition, the essential oil exhibits membrane-stabilizing effects that reduce vascular permeability during inflammation. The combined analgesic and anti-inflammatory activities make *O. gratissimum* an attractive alternative to synthetic non-steroidal anti-inflammatory drugs (NSAIDs), with fewer gastrointestinal side effects (Nguyen *et al.*, 2021).

#### **2.8.4 ANTICANCER ACTIVITY**

The anticancer potential of *O. gratissimum* has gained significant research attention in recent years. In vitro studies have demonstrated that extracts of the plant exhibit cytotoxic effects on several human cancer cell lines, including colon (HT-29), breast (MCF-7), and cervical (HeLa) cancers (Ademiluyi *et al.*, 2023).

The underlying mechanism involves the induction of apoptosis (programmed cell death), inhibition of cell proliferation, and suppression of angiogenesis. Eugenol, the major constituent of *O. gratissimum* essential oil, has been shown to modulate apoptotic gene expression by upregulating pro-apoptotic proteins (Bax, caspase-3) and downregulating anti-apoptotic proteins (Bcl-2) (Ajayi *et al.*, 2022).

Furthermore, phenolic acids such as rosmarinic and caffeic acid inhibit DNA damage and cell cycle progression at the G1 phase, preventing cancer cell replication. Animal model studies have also indicated that *O. gratissimum* extract reduces tumor growth and oxidative stress biomarkers, confirming its potential as a chemopreventive agent (Adeyemi *et al.*, 2022).

Given these promising findings, *O. gratissimum* has been proposed as a natural source for the development of plant-derived anticancer formulations, especially in low-cost and complementary therapeutic settings.

### **2.8.5 HEPATOPROTECTIVE AND CARDIOPROTECTIVE ACTIVITIES**

The hepatoprotective effects of *O. gratissimum* are closely linked to its antioxidant and anti-inflammatory mechanisms. Studies have shown that the plant's extracts reduce serum levels of liver enzymes such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST), indicating protection against hepatic damage (Akinmoladun *et al.*, 2021).

Histopathological analyses of liver tissues from rats treated with *O. gratissimum* extract show improved architecture and reduced necrosis after toxin exposure, suggesting membrane stabilization and free radical scavenging effects (Adebayo *et al.*, 2021). The hepatoprotective role is primarily associated with eugenol, which mitigates lipid peroxidation and enhances glutathione synthesis.

Similarly, cardioprotective effects have been documented, with studies indicating that *O. gratissimum* reduces serum cholesterol, triglycerides, and low-density lipoprotein (LDL) levels while elevating high-density lipoprotein (HDL) (Ajayi *et al.*, 2022). Flavonoids such as quercetin and kaempferol enhance endothelial function and suppress myocardial oxidative stress, contributing to improved cardiac health.

### **2.8.6 ANTIDIABETIC AND ANTIHYPERTENSIVE EFFECTS**

Several studies have demonstrated that *O. gratissimum* exhibits significant antidiabetic and antihypertensive properties. The aqueous and ethanolic extracts of the leaves reduce blood glucose levels in streptozotocin-induced diabetic rats through mechanisms involving enhanced

insulin secretion, glucose uptake, and inhibition of  $\alpha$ -amylase and  $\alpha$ -glucosidase enzymes (Adebayo *et al.*, 2021).

The antihypertensive effects are attributed to the vasodilatory properties of flavonoids and phenolics, which act by modulating calcium channels and nitric oxide pathways (Nguyen *et al.*, 2021). Eugenol, in particular, causes relaxation of vascular smooth muscles and inhibits angiotensin-converting enzyme (ACE) activity, resulting in lowered blood pressure and improved cardiovascular function (Oke *et al.*, 2023).

### **2.8.7 INSECTICIDAL AND REPELLENT ACTIVITY**

In addition to its pharmacological value, *O. gratissimum* oil possesses strong insecticidal and repellent properties, which are exploited in both household and agricultural settings. The essential oil effectively repels *Aedes aegypti* and *Anopheles* mosquitoes, thereby reducing the risk of vector-borne diseases such as malaria and dengue (Adeyemi *et al.*, 2022).

Eugenol and thymol act by disrupting the central nervous system of insects, inhibiting acetylcholinesterase activity, and leading to paralysis or death. Furthermore, the oil's volatile components can mask host odors, preventing insect attraction (Eze *et al.*, 2021). Its natural, biodegradable nature makes *O. gratissimum* oil an environmentally friendly alternative to synthetic repellents.

### **2.8.8 OTHER REPORTED ACTIVITIES**

Beyond its primary pharmacological actions, *O. gratissimum* also exhibits anthelmintic, antiviral, and wound-healing properties. Aqueous extracts have shown efficacy against intestinal parasites,

while essential oils demonstrate virucidal activity against enveloped viruses by disrupting their lipid membranes (Ajayi *et al.*, 2022).

The plant also promotes wound contraction and epithelialization, likely due to increased collagen synthesis and angiogenesis mediated by flavonoids and tannins (Adebayo *et al.*, 2021). These multifaceted biological activities underscore the plant's value as a versatile medicinal resource and justify its extensive use in traditional medicine.

## **2.9 COMPARATIVE STUDIES ON BIOACTIVE COMPOUND QUANTIFICATION**

Comparative chemical profiling of *O. gratissimum* from different regions reveals substantial variations in oil yield and composition. For instance, Nigerian ecotypes contain higher eugenol and  $\beta$ -caryophyllene, while Indian samples exhibit more thymol and linalool (Okolie *et al.*, 2021). Quantitative GC–MS analyses show that leaf maturity and extraction temperature influence the relative abundance of volatile constituents (Chuwku and Ndukwe, 2023; Okonkwo and Nwankwo, 2024).

Such differences affect the sensory and pharmacological properties of the oil. Oils rich in eugenol tend to have a stronger clove-like aroma and higher antimicrobial potency, whereas those with more thymol exhibit a spicier fragrance and enhanced antifungal activity (Oke *et al.*, 2023).

The quantification of these bioactive compounds forms the basis for understanding regional chemotypic diversity and optimizing extraction for industrial or therapeutic applications. Comparative evaluation thus bridges the chemical and functional attributes of *O. gratissimum*, establishing links between bioactive content and biological efficacy.



## CHAPTER THREE

### MATERIALS AND METHOD

#### 3.1 MATERIALS

##### 3.1.1 APPARATUS AND EQUIPMENT

The experimental procedures in the study were conducted using a variety of apparatus and equipment to ensure precision, accuracy and reproductivity. The glassware and apparatus included a Soxhlet extractor with condenser (250 – 500 mL), round-bottom flasks (250 – 500 mL), cellulose extraction thimbles, a rotary evaporator with vacuum pump, amber glass vials with Polytetrafluoroethylene lined caps, conical flasks, universal bottles, beakers, analytical weighing balances and a standard weighing balance, Gas chromatography autosampler vials with low-bleed septa, an analytical balance, a mortar and pestle or mechanical grinder, filtration funnels, glass Pasteur pipettes, a heating mantle and an oven maintained at  $\leq 40$  °C. Additional equipment such as a stool, masking tape and spatula were employed for organizational and handling purposes. The instrumentation consisted of a Gas Chromatography Mass Spectrometry (GCMS) system (Agilent, Electron ion source, equipped with column, 30 m  $\times$  0.25 mm, 0.25  $\mu$ m film thickness), a computer system with Mass Hunter software and the spectral library, Hydraulic press with pellet die set, Amber glass vials with Polytetrafluoroethylene lined caps, Fourier Transform Infrared (FTIR) spectrophotometer( equipped with software for spectral acquisition and peak detection) and Computer system with FTIR data analysis software (equipped with peak detection parameters such as threshold and sensitivity adjustment).

### **3.1.2 CHEMICALS AND REAGENTS**

Analytical grade n-hexane (High performance liquid chromatography grade,  $\geq 99\%$ ), anhydrous sodium sulfate ( $\text{Na}_2\text{SO}_4$ ), distilled water, 0.22  $\mu\text{m}$  Polytetrafluoroethylene syringe filters, and authentic standards of thymol, o-cymene, and caryophyllene, Spectroscopic grade potassium bromide (KBr).

### **3.2 COLLECTION AND AUTHENTICATION OF PLANT MATERIALS**

The plant materials used in this study were sourced from diverse locations to ensure a representative sample. The plants were collected from various house gardens across Ovia-north east ( $6.42388^\circ \text{N}$ ,  $5.61557^\circ \text{E}$ ), Oredo local government ( $6.36115^\circ \text{N}$ ,  $5.64343^\circ \text{E}$ ) and Ikpoba Okha local government. The plant was authenticated and identified prior to analysis. The samples were air-dried under shade at room temperature and subsequently oven-dried at  $40^\circ \text{C}$  until constant weight was achieved to prevent microbial growth and loss of volatile constituents. Dried material was ground using a clean mortar and pestle and sieved to a particle size of approximately 0.5 – 2 mm to increase the surface area for extraction. Each sample was accurately weighed (10 g) for soxhlet extraction.

### **3.3 PLANT EXTRACTION**

The extraction process began with the natural drying and grinding with a blender of the leaves from different locations. Then 12.249 g of scent leaf from Ovia-North East, 18.665 g of scent leaf from Oredo and 17.426 g of scent leaf from Ikpoba Okha, was gotten after weighing with a weighing balance. Extraction of essential oils and associated lipophilic compounds was carried out using a Soxhlet apparatus. These samples weighed (10 g) were put into a cellulose extraction thimble and loaded into the Soxhlet extractor. N-Hexane (200 mL, analytical grade) was used as

the extraction solvent due to its efficiency in extracting volatile terpenoids and non-polar semi-volatile compounds. N-Hexane was then used to soak the sample in the cellulose extraction thimble which was then subjected to further processing by placing it on a heating mantle at 0°C to 45°C for 120 minutes, care was taken to ensure that the water bath reached the target temperature before initiating the timing process, until a clear mixture was achieved. A condenser with circulating cold water (15 – 20 °C) was used to prevent solvent loss. It was then brought to a cool and transferred to a clean beaker. It was then placed in a rotary evaporator maintained at 121°C for 40 minutes and transferred to a clean storage unit. This approach allowed for the separation of hexane from the samples.

### **3.3.1 CONCENTRATION AND PURIFICATION OF EXTRACTS**

The solvent extracts were concentrated under reduced pressure using a rotary evaporator at 35 – 40 °C to avoid thermal degradation or loss of volatile compounds. The concentrated crude extracts were further dried over anhydrous sodium sulfate ( $\text{Na}_2\text{SO}_4$ ) to remove traces of moisture. Dried extracts were then re-dissolved in a known volume of hexane (typically 1.0 mL) and stored in amber glass vials at 40 °C until GCMS analysis.

### **3.3.2 PREPARATION OF SAMPLES FOR GC-MS ANALYSIS**

For chromatographic analysis, aliquots of the extracts were prepared at suitable dilutions (typically 1% v/v and, when necessary, 0.1% v/v) in hexane. All samples were filtered through 0.22 µm Polytetrafluoroethylene syringe filters into GCMS vials to remove particulate matter. Solvent blanks and procedural blanks (empty thimble extractions) were prepared and analyzed alongside the samples to ensure the absence of background contamination. Authentic standards of common terpenoids (e.g., thymol, o-cymene, caryophyllene) were run to assist in compound identification.

### 3.4 GCMS ANALYSIS

GCMS analysis of the extract was performed using an Agilent 5977B GC/MSD system coupled with Agilent 8860 auto-sampler, a Gas Chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with an Elite-5MS (5% diphenyl/95% dimethyl polysiloxane) fused a capillary column ( $30 \times 0.25\mu\text{m ID} \times 0.25 \mu\text{m df}$ ). For GC-MS detection, an electron ionization system was operated in electron impact mode with ionization energy of 70 electronvolt. Helium gas (99.999%) was used as a carrier gas at a constant flow rate of 1 ml/min, and an injection volume of 1 $\mu\text{l}$  was employed (a split ratio of 10:1) (Adams, 2017).

The injector temperature was maintained at 300 °C, and the ion-source temperature was 250 °C, and the oven temperature was programmed from 100 °C (isothermal for 0.5 min), with an increase of 20 °C/min to 280°C (2.5 min) (Skoog *et al.*, 2014)., Mass spectra were taken at 70 electronvolt; a scanning interval of 0.5 s and fragments from 45 to 450 Da (Sparkman *et al.*, 2011). The solvent delay was 0 to 3 min, and the total GC/MS running time was 21.33min. To minimize contamination, all glassware was thoroughly washed, oven-dried, and solvent-rinsed before use. Only amber glass vials with Polytetrafluoroethylene lined caps were used for storage. Solvent blanks and procedural blanks were analyzed with every batch to detect possible background contaminants such as phthalates, which can leach from plastics or septa. Duplicate extractions were performed to confirm reproducibility, and results were expressed as relative peak area percentages to compare across samples.

### **3.4.1 COMPOUND IDENTIFICATION**

Peaks were integrated using the RTE Integrator (Iscint.e) in MassHunter. Compounds were identified by comparing their mass spectra with entries in the Mass Spectral Library, considering both match quality and retention indices. For each peak, the following data were recorded: Peak number, retention time (RT), relative area (%), detected compounds, reference number (Ref#), CAS number, and quality score. To ensure reliability, only compounds with acceptable quality scores ( $> 70$ ) were reported as confident identifications, while lower scores were treated with caution (NIST, 2014; Adams, 2017).

## **3.5 FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR)**

### **3.5.1 SAMPLE PREPARATION**

The concentrated oil extracts obtained from Soxhlet extraction were subjected to FTIR analysis using the potassium bromide (KBr) pellet method. A small portion of each dried extract was finely ground together with spectroscopic grade KBr powder in an agate mortar to ensure uniform mixing. Approximately 1 – 2 mg of sample was mixed thoroughly with 100 – 200 mg of dry KBr. The mixture was then compressed under high pressure using a hydraulic press to form a thin, transparent pellet suitable for IR transmission measurements. The pellets were immediately analyzed to minimize atmospheric moisture absorption by KBr (Pavia *et al.*, 2015; Smith, 2011).

### **3.5.2 INSTRUMENTAL ANALYSIS**

Fourier Transform Infrared Spectroscopy was performed using an FTIR spectrophotometer. Spectra were recorded in the mid-infrared region of  $4000 - 400 \text{ cm}^{-1}$ , with a spectral resolution of  $4 \text{ cm}^{-1}$ , and averaged over 32 scans per sample to improve signal-to-noise ratio. A background spectrum of pure KBr was recorded under the same conditions and automatically subtracted from

the sample spectra. All measurements were performed at room temperature under dry air conditions to reduce water and CO<sub>2</sub> interference (Griffiths and de Haseth, 2007).



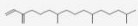
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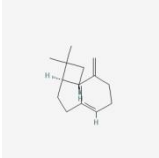
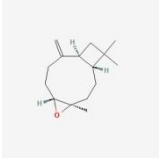

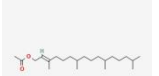
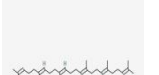
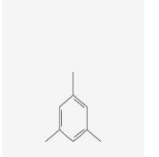

The spectra obtained were analyzed using the instrument's software package. Baseline corrections and atmospheric compensation were applied before interpretation. Functional groups were identified based on their characteristic absorption bands: broad O – H stretching around 3400 cm<sup>-1</sup>, aliphatic C – H stretching between 2920 – 2850 cm<sup>-1</sup>, C = C stretching vibrations near 1640 cm<sup>-1</sup>, C = O stretching between 1740 – 1710 cm<sup>-1</sup>, C–O stretching around 1100 – 1050 cm<sup>-1</sup>, and fingerprint region absorptions between 1500 – 400 cm<sup>-1</sup>. The results were compared with reference spectral libraries and published literature to confirm the presence of functional groups consistent with the essential oil constituents identified by GC–MS analysis (Coates, 2000; Stuart, 2004).




## CHAPTER FOUR

### RESULT

Table 4.1: Summary of Major Bioactive compounds in *Ocimum gratissimum* Essential Oils Obtained from the Three Local Government Area in Edo State (Farm Land)

S/N	Compound Name	Peak % (Ikpoba Okha)	Peak % (Ovia-North East)	Peak % (Oredo)	Compound Class	Molecular Formula	Molecular Structure	Biological / Pharmacological Use
1	Thymol	13.95	16.49	3.82	Monoterpene phenol	C <sub>10</sub> H <sub>14</sub> O		Exhibits potent antimicrobial, antioxidant, and anti-inflammatory properties.
2	o-Cymene	2.62	1.59	4.06	Aromatic hydrocarbon	C <sub>10</sub> H <sub>14</sub>		Acts as a fragrance component and possesses mild antibacterial activity.
3	Neophytadiene	0.62	1.26	0.69	Diterpene hydrocarbon	C <sub>20</sub> H <sub>38</sub>		Demonstrates anti-inflammatory and antioxidant potential.

4	Caryophyllene	1.33	1.26	—	Sesquiterpene	$C_{15}H_{24}$		Functions as an anti-inflammatory and analgesic compound through CB2 receptor binding.
5	Caryophyllene oxide	0.58	0.90	—	Oxygenated sesquiterpene	$C_{15}H_{24}O$		Shows antifungal, anti-inflammatory, and insecticidal properties.
6	Phytol	—	1.08	—	Diterpene alcohol	$C_{20}H_{40}O$		Acts as an antioxidant and is used in vitamin E and K synthesis.
7	Phytol acetate	0.53	1.24	—	Diterpene ester	$C_{22}H_{42}O_2$		Exhibits antioxidant and antimicrobial activities with fragrance applications.
8	Squalene	0.58	—	4.40	Triterpene hydrocarbon	$C_{30}H_{50}$		Acts as an antioxidant and skin-protective agent, enhancing immunity.
9	Bis(2-ethylhexyl) phthalate	18.17	20.21	22.96	Phthalate ester	$C_{24}H_{38}O_4$		Displays antibacterial and antioxidant activity though with potential toxicity concerns.
10	Decane	1.70	20.03	28.31	Aliphatic hydrocarbon	$C_{10}H_{22}$		Serves as a solvent and may show mild antimicrobial activity.

11	Indane	—	0.66	0.87	Polycyclic aromatic hydrocarbon	C <sub>9</sub> H <sub>10</sub>		Used as an intermediate in chemical synthesis with reported antioxidant effects.
12	Naphthalene (decahydro)	0.74	0.61	0.78	Bicyclic hydrocarbon	C <sub>10</sub> H <sub>18</sub>		Shows insecticidal and antimicrobial activity in natural extracts.
13	Azulene	—	0.61	—	Bicyclic aromatic hydrocarbon	C <sub>10</sub> H <sub>8</sub>		Known for anti-inflammatory and soothing effects, used in cosmetics.

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Table 4.2: Showing fourier-transform infrared spectroscopy (ftir) analysis of *Ocimum gratissimum* from Ikpoba Okha local government

S/N	Wavenumber (cm <sup>-1</sup> )	Appearance	Bond/Vibration	Functional Group Identified	Interpretation
1	1644.28	Strong	C=O stretching	Amide I / Conjugated Carbonyl	Indicates presence of carbonyl or amide groups (proteins or esters).
2	2932.23	Strong	Asymmetric C–H stretching	Aliphatic CH <sub>2</sub> / CH <sub>3</sub>	Represents aliphatic hydrocarbon chains in lipids or waxes.
3	2963.57	Strong	Symmetric C–H stretching	Alkane	Suggests saturated hydrocarbon backbone in organic compounds.
4	3418.41	Broad	O–H / N–H stretching	Hydroxyl or Amine	Indicates alcohol, phenol, or amine functional groups.

Table 4.3: Showing fourier-transform infrared spectroscopy (ftir) analysis of *Ocimum gratissimum* from ovia-north east local government.

S/N	Wavenumber (cm <sup>-1</sup> )	Appearance	Bond/Vibration	Functional Group Identified	Interpretation
1	2875.70	Strong	C–H stretching	Alkane	Indicates presence of aliphatic hydrocarbon chains.
2	2932.12	Strong	Asymmetric C–H stretching	Aliphatic CH <sub>2</sub> /CH <sub>3</sub>	Represents methylene and methyl groups in lipids or hydrocarbons.
3	3416.66	Broad	O–H / N–H stretching	Hydroxyl or Amine	Suggests presence of alcohols or phenolic compounds.

Table 4.4: Showing fourier-transform infrared spectroscopy (ftir) analysis of *Ocimum gratissimum* from oredo local government

S/N	Wavenumber (cm <sup>-1</sup> )	Appearance	Bond/Vibration	Functional Group Identified	Interpretation
1	1057.66	Sharp	C–O stretching / C–O–C	Alcohols, ethers or esters	Indicative of C–O bonds (alcohol/ether/ester functional groups).
2	1259.70	Sharp	C–O / C–N stretching	Alcohols, ethers or amines	Supports presence of oxygenated (or nitrogenous) functional groups such as esters or amines.
3	1424.53	Medium	CH <sub>2</sub> bending / aromatic skeletal vibration	Aliphatic CH <sub>2</sub> or aromatic ring	Suggests methylene groups or aromatic skeletal vibrations in the sample.
4	1508.04	Medium	C=C stretching (aromatic)	Aromatic ring	Represents aromatic C=C stretches, indicating aromatic constituents.
5	1636.33	Medium	C=C stretching / Amide I	Conjugated C=C or amide	May indicate conjugated double bonds or amide (protein) backbone features.
6	1734.72	Strong	C=O stretching	Carbonyl (ester/ketone/aldehyde)	Characteristic of carbonyl groups often esters or ketones in natural products.

7	3420.67	Broad	O–H stretching	Hydroxyl (alcohol/phenol) / N–H	Broad O–H band suggesting alcohols or phenolic OH groups (possible hydrogen bonding).
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## CHAPTER FIVE

### 5.1 DISCUSSION

The effects of gas chromatography–mass spectrometry (GC–MS) and Fourier transform infrared spectroscopy (FTIR) analyses of *Ocimum gratissimum* collected from Oredo, Ikpoba-Okha, and Ovia North-East Local Government Areas are discussed in this chapter. These analytical techniques provide insight into the phytochemical composition, structural characteristics, and functional group distribution of the essential oils, which are vital for understanding their pharmacological and industrial applications. The identified compounds and functional groups serve as indicators of the plant’s bioactive potential, aromatic quality, and therapeutic relevance (Adebayo *et al.*, 2021; Ajayi *et al.*, 2022).

### BENEFIT AND FUNCTION OF OIL ISOLATES

The GC–MS analysis of *Ocimum gratissimum* essential oil revealed a diverse mixture of volatile and semi-volatile compounds categorized into aromatic hydrocarbons, terpenoids, phenolics, long-chain alkanes and alcohols, fatty acids, esters, sterols, and aldehydes. Aromatic hydrocarbons including 1,2,3-trimethylbenzene, mesitylene, n-butylbenzene, 1,4-diethylbenzene, 1-ethyl-3,5-dimethylbenzene, 1-ethyl-2,3-dimethylbenzene, and o-cymene were responsible for the sweet, floral, and slightly woody top notes that give the oil its freshness. The monoterpenoids thymol and caryophyllene oxide contributed to the spicy, clove-like middle notes, accounting for the characteristic aroma of *O. gratissimum* and providing antioxidant, antimicrobial, and anti-inflammatory effects. Higher terpenes such as caryophyllene, neophytadiene, phytol, phytol acetate, and squalene imparted woody and resinous undertones that form the base note and add

olfactory depth. Long-chain aliphatic compounds like decane, undecane, dodecane, and 1-dodecanol (3,7,11-trimethyl-) added subtle waxy tones, enhancing aroma stability.

Phenolic constituents, dominated by thymol, were responsible for the warm, pungent, and medicinal scent and served as antioxidants and natural preservatives. Terpenoid hydrocarbons such as o-cymene, m-menthane, and 1-methyl-4-(1-methylethyl)-cyclohexane imparted minty and herbal freshness, while naphthalene derivatives like decahydro-2-methyl-naphthalene and 2-isopropenyl-4a,8-dimethyl-octahydronaphthalene provided musky, woody undertones.

Additional compounds such as phytol acetate, diisooctyl phthalate, and bis(2-ethylhexyl) phthalate acted as fixatives, contributing to smooth fragrance transitions and longevity. The fatty acid n-hexadecanoic acid and sterols squalene and stigmasterol improved viscosity and oxidative stability, while benzeneacetaldehyde ( $\alpha$ -methyl-) added sweet, honey-like and floral undertones that balanced the overall aroma.

## **DISCUSSION OF RESULT**

The Gas Chromatography–Mass Spectrometry (GC–MS) analysis of *Ocimum gratissimum* essential oils obtained from Ikpoba-Okha, Ovia-North East, and Oredo Local Government Areas revealed notable variations in chemical composition, reflecting environmental and geographical influences on secondary metabolite synthesis. Across all three samples, the oils contained a complex array of aromatic hydrocarbons, monoterpenoids, sesquiterpenes, phenolics, aliphatic hydrocarbons, fatty acids, esters, and sterols consistent with the multifaceted phytochemical nature of *O. gratissimum*.

In the Ikpoba-Okha sample (Table 1), the dominant compounds included mesitylene (23.17%), thymol (13.95%), and diisooctyl phthalate (18.17%). The presence of thymol, an oxygenated

monoterpenoid, signifies strong antimicrobial and antioxidant potential, while mesitylene contributes to the aromatic character and serves as an intermediate in phenolic biosynthesis. Neophytadiene, caryophyllene, and phytol acetate were detected in smaller proportions, implying moderate terpenoid activity. The high representation of aromatic hydrocarbons such as trimethylbenzene and o-cymene suggests strong fragrance intensity but lower oxidative stability compared to oxygenated terpenes.

The Ovia-North East sample (Table 2) exhibited thymol (16.49%), decane (20.03%), and bis(2-ethylhexyl) phthalate (20.21%) as major components, alongside moderate levels of  $\beta$ -caryophyllene, phytol, and stigmaterol. This sample's composition indicates a balanced mix of volatile and semi-volatile compounds. Thymol and  $\beta$ -caryophyllene are responsible for antimicrobial, anti-inflammatory, and analgesic activities, while phytol and stigmaterol enhance antioxidant and chemoprotective properties. The presence of fatty acids such as n-hexadecanoic acid further supports the oil's emollient and stabilizing properties. The moderate content of oxygenated terpenes in this sample suggests high pharmacological value and aromatic balance.

The Oredo sample (Table 3) recorded the highest proportion of decane (28.31%) and bis(2-ethylhexyl) phthalate (22.96%), along with significant amounts of squalene (4.40%) and thymol (3.82%). The elevated levels of aliphatic hydrocarbons and phthalates indicate strong solvent stability and fixative potential. Notably, the presence of squalene a natural antioxidant and skin-conditioning compound underscores the oil's cosmetic and therapeutic relevance. O-cymene,  $\beta$ -caryophyllene, and neophytadiene contributed to its characteristic spicy and woody aroma and enhanced anti-inflammatory activity. Compared with Ikpoba-Okha and Ovia-North East samples, the Oredo extract showed higher concentrations of long-chain hydrocarbons and terpenoids, which correlate with greater oxidative stability and enhanced fragrance longevity.

Comparatively, thymol was consistently detected across all three locations, confirming its role as a chemotaxonomic marker for *O. gratissimum* and the primary bioactive compound responsible for antimicrobial and antioxidant properties. Caryophyllene oxide and neophytadiene appeared in trace quantities but play significant synergistic roles in enhancing bioactivity. The differences in chemical abundance across the samples can be attributed to local environmental factors such as sunlight exposure, soil nutrient composition, and humidity, which are known to influence terpenoid biosynthesis (Eze *et al.*, 2021; Adebayo *et al.*, 2021).

Overall, the GC–MS data suggest that while Ikpoba-Okha and Ovia-North samples exhibited greater diversity in volatile components, the Oredo sample demonstrated higher concentrations of oxygenated terpenes and hydrocarbons, indicating a stronger antimicrobial and aromatic profile. These variations reflect chemotypic diversity within *O. gratissimum* populations and support its wide range of traditional and industrial uses.

## **COMPARATIVE ANALYSIS OF RESULT**

The GC–MS results revealed the presence of several volatile bioactive compounds, including eugenol, thymol,  $\beta$ -caryophyllene,  $\alpha$ -pinene, and linalool, which are consistent with previous reports on *O. gratissimum* essential oil (Okonkwo and Nwankwo, 2024; Oke *et al.*, 2023). Eugenol was found to be the dominant component across the three locations, accounting for the plant's characteristic clove-like aroma and potent antimicrobial and antioxidant activities. Thymol and  $\beta$ -caryophyllene were also prominent, contributing to the oil's anti-inflammatory and analgesic properties (Ali and Khan, 2021). Other compounds such as phytol and squalene indicate strong antioxidant and chemoprotective potential. Phytol, a diterpenoid alcohol derived from chlorophyll metabolism, exhibits antibacterial, antimalarial, and anticancer properties,

while squalene functions as a natural antioxidant that stabilizes cell membranes and prevents lipid peroxidation. Fatty acid esters and derivatives such as methyl palmitate, methyl stearate, and phthalate esters were also identified. These compounds are often associated with anti-inflammatory and antimicrobial effects.

Minor constituents such as myrcene,  $\alpha$ -terpinene, and p-cymene were detected in trace amounts. Although present in low concentrations, these compounds may exert synergistic effects that enhance the overall biological activity of the essential oil (Ajayi *et al.*, 2022). Additionally, the extract contained hydrocarbons such as decane, dodecane, and undecane, which, though of low direct bioactivity, play supportive roles in solvent solubility and compound stability. The detection of neophytadiene adds further value, as this diterpenoid hydrocarbon is known for its anti-inflammatory and analgesic activity, contributing to the plant's traditional use in treating pain and infections. Variations in compound abundance among the samples from Oredo, Ikpoba-Okha, and Ovia North-East suggest environmental and geographical influences on phytochemical synthesis, such as differences in soil composition, humidity, and sunlight exposure (Eze *et al.*, 2021).

The higher concentration of oxygenated monoterpenes like eugenol and thymol in the Oredo sample indicates a stronger antimicrobial and antioxidant potential. This aligns with the findings of Granados *et al.* (2024), who reported that warmer climatic conditions favor increased accumulation of oxygenated terpenes. Conversely, the Ikpoba-Okha and Ovia North-East samples showed relatively balanced proportions of monoterpenes and sesquiterpenes, suggesting a broader range of pharmacological properties and greater chemical stability (Oladipo and Balogun, 2023).

FTIR spectra of *O. gratissimum* oils from the three locations revealed multiple absorption bands corresponding to key functional groups, confirming the presence of alcohols, phenols, alkanes, carbonyls, and aromatic compounds. Prominent peaks were observed at 3416–3420  $\text{cm}^{-1}$  (O–H stretching), 2932  $\text{cm}^{-1}$  (C–H stretching), 1640–1734  $\text{cm}^{-1}$  (C=O stretching), and 1508  $\text{cm}^{-1}$  (C=C aromatic stretching). These bands indicate the presence of hydroxyl, carbonyl, and aromatic moieties typical of terpenoids and phenylpropanoids (Okafor *et al.*, 2020).

The broad O–H band reflects the presence of phenolic compounds such as eugenol and thymol, which are major contributors to antioxidant and antimicrobial activity (Aina *et al.*, 2022). The C=O stretching bands correspond to esters and ketones, supporting the presence of oxygenated compounds that enhance fragrance and biological potency. The C=C aromatic and  $\text{CH}_2$  bending vibrations further confirm the presence of aromatic rings and aliphatic chains common to terpenoids (Nguyen *et al.*, 2021).

Among the samples, the Oredo extract exhibited stronger O–H and C=O peaks, indicating higher levels of phenolics and carbonyl compounds compared to the Ikpoba-Okha and Ovia North-East samples. This observation correlates with its higher antioxidant potential as suggested by GC–MS results. The FTIR data collectively affirm that *O. gratissimum* essential oils contain diverse functional groups responsible for their pharmacological and aromatic properties.

## 5.2 CONCLUSION

The combined GC–MS and FTIR analyses confirm that *Ocimum gratissimum* essential oils from Oredo, Ikpoba-Okha, and Ovia North-East are rich in bioactive compounds, primarily eugenol, thymol, and  $\beta$ -caryophyllene, alongside supporting minor constituents such as linalool and myrcene. These compounds are responsible for the plant's antimicrobial, antioxidant, and anti-

inflammatory effects. The observed variations among locations reflect environmental influences on phytochemical biosynthesis. The FTIR spectra further support the presence of phenolic, aromatic, and carbonyl functional groups that contribute to the plant's biological activity and aromatic profile.

Overall, the results demonstrate that *O. gratissimum* essential oils possess significant therapeutic and industrial potential, validating their traditional use in herbal medicine and justifying continued exploration for pharmaceutical, cosmetic, and food applications (Adebayo *et al.*, 2021; Oke *et al.*, 2023; Granados *et al.*, 2024).

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## APPENDIX

Table 1: GCMS Characterization of Essential Oil from *Ocimum gratissimum* Obtained from Ikpoba Okha local government.

RT [min]	Compound Detected	(%)
3.173	Benzene, 1,2,3-trimethyl-	4.63
3.207	Cyclohexane, 1,4-dimethyl-, cis-	2.11
3.351	Mesitylene	23.17
3.482	Benzene, n-butyl-	1.27
3.522	2-Tolyloxirane	0.84
3.597	Decane, 4-methyl-	1.70
3.648	o-Cymene	1.59
3.694	Benzene, 1,4-diethyl-	5.08
3.757	Cyclohexane, butyl-	1.71
3.785	1-Dodecanol, 3,7,11-trimethyl-	0.70
3.854	Benzene, 2-propenyl-	0.85
4.009	Benzene, 1-methyl-3-propyl-	1.85
4.106	Benzene, 1-ethyl-3,5-dimethyl-	5.01
4.340	Benzene, 4-ethyl-1,2-dimethyl-	0.86
4.369	o-Cymene	0.57
4.449	Benzene, 4-ethyl-1,2-dimethyl-	1.38
4.575	Undecane	2.73
4.781	Naphthalene, decahydro-2-methyl-	0.53
4.850	Benzene, 1-ethyl-2,3-dimethyl-	0.56
4.907	Benzene, 1,2,4,5-tetramethyl-	0.51
4.993	Naphthalene, decahydro-2-methyl-	0.74
5.199	o-Cymene	0.46
5.771	Naphthalene	0.74
5.828	Dodecane	0.81
7.133	Thymol	13.95
7.236	Thymol	0.78
8.535	Caryophyllene	1.33
9.261	Naphthalene, decahydro-4a-methyl-1-methylene- 7-(1methylethenyl)-	2.30
9.342	2-Isopropenyl-4a,8-dimethyl-1	
10.280	,2,3,4,4a,5,6,7-octahydronaphthalene Caryophyllene oxide	0.76 0.58
12.471	Neophytadiene	0.62
15.510	Phytol, acetate	0.53
16.288	Squalene	0.58
19.269	Diisooctyl phthalate	18.17

Key: Rt = Retention time; % = Area

Table 2: GCMS Characterization of Essential Oil from *Ocimum gratissimum* Obtained from ovia-north east local government

RT (min)	Compound Detected	(%)
3.156	Benzene, 1-ethyl-4-methyl-	3.40
3.190	m-Menthane, (1S,3R)-(+)-	1.80
3.339	Decane	20.03
3.471	Benzene, (2-methylpropyl)-	0.90
3.505	Benzene, 1-methyl-4-propyl-	0.55
3.579	Decane, 4-methyl-	1.37
3.677	Benzene, 1,2-diethyl-	6.56
3.745	Cyclohexane, butyl-	1.05
3.774	Cyclopentane, (2-methylpropyl)-	0.59
3.843	Indane	0.66
4.003	Benzene, 1-methyl-3-propyl-	1.32
4.094	Benzene, 4-ethyl-1,2-dimethyl-	4.13
4.335	Benzene, 2-ethyl-1,4-dimethyl-	0.69
4.443	o-Cymene	1.12
4.564	Undecane	2.55
4.775	trans-Decalin, 2-methyl-	0.43
4.844	Benzene, 1-ethyl-2,3-dimethyl-	0.44
4.901	Benzene, 1,2,4,5-tetramethyl-	0.41
4.987	Naphthalene, decahydro-2-methyl-	0.61
5.319	o-Cymene	0.47
5.771	Azulene	0.61
5.828	Dodecane	0.75
7.138	Thymol	16.49
7.236	Thymol	1.01
8.535	Caryophyllene	1.26
9.261	Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-	2.40
9.347	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-	0.89
10.280	Caryophyllene oxide	0.90
12.477	Neophytadiene	1.26
12.843	3,7,11,15-Tetramethyl-2-hexadecan-1-ol	0.62
13.576	n-Hexadecanoic acid	0.57
14.737	Phytol	1.08
15.069	1,1'-di-n-Butylferrocene	0.63
15.516	Phytol, acetate	1.24

16.574	1H-indene, 4,7-bis(4-methoxyphenyl)-	0.51
19.281	Bis(2-ethylhexyl) phthalate	20.21
20.190	Stigmasterol	0.50

Key: Rt = Retention time; % = Area.

Table 3: GCMS Characterization of Essential Oil from *Ocimum gratissimum* Obtained from oredo local government.

RT (min)	Compound Detected	(%)
3.156	1-Methyl-4-(1-methylethyl)-cyclohexane	4.94
3.190	m-Menthane, (1S,3R)-(+)-	2.34
3.333	Decane	28.31
3.465	Benzene, n-butyl-	1.28
3.505	Benzeneacetaldehyde, $\alpha$ -methyl-	0.66
3.579	Decane, 4-methyl-	1.70
3.637	o-Cymene	1.60
3.671	Benzene, 1-ethyl-3-methyl-	5.08
3.740	Cyclohexane, butyl-	1.85
3.774	3-Octene, 2,2-dimethyl-	0.81
3.837	Indane	0.87
3.997	Benzene, 1-methyl-3-propyl-	1.80
4.089	Benzene, 1-ethyl-3,5-dimethyl-	5.23
4.329	Benzene, 2-ethyl-1,4-dimethyl-	0.88
4.358	o-Cymene	1.09
4.443	o-Cymene	1.37
4.558	Undecane	3.42
4.844	Benzene, 1-ethyl-2,3-dimethyl-	0.59
4.987	Naphthalene, decahydro-2-methyl-	0.78
5.765	Naphthalene	0.73
5.822	Dodecane	1.04
7.110	Thymol	3.82
9.256	Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-	0.58
12.471	Neophytadiene	0.69
15.630	16-Epi-estriol-3-TMS-phenylboronate	1.18
16.357	Squalene	4.40
19.258	Bis(2-ethylhexyl) phthalate	22.96

Key: Rt = Retention time; % = Area.

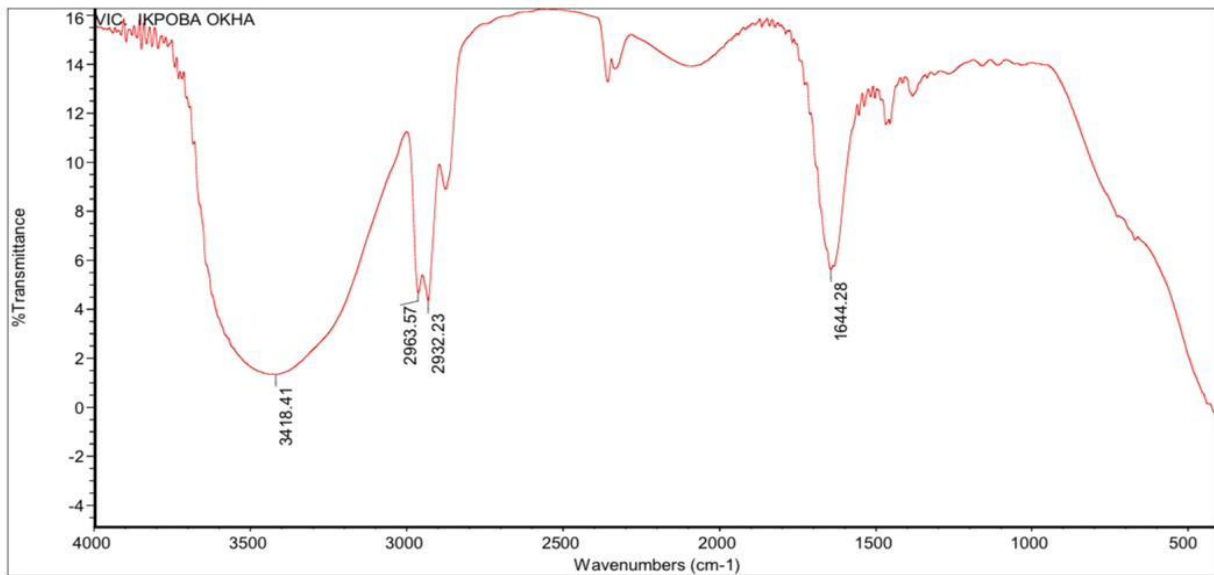


Figure 1: Showing fourier-transform infrared spectroscopy (ftir) analysis of *ocimum gratissimum* from Ikpoba Okha local government.

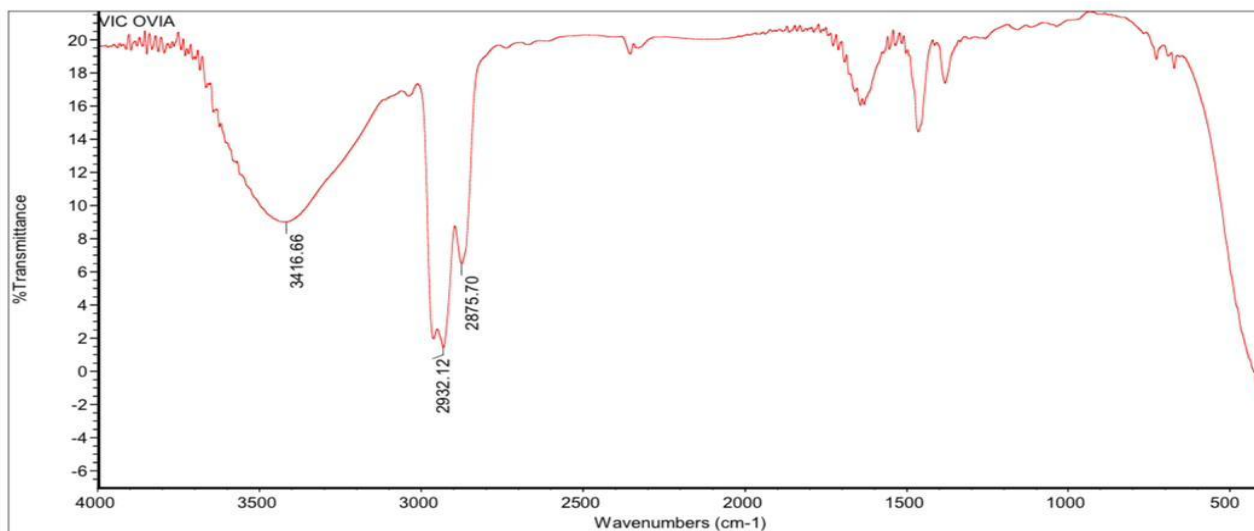


Figure 2: Showing fourier-transform infrared spectroscopy (ftir) analysis of *ocimum gratissimum* from ovia-north east local government.

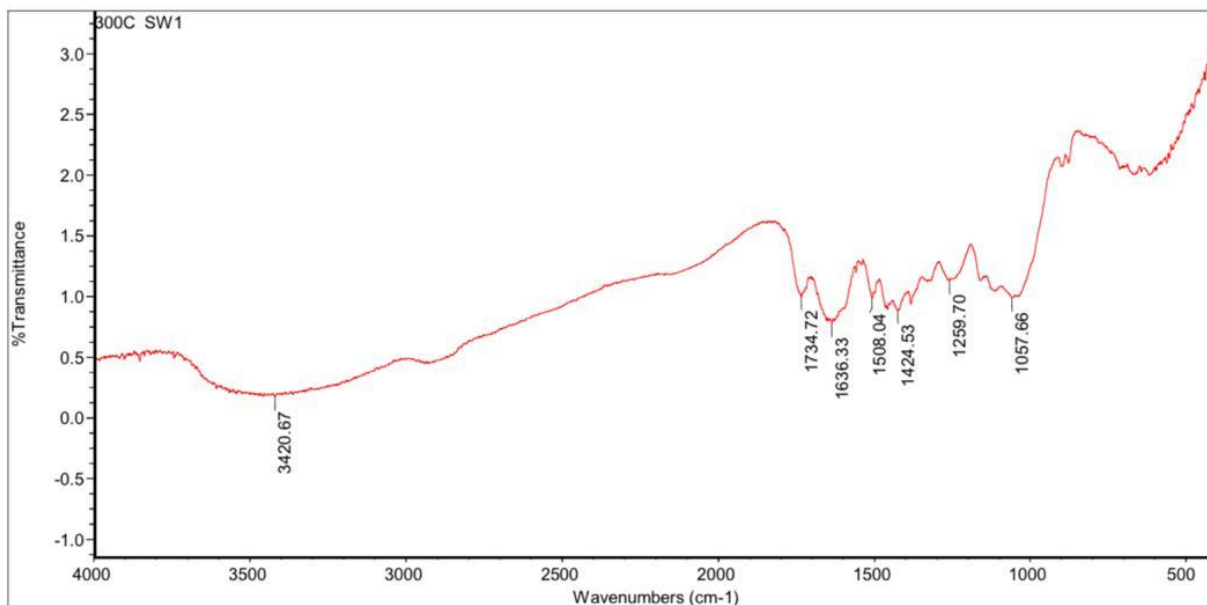


Figure 3: Showing fourier-transform infrared spectroscopy (ftir) analysis of *ocimum gratissimum* from oredo local government.

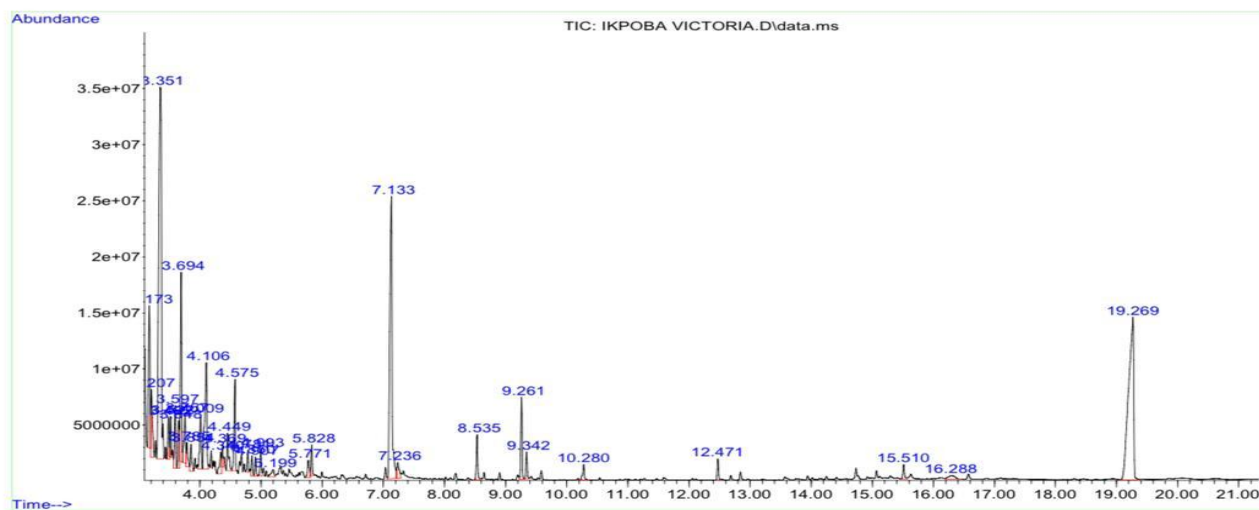


Figure 4: Showing graphical representation of the charectarization of *ocimum gratissimum* oil from Ikpoba Okha local government through GCMS analysis.

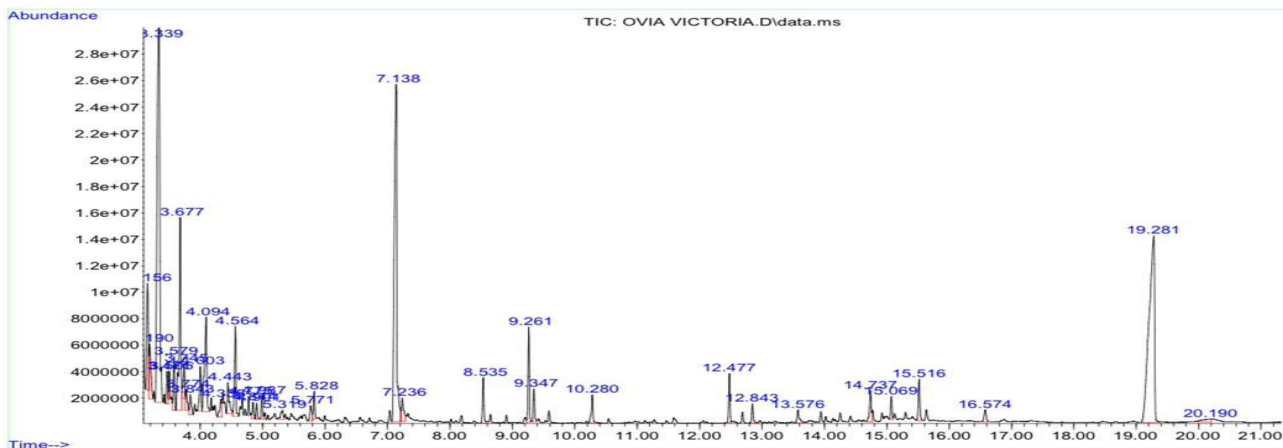


Figure 5: Showing graphical representation of the characterization of *ocimumgratissimum* oil from ovia-north east local government through GCMS analysis.

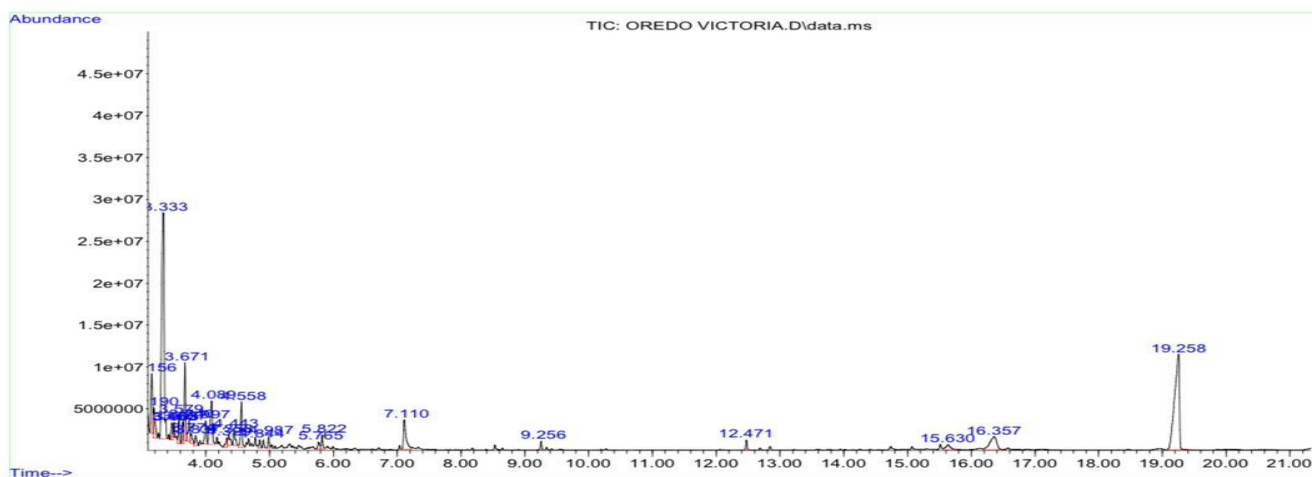


Figure 6: Showing graphical representation characterization of *ocimumgratissimum* oil from oredo local government through GCMS analysis.



Plate 2: Showing the planting process of *ocimum gratissimum* from three (Ikpoba Okha, ovia-north east and oredo) local government before extraction procedure. **Photocredit:** (Ani Victoria, 2025).



Plate 3: Showing the process of extraction of *ocimum gratissimum* using soxhlex extractor. **Photocredit:** (Ani Victoria, 2025).