# THE EFFECT OF BUILTUP MICROENVIRONMENT AREA ON THE CHEMOTYPE OF *Ocimum gratissimum* ESSENTIAL OIL HARVESTED FROM THREE LOCAL GOVERNMENT AREA IN EDO STATE


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**A 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 THE AWARD OF BACHELOR DEGREE (BSc.) IN SCIENCE LABORATORY TECHNOLOGY (BIOTECHNOLOGY TECHNIQUES).**

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## CERTIFICATION

This is to certify that this project work, titled "THE EFFECT OF BUILTUP AREA MICROENVIRONMENT ON THE CHEMOTYPE OF *Ocimum gratissimum* ESSENTIAL OIL HARVESTED FROM THREE LOCAL GOVERNMENT AREA IN EDO STATE**"** was carried out by Blesssing Osa IYAHEN with matriculation number LSC2009905 of the Department of Science Laboratory Technology (Biotechnology Techniques), Faculty of Life Sciences, University of Benin City, Edo State, under the supervision of Prof. E. O OSHOMOH.

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## DEDICATION

I dedicate this project work to God Almighty, my family and friends who have shown constant support and encouragement throughout this journey.

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**ABSTRACT**

The use of medicinal plants such as *Ocimum gratissimum* (scent leaf) is widespread, but the therapeutic capability of its essential oil is highly dependent on its chemical profile, or chemotype. Global urbanization introduces specific microenvironmental stresses (e.g., pollution, heat, and soil alteration) that can disrupt plant metabolism, leading to inconsistent oil composition and quality. This study investigated the effect of built-up microenvironments on the chemotype of *O. gratissimum* essential oil harvested from three distinct Local Government Areas (LGAs) in Edo State, Nigeria: Oredo, Egor, and Ovia North-East. Fresh *ocimum. gratissimum* leaves were collected from built-up microenvironments in the three selected LGAs. Essential oils were extracted using the Soxhlet method with 99% HPLC-grade hexane, and their chemical compositions were determined using Gas Chromatography–Mass Spectrometry (GC–MS) and Fourier Transform Infrared Spectroscopy (FTIR). GC–MS analysis showed that the essential oil from all three LGAs belonged to a thymol-rich chemotype. However, the concentration of the key active compound, thymol, varied across locations: Egor had the highest thymol content (7.75%), while Oredo was richest in its precursor, *o*-cymene (12.89%). Ovia North-East exhibited the lowest thymol concentration (5.12%). The presence of high percentages of non-essential oil components (decane and bis(2-ethylhexyl) phthalate) was noted as a potential artifact of the extraction process. FTIR analysis further revealed the consistent presence of nitrogen-containing amine functional groups across all samples, suggesting a stress-induced shift in secondary metabolism. The built-up microenvironment significantly influences the chemical profile of *O. gratissimum* essential oil. The variation in the *o*-cymene/thymol ratio and the presence of nitrogenous compounds suggest that local environmental stressors (such as pollution or soil conditions) may disrupt the final stages of the monoterpene biosynthetic pathway. These findings highlight the critical need for standardizing growing conditions in pharmacologically driven cultivation to ensure a consistent and high-quality essential oil chemotype.

## CHAPTER ONE

## INTRODUCTION

### 1.1 Background of study

The use of medicinal plants for treating, controlling, and preventing illness is a practice with ancient origins, dating back to the earliest human societies (Yuan *et al.,* 2016; Ekweogu *et a*l., 2019). The growing interest in herbal treatments over conventional drugs stems from the proven therapeutic benefits of their natural components, along with the fact that they are generally available, affordable, accessible, and thought to have fewer toxic side effects (Ikpeazu *et al.,* 2018; Ijioma *et al.,* 2021). Consequently, scientists have recently paid substantial attention to these plants and their chemical byproducts (secondary metabolites) because of their promise in addressing chronic and life-threatening diseases (Sofowora *et al.*, 2013; WHO, 2019).

*Ocimum gratissimum* is a tropical herb in the Lamiaceae family, known by many common names, including scent leaf, African basil, camphor basil, basil leaf, or ram tulsi (Kalita and Narzary, 2023; Moneme *et al.,* 2024b). This plant originated in Africa, Madagascar, southern Asia, and the Bismarck Archipelago, but has since become established in many other areas, such as Polynesia, Hawaii, Mexico, Panama, the West Indies, Brazil, and Bolivia. Its leaves are popularly called "scent leaf" because of their distinct mint-like fragrance. They are traditionally used as a spice in cooking, especially for soups (Akinjogunola *et al*., 2009). Furthermore, the leaves function as a natural flavor enhancer, condiment, and leafy vegetable in preparing various meals, including fish, meat, soups, and stews. Besides its culinary uses, *Ocimum gratissimum* is an important part of traditional medicine, used to treat numerous health issues like cough, pneumonia, fever, inflammation, anemia, diarrhea, pain, and fungal or bacterial infections (Akara *et al*., 2021).

Essential oils are natural, complex, and volatile compounds with a distinct aroma, produced by aromatic plants as a type of secondary metabolite (Mas et al., 2019). Chemically, Essential oils are classified as terpenes, which are among the most abundant groups of plant secondary metabolites (Singh and Sharma, 2015).Every plant species yields a unique combination of essential oils (Blowman *et al*., 2018). Consequently, the phytochemical components of *Ocimum gratissimum* essential oil differ depending on the geographical source of the leaves and the season they're collected. Since these phytochemical components dictate the medicinal effects, the traditional uses of the plant can vary by location (Sharma *et al*., 2022). The essential oil of *Ocimum gratissimum* is known to contain many chemicals, such as Eugenol and Thymol (Borges *et al.*, 2012), \gamma-muurolene and \beta-caryophyllene (Silva *et al.*, 2010), \alpha-bisabolene and \gamma-terpinene (Franco, 2007), 1.8-cineole and \beta-selinene (Benitez *et al*., 2009), and Geraniol and p-cymene.

## 1.2 Aim:

The aim of this study is to investigate the effect of built-up area microenvironments on the chemotype of *Ocimum gratissimum* essential oil harvested from three Local Government Areas (LGAs) in Edo State.

## 1.3 Objectives of study

The objectives of this study are to:

i. obtain and authenticate samples of *Ocimum gratissimum* from three chosen Local Government Areas (LGAs) in Edo State, each representing distinct urbanized settings.

ii. isolate the essential oils from the gathered plant material using Soxhlet extraction method.

iii. determine the precise chemical makeup (chemotype) of the extracted essential oils via advanced analytical methods, such as Gas Chromatography–Mass Spectrometry (GC-MS) and Fourier Transform Infrared Spectroscopy (FTIR)

iv. evaluate and contrast the differences observed in the essential oil composition among the three selected LGAs.

v. determine how much urban-related microenvironmental elements (like pollution, soil condition, temperature, and overall urbanization) affect the final quality and quantity (yield) of the *Ocimum gratissimum* essential oil.

# CHAPTER TWO
# LITERATURE REVIEW

## 2.1 INTRODUCTION

In human civilization, nature has played a vital role in the prevention and treatment of diseases. Early people instinctively used observation and trial-and-error to treat different health problems (Korkmaz *et al*., 2021; Unal *et al*., 2022). By systematically studying organisms in the natural world, humans developed methods for preventing and treating illness, which led to the identification of bioactive compounds with therapeutic potential (Mohammed *et al.,* 2021a; Bal *et al.,* 2023). The natural environment has consistently supplied vital resources for human survival, including a wide variety of medicines sourced from plants, animals, and fungi (Mohammed *et al.,* 2018; Krupodorova and Sevindik, 2020). For many developing nations, traditional medicine is still the primary and initial source of healthcare (Mohammed *et al*., 2020a).

The plant *Ocimum gratissimum*, known as "Tulsi," is highly regarded in Ayurveda as a "Golden Remedy" because of its vast healing potential (Priyanka *et al.*, 2018). It holds profound spiritual and cultural importance in India, where it's commonly grown in homes. Its leaves, both fresh and dried, are used in cooking and for treating various health issues (Priyanka *et al.,* 2018). Plants belonging to the *Ocimum* genus are rich in a variety of phytochemicals, such as tannins, phenolic acids, anthocyanins, phytosterols, and policosanols, all of which display significant bioactive properties beneficial to human health. Beyond its medical applications, *Ocimum* has substantial economic value because its essential oil and its chemical derivatives are extensively used in the food, pharmaceutical, herbal, perfumery, and flavoring sectors (Priyanka *et al.,* 2018).

Plate 1: *Ocimum gratissimum*. Source: (Adebayo *et al*., 2019)

## 2.2 Taxonomy

Table 2.1: Taxonomical classification of *Ocimum gratissimum*

| | |
|---|---|
| Scientific name | *Ocimum gratissimun L*. |
| Kingdom | Plantae |
| Division | Magnoliophyta |
| Class | Magnoliopsida |
| Order | Lamiales |
| Family | Lamialees |
| Genius | *Ocimum* |
| Species | *O. gratissimum* L. |

(Ashokkumar *et al*., 2020 and Pandian *et al*., 2021).

## 2.3 Phytochemical constituents of *Ocimum gratissimum.*

### 2.3.1 Chemical Diversity in *Ocimum gratissimum* Essential Oil

The phytochemical makeup of *Ocimum gratissimum* shows significant variation based on location and time (Joshi, 2021), which is expected given its wide presence across tropical regions. For example, harvesting the plant during different seasons has been shown to change its chemical components, with noticeable differences between summer and winter collections (Joshi, 2021). Despite this variability, some compound groups like tannins, flavonoids, and saponins are consistently present in the essential oil, regardless of where or when the plant is harvested. Conversely, the amounts of other constituents depend entirely on the specific location and harvest period used for oil extraction. Since the plant is broadly distributed in tropical climates, most published studies come from India and Nigeria. A key finding across these regions is that plant extracts from the Indian subcontinent contain a significantly higher concentration of the phenolic compound eugenol compared to those from African sources (Gilles *et al*., 2023). Eugenol, an aromatic oil with a spicy scent, is a versatile chemical used extensively in flavorings, seasonings, fragrances, antiseptics, and insecticides.

**Key Secondary Metabolites**

Tannins are polyphenolic substances known for their ability to constrict body tissues and easily combine with proteins and other large molecules. In contrast, flavonoids are common in the human diet. While they don't possess direct antioxidant activity themselves, their antioxidant properties are attributed to a process called depolymerization after ingestion, which results in the creation of uric acid (Cornell, 2014; Bhavani et al., 2019). Beyond this fu nction, flavonoids have been associated with potential anticancer effects and a reduction in both blood pressure and overall cardiovascular risk. Notable flavonoids found in *Ocimum gratissimum* include nepetrin, quercetin, and rutin (Cornell, 2014).

### Other Identified Compounds

The essential oil of *Ocimum gratissimum* also contains other important compounds, such as thymol, gratissimol, and various terpenes (Bhavani *et al.,* 2019). When using methanol for extraction, additional components like sinapic acid, rosmarinic acid, luteolin, hymenoxin, and oleanolic acid have been isolated (Venuprasad *et al.,* 2014). More recent studies have also identified compounds such as ephedrine, sparteine, lectin, resveratrol, and proanthocyanin in *Ocimum gratissimum* extracts (Idigo *et al*., 2022).

## 2.4 Ethnopharmalogical and Traditional uses

*Ocimum gratissimum* is extensively documented in ethnopharmacological research as an easily available plant used frequently to treat many different illnesses (Ajayi *et al.,* 2017a, b, c). This aromatic, perennial species is found worldwide and is well-known for its medicinal qualities.Throughout Africa, its therapeutic uses are broad and differ depending on local cultural traditions and geography (Kpoviessi *et al.,* 2014).

In Cameroon, leaf infusions are given as general tonics and pectorals, and the juice extracted from the leaves is traditionally used for symptoms like dizziness, headaches, colds, and coughs. In Côte d'Ivoire, preparations are applied for treating ear infections, skin conditions (dermatoses), and eye problems (ophthalmic conditions) (Kpoviessi *et al*., 2014). In Nigeria, it is recommended for treating diarrhea (Kpoviessi *et al*., 2014). It is also noted its use for respiratory disorders and as a treatment for intestinal worms (Sofowora,1970). Locally, it is also used for headaches, fevers, eye and skin problems, and pneumonitis. In Togo, leaf infusions are drunk for their cough-suppressing (antitussive) effects, fresh leaf juice is used against diarrhea and dysentery, and aqueous macerations are employed to treat bloody urine (haematuria) and pus-filled urethritis (Kpoviessi *et al.,* 2014). In the Benin Republic, aqueous extracts from the plant's above-ground parts are administered for conditions including dystopias, pelvic pain, colic, candidiasis, painful menstruation (dysmenorrhea), vomiting, hemorrhoids, and diarrhea. Furthermore, decoctions (boiled extracts) of the stem are used to treat hepatitis, cough, asthma, and infected wounds (Chah *et al.*, 2006; Kpoviessi *et al.*, 2014). Additionally, the leaf juice is traditionally applied for angina, headaches, fever, and malnutrition, while the plant's flower clusters (inflorescences) are utilized as flavoring agents in cooking.

### 2.4.1 Pharmacological Properties

#### Anti-inflammatory and Pain Relief

*Ocimum gratissimum* demonstrates notable anti-inflammatory capabilities, suggesting it could be a natural source for developing pain relievers similar to drugs like ibuprofen and aspirin. Extracts from the leaves are reported to ease pain and inflammation associated with conditions such as ulcer discomfort, ear infections, and fever (Amengialue *et al.*, 2013; Okoye *et al*., 2014; Elizabeth *et al*., 2019).

#### Management of Respiratory Disorders

Leaf extracts of *Ocimum gratissimum* have proven effective at regulating markers of irritation such as protein kinases, interleukins, and leukocytes/eosinophils in experimental models of airway hypersensitivity. Both lab tests (using airway epithelial cells) and animal studies (in rodents) suggest that these extracts could be beneficial in treating respiratory conditions (Jiao *et al.*, 2013).

#### Regulation of Blood Levels and Circulation

The plant contains cinnamic acid, a bioactive compound known to both improve blood circulation and help stabilize blood glucose levels. Furthermore, this compound has been linked to better respiratory function for people with breathing difficulties (Abhay *et al*., 2014).

**Gastrointestinal and Respiratory Remedies**

Historically, *Ocimum gratissimum* leaves have been used to manage various stomach and digestive issues, including general upset and gastroenteritis. Chewing the fresh leaves is reported to alleviate symptoms of the common cold and flu. Decoctions (boiled extracts) of the leaves, when prepared with ginger and honey, are used to effectively treat cough, fever, asthma, bronchitis, and influenza. Additionally, the juice extracted from the leaves is a traditional remedy for diarrhea (Okoye *et al.,* 2014).

**Antimicrobial and Antibacterial Properties**

The antimicrobial potential of *Ocimum gratissimum* makes it a valuable natural agent for both medicine and food preservation. Its active compounds can inhibit microbial growth, which allows its use in treating oral and fungal infections, including tooth decay, mouth sores, bad breath, and other related issues. Scientific evidence further shows that *Ocimum gratissimum* extracts are effective against pathogenic bacteria such as *Salmonella enteritidis, Staphylococcus aureus*, and *Escherichia coli* (Nakamura *et al*., 1999; Ojewumi *et al.,* 2021c).

**Anti-sickling Activity**

The presence of ursolic acid in *Ocimum gratissimum* provides its anti-sickling properties, which are important for managing sickle cell anaemia. This compound works by preventing the deformation of red blood cells into abnormal shapes, thereby reducing the complications associated with the disease (Tshilanda *et al.*, 2015).

**Cardioprotective Effects**

Compounds found in *Ocimum gratissimum*, particularly magnesium, support cardiovascular health by boosting blood circulation and decreasing cholesterol levels. Animal studies indicate that water extracts of the plant can alleviate heart problems related to liver fibrosis in rats (Li *et* *al.,* 2012). Furthermore, scent leaf extracts have been reported to offer protection against damage caused by cobalt-chloride exposure, including normalizing blood pressure changes and preventing heart and blood vessel abnormalities (Li *et al.,* 2012).

**Insect and Mosquito Repellent**

*Ocimum gratissimum* exhibits larvicidal properties, meaning it can disrupt insect growth during the larval stage. This activity is primarily attributed to key components such as camphor, cineole, and limonene. Traditionally, the leaves and other parts of the plant have been used as natural repellents against a variety of pests, including mosquitoes, houseflies, bedbugs, and other crawling and flying insects (Kin *et al.,* 2018; Ojewumi *et al*., 2017a; 2018a, b).

## 2.5 Microenvironment in plant science

The microenvironment is defined as the combination of localized living and non-living factors that directly affect an individual plant or organism, often creating conditions significantly different from the larger surrounding area, or macroenvironment (Anderson, 2009; Anderson *et al.,* 2009). This small-scale variation is essential because it helps structure plant communities (Ray *et al*., 2023). When plant species change local conditions such as soil moisture, nutrient availability, and the amount of light these microenvironments profoundly impact the overall species composition and diversity of a community, as well as the physical traits (phenotypic expression) of individual plants. Ultimately, these localized effects control where plants grow and how they are spatially distributed (Anderson, 2009; Anderson *et al.*, 2009; Ray *et al*., 2023).

## 2.6 CHEMOTYPE

In plant science, an essential oil chemotype describes a chemically unique variation within a plant species. These plants look alike (morphologically similar) but produce essential oils with different dominant compounds. This distinction is a result of differences in genetic makeup, environmental factors, or ecological conditions, all of which influence the makeup and quantity of secondary metabolites like terpenes and phenolics. Consequently, plants belonging to the same species can produce oils with differing chemical signatures their chemotypes which ultimately dictate their specific biological activity, aroma, and practical uses (Baser and Buchbauer, 2015; Juliani *et al*., 2008).

### 2.6.1 Essential oil and chemotype variations in plant

Essential oils are natural chemical substances synthesized by plants for vital biological roles, including acting as a defense mechanism against predators and pests, as well as helping with pollination and seed dispersal (Wink, 2018). These oils are intricate blends of volatile, fat-soluble (lipophilic) chemicals that typically weigh less than 300 daltons, giving each plant its characteristic flavor and scent (Sell, 2010). Essential oils are housed in specialized plant structures, such as glandular hairs or secretory canals, and can be found in different parts of the plant, including the leaves, flowers, seeds, roots, and bark (Baser, 2010; Rehman *et al.*, 2016). They are clear, non-water-based liquids that can be removed for commercial uses using organic solvents like ethanol or acetone (Adorjan and Buchbauer, 2010; Sell, 2010). The final aroma, flavor, and color of the oil are determined by the specific compounds they contain, such as oxygenated monoterpenes (Shakeel *et al*., 2018; Parthasarathy *et al*., 2008).

### 2.6.2 Factors influencing chemical variability and efficacy of essential oils.

**Endogenous Factors**

These are factors inherent to the plant, specifically: the part of the plant where the essential oils are produced and stored, the plant's age at the time of oil collection, and the genetic characteristics that govern how the essential oil is secreted.

## Age of the plant and the tissues generating the essential oil

The synthesis of essential oil from the plants varies as the plant and the tissue ages. Same plant organs but at different growth stages produce essential oils with different chemical compounds (Shokrpour, 2019). Several studies have reported the influence of plant age on the chemical composition of essential oils and their efficacy in pest and disease control.

## Part of the Plant Producing and Storing the Oil

Plant essential oils can be extracted from either the roots, stems, flowers, leaves or seeds. Different plant species store their essential oils in different parts. For example, plants of Lamiaceae family store their essential oils in the aerial parts while Citrus family store their oils in fruit peels and leaves (Barra, 2009).

## Exogenous Factors

Environmental factors influence the growth, yield, chemical makeup, and efficacy of essential oils. These include light, rainfall, geographical location (altitude and latitude), and soil conditions (pH and components).

## Light intensity and temperature

Research has pointed out that light is responsible for variation in chemical compounds of essential oils of similar plant species growing in environments with different light intensity. This is because light intensity influences plant metabolic activities that results to synthesis of the chemical compounds. For example, it was noted that high light intensity was responsible for increased concentrations of phenyl propanes and monoterpenes in *Ocimum basilicum* and *Satureja Douglasii* (Singh and Sharma, 2015; Machado, 2015).

**Water availability**

Water availability influences chemical composition of essential oils by influencing the secondary metabolism since biosynthetic reactions occur in an aqueous medium. In relation to this, research has shown that increase in water availability increased monoterpenes production in essential oils (Palà-Paùl *et al.*, 2001 ; Taveira *et al.*, 2003)

**Soil Type and Composition**

The soil type, pH and soil constituents influences the yield, efficacy and chemical composition of the essential oils because the plants depend on the soil for nutrients and the pH influences the nutrient absorption from the soil by the plants.

In connection to this,a research carried by (Barra,2009) showed that Rosmarinus officinalis essential oil chemical composition varied significantly in different pH of the soil in the same altitude.

**Seasonal Variations**

The yield and chemical composition of essential oils from the same plants may vary from season to season and this is influenced by variation in precipitation during the dry and the rainy season and temperature changes within seasons. It was reported that in *Salvia officinallis* essential oil, monoterpene hydrocarbons significantly increased while the oxygenated monoterpenes decreased significantly in winter (Santos-Gomes and Fernandes-Ferreira 2001). This brings about variability in efficacy between essential oils from plants grown in winter and those grown in summer.

**The method of oil extraction**

There are various methods of essential oil extraction. These include, low temperature distillation for compounds that require low temperatures and steam distillation for those that require high temperatures (Angioni,2006], Extraction with solvents and hydro-distillation (Singh *et al.* 2008) microwave assisted distillation, expression, supercritical carbon dioxide extraction, enfleurage, phytonic process and ultrasonic extraction (Okoh *et al.,* 2010; Farhat *et al*., 2010). The method of essential oil extraction determines the chemical composition hence the quality of essential oils.

### 2.7 Effects of Built-up Microenvironment on the Chemotype of *Ocimum Gratissimum*

The chemotype, which is defined as the dominant chemical profile of *Ocimum gratissimum* essential oil, is strongly affected by external environmental conditions. Specifically, the abiotic stresses found in urban and semi-urban microenvironments cause shifts in the plant's metabolic pathways responsible for synthesizing essential oils, which ultimately results in a change in the chemotype.

#### 2.7.1 Key Environmental Determinants of Chemotypic Change

Several major environmental factors associated with developed areas contribute to this diversity in chemical profile:

- Thermal Stress (Urban Heat Island Effect): The higher temperatures in urban areas significantly accelerate the plant's metabolism and activate physiological responses linked to stress. Evidence suggests this heat stress promotes the production of volatile terpenoids, directly leading to chemotypic changes in aromatic species like *Ocimum* (Chapin *et al.*, 2011; Oke, 1987).
- Light Regime Modification: Urban structures change how much light is available through processes like shading, reflection, and altering the light's color spectrum. Since light intensity is crucial for regulating the synthesis of terpenes and phenylpropanoids, *Ocimum gratissimum* grown under these varying light conditions often exhibits a chemotype distinct from plants grown in less urban, rural settings (Kumar *et al*., 2020).

- Soil and Nutrient Limitation: Developed areas often have compacted and less fertile soil. These conditions create nutrient stress, which triggers the plant's defense system to produce secondary metabolites. As a result, *Ocimum gratissimum* under this stress tends to build up protective compounds like eugenol, thymol, and carvacrol, thereby altering the essential oil's profile (Sangwan *et al.*, 2001; Pickett *et al.*, 2017).
- Water Deficit: The limited water supply and reduced humidity typical of urban microenvironments worsen the chemotypic variation. Water stress has been shown to boost the synthesis of both phenolic compounds and certain volatile oils, impacting the total yield and chemical composition of *Ocimum gratissimum* essential oil constituents (Bhattacharya *et al.,* 2008).
- Pollution Load: Exposure to atmospheric pollutants, such as heavy metals and particulate matter, causes oxidative stress. This condition stimulates the plant to accumulate antioxidant metabolites, consequently influencing both the total amount and the specific chemical composition of the essential oils (Sharma and Agrawal, 2005).

# CHAPTER THREE
# MATERIALS AND METHODS

## 3.1 Research Design

This study employed an experimental design to investigate the effect of built-up area microenvironments on the constituents of *Ocimum gratissimum* essential oil. Plant leaves were collected from three different built-up local government areas (LGAs) in Benin City, Edo State, and subjected to standardized laboratory extraction and chemical analysis.

## 3.2 Study Area

These locations were chosen based on their urbanized characteristics to assess the effect of built-up microenvironments on essential oil composition. The geographical coordinates of the sampling sites are presented in Table 3.1.

Table 3.1: Samples  Locations and Coordinates

| Local Government Area | Coordinates |
|---|---|
| Egor | 6.38165° N, 5.61369° E |
| Oredo | 6.33961° N, 5.61718° E |
| Ovia North-East | 6.43014° N, 5.61647° E |

### 3.3 Sample Collection and Preparation

Fresh leaves of *Ocimum gratissimum* were harvested from the three LGAs (Egor, Oredo, and Ovia North-East) in July. The samples were collected from built-up areas within each LGA to ensure consistency of microenvironmental influence. The leaves were air dried away in the laboratory drying room for two weeks to prevent photodegradation of volatile compounds. The dried samples were then blended into fine powder using an electrical blender. Each powdered sample was weighed using an analytical balance.

### 3.4 Extraction of Essential Oils

The Soxhlet extraction method was employed using 99% HPLC grade hexane as the solvent. The Soxhlet apparatus was assembled, and the powdered leaf samples were carefully placed inside a thimble and placed in the Soxhlet apparatus.125 ml volume of hexane was measured and poured into the round-bottom flask. The flask was then positioned in the heating mantle, which was maintained at 100 °C for 2 hours. The extraction process was monitored until sufficient oil-solvent mixture was collected in the extraction chamber. The obtained extract was carefully transferred into clean jar bottles for further processing.

### 3.5 Solvent Recovery

To remove and recover the hexane, the crude extract was subjected to a rotary evaporator. The rotary evaporator was operated at 75 °C for 45 minutes under reduced pressure conditions. After complete solvent recovery, the essential oil residues were obtained. The oil samples were then stored in amber vial bottles to prevent degradation by light or oxidation until chemical analysis was carried out.

### 3.6 Chemical Analysis of Essential Oils

#### 3.6.1 Gas Chromatography–Mass Spectrometry (GC–MS) Analysis

GC-MS 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 × 0.25μm ID × 0.25 μm df). For GC-MS detection, an electron ionization system was operated in electron impact mode with an ionization energy of 70 eV. Helium gas (99.999%) was used as a carrier gas at a constant flow rate of 1 ml/min, and an injection volume of 1μl was employed (a split ratio of 10:1).

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), Mass spectra were taken at 70 eV; a scanning interval of 0.5 s and fragments from 45 to 450 Da. The solvent delay was 0 to 3 min, and the total GC/MS running time was 21.33min.

#### 3.6.2 Fourier Transform Infrared Spectroscopy (FTIR) Analysis

FTIR spectroscopy was used to identify functional groups present in the essential oils. A small volume of oil was placed on the FTIR sample holder, and spectra were recorded across a wavelength range of 4000–400 $cm^{-1}$. Peaks were analyzed to confirm the presence of chemical bonds associated with terpenoids, phenylpropanoids, and other volatile compounds.

# CHAPTER FOUR

# RESULTS

## 4.1 Essential Oil Yield

The essential oil yields of Ocimum gratissimum leaves collected from Oredo, Egor, and Ovia North East LGAs are presented in Table 4.1.

Table 4.1: Essential oil yield of *Ocimum gratissimum* leaves from three LGAs in Edo State

| Local Government Area | Weight (g) | Oil yield |
|---|---|---|
| Oredo | 53.222 | 2.37 |
| Egor | 28.027 | 1.05 |
| Ovia North-East | 21.052 | 0.89 |

### 4.2 Essential Oil Composition (GC–MS Analysis)

The GC–MS analysis identified a wide range of volatile compounds in the essential oil, including monoterpenes, sesquiterpenes, phenylpropanoids, hydrocarbons, and oxygenated derivatives. The relative abundance of these compounds varied significantly with Egor, Ovia North East, Oredo local government area. (Tables 4.2 – 4.4). A summary table is showed in Table 4.5.

Table 4.2**:** GC-MS Profile of compounds in *Ocimum gratissimum* Essential oil from Oredo

| Retention time | Compound | Area% |
|---|---|---|
| 3.327 | Decane | 22.62 |
| 19.217 | Bis (2-ethylhexy1) phthalate | 14.97 |
| 3.682 | o-Cymene | 12.01 |
| 7.104 | Thymol | 6.32 |
| 9.267 | Naphthalene, decahydro-4a-methy1-1 -methylene-7- (1-methyletheny1)-, [ 4aR-(4a. alpha.,7. alpha.,8a.beta.)] | 6.10 |
| 4.088 | Benzene, 1-ethyl-3,5-dimethyl- | 4.27 |
| 3.150 | Benzene, 1,2,3-trimethyl- | 3.84 |
| 4.558 | Undecane | 2.56 |
| 3.184 | Cyclohexane, 1,4-dimethyl-, cis- | 2.34 |
| 8.534 | Caryophyllene | 2.28 |
| 3.739 | Cyclohexane, butyl- | 2.00 |
| 9.347 | 2-Isopropeny1-4a,8-dimethy1-1,2,3, 4,4,5,6,8a-octahydronaphthalene | 1.86 |
| 3.579 | Decane, 4-methyl- | 1.50 |
| 3.997 | Benzene, 1-methy1-3-propyl- | 1.46 |
| 4.443 | Benzene, 4-ethyl-1,2-dimethy1- | 1.33 |
| 14.731 | Phytol | 1.13 |
| 3.465 | Benzene, n-butyl- | 1.06 |
| 4.231 | Bicyclo[3.1.0]hexan-2-ol, 2-methyl -5- (1-methylethy1)-, (1.alpha.,2.a 1pha.,5.alpha.)- | 0.90 |
| 4.357 | o-Cymene | 0.88 |
| 3.774 | 1-Hexacosanol | 0.82 |
| 5.822 | Tridecane | 0.77 |
| 3.842 | (Z) -1-Phenylpropene | 0.69 |
| 7.236 | Thymol | 0.68 |
| 9.582 | (2S,4aR, 8aR)-4a,8-Dimethy1-2- (prop -1-en-2-y1) -1, 2,3,4,4a,5,6,8a-octa hydronaphthalene | 0.67 |
| 12.471 | Neophytadiene | 0.64 |
| 3.774 | Bicyclo[3.1.0]hexan-3-one, 4-methy 44 m1-1-(1-methylethy1)- | 0.60 |

| | | |
|---|---|---|
| 4.987 | trans-4a-Methy1-decahydronaphthalene | 0.60 |
| 15.630 | 3-Hydroxy-4-methoxybenzy alcohol, 255459 1000374-85-4 43 di (pentafluoropropionate) | 0.60 |
| 4.329 | Benzene, 2-ethy1-1,4-dimethy1- | 0.59 |
| 3.505 | 2-Tolyloxirane | 0.59 |
| 8.185 | Cyclohexane, 1-etheny1-1-methy1-2, 4-bis(1-methyletheny1)-, [15-(1.al Pha.,2.beta.,4.beta.)] | 0.56 |
| 10.274 | Caryophyllene oxide | 0.53 |
| 8.649 | trans-.alpha. -Bergamotene | 0.53 |
| 9.210 | 4a, 8-Dimethy1-2-(prop-1-en-2-y1) -1 ,2, 3,4,4a,5,6,7-octahydronaphthalene | 0.51 |
| 6.314 | Benzene, 2-methoxy-4-methy1-1-1-methylethy1)- | 0.48 |
| 8.901 | Humulene | 0.46 |
| 4.844 | Benzene, 1-ethyl-2,3-dimethyl- | 0.46 |

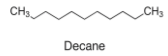
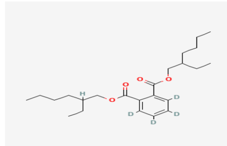
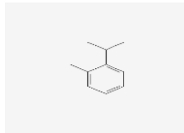
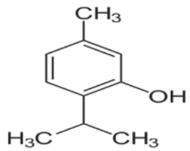
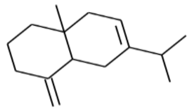
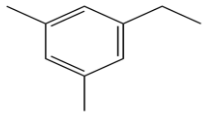
Table 4.3 GC-MS Profile of compounds in *Ocimum gratissimum* Essential oil from Egor

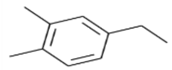
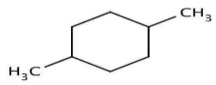
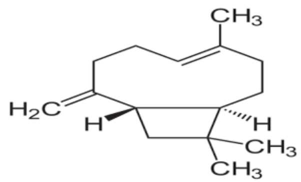
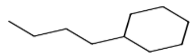
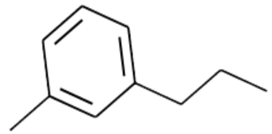
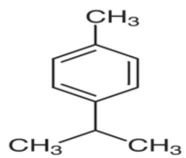
| Retention time | Compound | Area % |
|---|---|---|
| 3.333 | Decane | 26.54 |
| 19.235 | Bis(2-ethylhexy1) phthalate | 18.11 |
| 3.677 | o-Cymene | 7.40 |
| 7.104 | Thymol | 7.14 |
| 4.094 | Benzene, 1-ethy1-3,5-dimethyl- | 5.19 |
| 3.156 | Benzene, 1,2,3-trimethyl- | 4.85 |
| 4.558 | Undecane | 3.20 |
| 3.190 | Cyclohexane, 1-ethy1-1-methyl- | 2.50 |
| 9.256 | Naphthalene, | 2.04 |
| 8.529 | Caryophyllene | 1.96 |
| 3.740 | Cyclohexane, butyl- | 1.96 |
| 4.003 | Benzene, 1-methy1-3-propyl- | 1.78 |
| 3.579 | Decane, 4-methyl- | 1.77 |
| 3.637 | P-Cymene | 1.48 |
| 4.443 | Benzene, 4-ethy1-1,2-dimethyl- | 1.41 |
| 3.471 | Benzene, n-butyl- | 1.25 |
| 14.732 | Phytol | 1.08 |
| 4.335 | Benzene, 2-ethy1-1,4-dimethyl- | 0.93 |
| 3.843 | Indane | 0.88 |
| 5.822 | Dodecane | 0.88 |
| 10.280 | Caryophyllene oxide | 0.87 |
| 16.282 | Squalene | 0.80 |
| 3.774 | 3-Methy1-2-(2-oxopropy1)furan | 0.76 |
| 3.505 | : 2-Phenylpropanal | 0.75 |
| 4.987 | Naphthalene, decahydro-2-methyl- | 0.74 |
| 5.765 | Naphthalene | 0.71 |
| 9.341 | Naphthalene, 1,2,3,4,4a, 5,6,8a-oct ahydro-4a,8-dimethy1-2-(1-methylet heny1)-, [2R-(2.alpha.,4a.alpha.,8 a.beta.)]- | 0.66 |
| 7.236 | Thymol | 0.61 |
| 12.471 | Neophytadiene | 0.61 |
| 4.844 | Benzene, 1-ethy1-2,3-dimethyl- | 0.59 |
| 4.775 | Naphthalene, decahydro-2-methyl- | 0.54 |

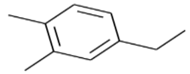
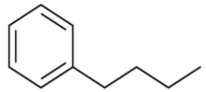
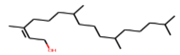
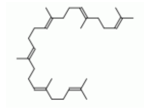
Table 4.4 GC-MS Profile of compound in *O. gratissimum* essential oil from Ovia north east.

| Retention time | Compound | Area % |
|---|---|---|
| 3.333 | Decane | 28.28 |
| 19.212 | Bis(2-ethylhexy1) phthalate | 17.39 |
| 3.676 | Benzene, 1,2-diethyl- | 7.90 |
| 4.088 | Benzene, 1-ethy1-3,5-dimethyl- | 5.34 |
| 7.104 | Thymol | 5.12 |
| 3.150 | Benzene, 1-ethy1-4-methyl- | 4.94 |
| 4.558 | Undecane | 3.26 |
| 3.190 | Cyclohexane, 1,4-dimethy1- | 2.87 |
| 3.739 | Cyclohexane, butyl- | 1.94 |
| 16.288 | Squalene | 1.92 |
| 3.997 | Benzene, 1-methy1-3-propyl- | 1.87 |
| 9.255 | Naphthalene, decahydro-4a-methy1-1 -methylene-7- (1-methyletheny1)-, [4aR-(4a. alpha.,7 .alpha.,8a.beta.)] | 1.89 |
| 3.579 | Decane, 4-methyl- | 1.82 |
| 8.529 | Caryophyllene | 1.68 |
| 3.636 | p-Cymene | 1.65 |
| 4.443 | o-Cymene | 1.44 |
| 3.465 | Benzene, n-butyl- | 1.35 |
| 4.357 | o-Cymene | 1.08 |
| 5.822 | Dodecane | 0.90 |
| 3.842 | Indane | 0.88 |
| 4.329 | Benzene, 4-ethy1-1,2-dimethyl- | 0.84 |
| 3.774 | 1-Hexacosanol | 0.82 |
| 14.731 | Phytol | 0.82 |
| 3.505 | Benzeneace-atey-e, alpha. | 0.77 |
| 4.987 | Naphthalene, decahydro-2-methyl- | 0.74 |
| 5.771 | Naphthalene | 0.70 |
| 9.341 | Naphthalene, 1,2,3,4,4a,5,6,8-oct ahydro-4a,8-dimethy1-2-(1-methylet a.beta; 28-(2. | 0.61 |
| 10.274 | Caryophyllene oxide | 0.59 |
| 4.844 | Benzene, 4-ethy1-1,2-dimethyl- | 0.58 |

Table 4.5: Summary of GC-MS Profile of Compounds in *Ocimum gratissimum* Essential Oils from Three LGA

| S/N | Compound | Oredo % | Egor % | Ovia North East % | compound class | Molecular weight | Molecular formula | Molecular structure |
|---|---|---|---|---|---|---|---|---|
| 1 | Decane | 22.62 | 26.54 | 28.28 | Alkane | 142.29 | $C_{10}$ $H_{22}$ |   |
| 2 | Bis(2-ethylhexy1) phthalate | 14.97 | 18.11 | 17.39 | Phthalate (Contaminant/Plasticizer) | 390.56 | $C_{24}H_{38}O_4$ |  |
| 3 | o-Cymene | 12.01 | 7.40 | 1.44 | Monoterpene/Aromatic | 134.22 | $C_{10}H_{14}$ |  |
| 4 | Thymol | 7.00 | 7.75 | 5.12 | Monoterpenoid/Phenol | 150.22 | $C_{10}H_{14}O$ |   |
| 5 | Naphthalene, decahydro-4a-methy1-1-methylene-7-(1-methyletheny1)-... | 6.10 | 2.04 | 1.89 | Sesquiterpene | 204.35 | $C_{15}H_{24}$ |  |
| 6 | Benzene, 1-ethyl-3,5-dimethyl- | 4.27 | 5.19 | 5.34 | Aromatic | 134.22 | $C_{10}H_{14}$ |  |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 7 | Benzene, 1,2,3-trimethyl- | 3.84 | 4.85 | 4.94 | Aromatic | 120.19 | $C_9H_{12}$ |  |
| 8 | Undecane | 2.56 | 3.20 | 3.26 | Alkane | 156.31 | $C_{11}H_{24}$ |  |
| 9 | Cyclohexane, 1,4-dimethyl-, cis- | 2.34 | —— | 2.87 | Alkane/Alicyclic | 112.21 | $C_8H_{16}$ |  |
| 10 | Caryophyllene | 2.28 | 1.96 | 1.68 | Sesquiterpene | 204.35 | $C_{15}H_{24}$ |  |
| 11 | Cyclohexane, butyl- | 2.00 | 1.96 | 1.94 | Alkane/Alicyclic | 140.27 | $C_{10}H_{20}$ |  |
| 12 | Benzene, 1-methy1-3-propyl- | 1.46 | 1.78 | 1.87 | Aromatic | 134.22 | $C_{10}H_{14}$ |  |
| 13 | Decane, 4-methyl- | 1.50 | 1.77 | 1.82 | Alkane | 156.31 | $C_{11}H_{24}$ |  |
| 14 | p-Cymene | —— | 1.48 | 1.65 | Monoterpene/Aromatic | 134.22 | $C_{10}H_{14}$ |  |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 15 | Benzene, 4-ethyl-1,2-dimethy1- | 1.33 | 1.41 | 0.84 | Aromatic | 134.22 | $C_{10}H_{14}$ |  |
| 16 | Benzene, n-butyl- | 1.06 | 1.25 | 1.35 | Aromatic | 134.22 | $C_{10}H_{14}$ |  |
| 17 | Phytol | 1.13 | 1.08 | 0.82 | Diterpene/Alcohol | 296.53 | $C_{20}H_{40}O$ |  |
| 18 | Squalene | —— | 0.80 | 1.92 | Triterpene | 410.68 | $C_{30}H_{50}$ |  |

## 4.3 FOURIER TRANSFORM INFRARED (FTIR) SPECTROSCOPY ANALYSIS

Table 4.6: Functional group spectrum in *Ocimum grastissimum* from Oredo

| Frequency | Appearance | Bond | Compound |
|---|---|---|---|
| 3440.92 | Broad Asorption brand | NH-Stretch | Amine group |
| 2963.48 | Medium Strong | CH-stretch | Alkyl groups |
| 1642.95 | Strong broad | NH-Bend | Secondary Amine (R2NH) |
| 1461.34 | Strong | CH-Bend | Alkyl group |
| 727.78 | Strong Sharp | Methylene rocking | Alkyl group |

Table 4.7: Functional group spectrum in Ocimum grastissimum from Egor

| Frequency | Appearance | Bond | Compound |
|---|---|---|---|
| 3420.79 | Strong broad | NH-Stretch | Amine group |
| 1643.86 | Medium strong | NH-Bend | Amine group |

Table 4.8: Functional group spectrum in *Ocimum grastissimum* from Ovia North East

| Frequency | Appearance | Bond | Compound |
|---|---|---|---|
| 3440.76 | Broad | NH-Stretch | Amine group |
| 3032.60 | Medium | CH-Stretch | Alkene group |
| 2967.46 | Medium Strong | CH-Stretch | Alkyl group |
| 1462.33 | Strong | CH-Bend | Alkyl group |
| 1380.00 | Strong | Aliphatic Nitrogen compound | Hetero-Oxy compound |
| 727.81 | Sharp Strong | Methylene rocking | Alkyl group |

# CHAPTER FIVE

# DISCUSSION

This chapter discuses the results of the gas chromatography-mass spectrometry (GC-MS) and Fourier-Transform infrared spectroscopy (FTIR) analysis of the essential oils extracted from *Ocimum gratissimum* leaves harvested from three different local government areas (LGAs) in Edo State: Oredo, Egor, and Ovia North-East. The LGAs represent different built-up microenvironments, and the analysis aims to determine the effect of these environments on the chemical composition (chemotype) of the essential oil.

## 5.1 Major Constituent of *Ocimum gratissimum* Eseential oil

### Decane

This is a simple hydrocarbon a straight-chain alkane made of ten carbon atoms. Although decane itself isn't very reactive, it helps give essential oil its smooth texture and volatility, making it easier for other compounds to evaporate and release aroma. In nature, it plays more of a supportive role than a medicinal one, though in industries it's used as a solvent and fuel additive.

### Bis(2-ethylhexyl) phthalate (DEHP)

This compound is an ester, often used in plastics to make them flexible. Interestingly, it's sometimes found in plant extracts either because the plant absorbed it from its environment or because of contamination from lab plastics. However, when it appears naturally, it can show mild antimicrobial and antioxidant properties, helping the plant fight microorganisms. In research, it has been associated with insecticidal and anti-inflammatory effects. In *O. gratissimum*, its presence might be partly environmental, but it could still contribute to the plant's biological defense system.

**o-Cymene**

o-Cymene is a fragrant monoterpene that gives off a sweet, woody, and slightly citrus scent. It's a close relative of p-cymene (another common aromatic compound). In the plant, o-cymene plays a key role in attracting pollinators and repelling harmful insects. For humans, it's known for anti-inflammatory, antioxidant, and antimicrobial properties. This compound often works hand in hand with thymol, strengthening its antibacterial action.

**Thymol**

Thymol is one of the main active ingredients in *O. gratissimum* oil. It's a natural phenolic compound responsible for the plant's strong, clove-like aroma. Thymol is highly valued for its antibacterial, antifungal, antiviral, and antioxidant properties. It damages the cell membranes of harmful microbes, making it a natural disinfectant. In traditional medicine, *O. gratissimum* leaves rich in thymol are used to treat coughs, skin infections, stomach pain, and respiratory issues. It's also used in toothpaste, mouthwash, and herbal antiseptics.

**Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-…**

This long chemical name refers to a sesquiterpene, most likely related to cadinene or its derivatives. These compounds have a woody, earthy aroma and are found in many aromatic plants. In nature, they help plants defend themselves from insects and pathogens, and for humans, they show antifungal, antimicrobial, and insect-repellent activities. In *O. gratissimum*, it adds a deep, warm scent and contributes to the oil's protective and healing qualities.

**Benzene, 1-ethyl-3,5-dimethyl-**

This is a simple aromatic hydrocarbon that contributes to the sweet and resinous odor of the oil.

Though it doesn't have strong biological effects on its own, it adds to the overall fragrance complexity of the essential oil and may support the evaporation and blending of other active compounds.

**Benzene, 1,2,3-trimethyl- (Hemimellitene)**

Another aromatic compound, hemimellitene gives off a light floral scent. It's naturally present in small amounts and helps create the characteristic aroma balance of the essential oil. While it isn't a medicinal component, its role in the olfactory appeal of *O. gratissimum* oil makes it valuable for cosmetic and aromatic applications.

**Undecane**

Undecane is another long-chain hydrocarbon, similar to decane but slightly heavier. It helps in stabilizing and preserving the oil's composition by maintaining its physical consistency. Although undecane doesn't directly contribute to medicinal effects, it plays a part in how the oil disperses and evaporates, influencing how long the scent lasts and how evenly it spreads.

**Cyclohexane, 1,4-dimethyl-, cis-**

This compound is a cyclic hydrocarbon with a mild, sweet odor. It is often found in essential oils as a minor volatile that balances heavier terpenes. Its main contribution is aromatic,it gives the oil a smooth, rounded scent. It may also serve a solvent-like function, helping dissolve other organic components within the oil.

**Caryophyllene (β-Caryophyllene)**

Caryophyllene is a sesquiterpene and one of the most interesting compounds found in essential oils. It has a peppery, woody aroma and is widely known for its medicinal properties.

What makes it special is that it can bind to cannabinoid (CB2) receptors in the human body — giving it natural pain-relieving and anti-inflammatory effects without causing psychoactive reactions. In *O. gratissimum*, it contributes to the therapeutic value of the oil and works alongside thymol to enhance antioxidant and antimicrobial activity.

**Cyclohexane, butyl-**

This is a simple cyclic hydrocarbon that acts mainly as a carrier compound within the oil mixture. While not biologically active, it helps in oil stability and ensures that volatile molecules evaporate at a consistent rate. It contributes subtly to the fragrance and texture of the essential oil.

**Benzene, 1-methyl-3-propyl- (Propylbenzene)**

Propylbenzene is a fragrant aromatic compound often found in small quantities in plant volatiles. It adds to the sweet and spicy scent of O. gratissimum and has been linked to mild antimicrobial and antioxidant properties. Its main role is enhancing the overall aroma complexity of the oil.

**Decane, 4-methyl-**

This is a branched alkane, a structural isomer of decane. It's mainly involved in maintaining the oil's physical stability and influencing its evaporation characteristics. Though chemically simple, it helps in balancing the consistency of the essential oil and supporting other active ingredients.

**p-Cymene**

p-Cymene is one of the key aromatic monoterpenes in *O. gratissimum.* It's closely related to thymol and is part of the same biosynthetic pathway. It gives the oil its warm, spicy aroma and shows strong antimicrobial, antioxidant, and anti-inflammatory properties. p-Cymene also enhances the effectiveness of other antimicrobial compounds, making it an important synergist. It contributes to the distinctive scent that makes African basil easily recognizable.

**Benzene, 4-ethyl-1,2-dimethyl-**

This aromatic compound helps round out the sweet, warm scent of the essential oil. While not strongly medicinal, it adds depth and persistence to the oil's aroma and may contribute to its mild antimicrobial effects.

**Benzene, n-butyl-**

n-Butylbenzene is another aromatic hydrocarbon known for its pleasant, solvent-like odor. In essential oils, it enhances the fragrance diffusion and acts as a volatile support compound. It also plays a small role in repelling insects, giving the plant a bit of natural protection.

**Phytol**

Phytol is a diterpene alcohol and one of the bioactive compounds derived from chlorophyll breakdown. It has several biological benefits.it acts as an antioxidant, antimicrobial, anti-inflammatory, and anticancer agent. It also serves as a precursor for vitamins E and K, which are vital for human health. In *O. gratissimum*, phytol contributes to the healing, anti-aging, and skin-protective properties of the oil, making it valuable in cosmetic and pharmaceutical formulations.

**Squalene**

Squalene is a triterpene hydrocarbon that's naturally found in plants, animals, and even human skin. It plays a key role in the biosynthesis of sterols, including cholesterol and hormones. In *O. gratissimum*, squalene acts as a powerful antioxidant, protecting both plant tissues and human cells from oxidative stress. It's widely used in cosmetic, pharmaceutical, and nutraceutical industries for its skin-moisturizing, anti-aging, and immune-boosting effects.

## 5.2 GC-MS COMPARATIVE ANALYSIS OF *Ocimum gratissimum* ESSENTIAL OIL FROM BUILTUP AREA IN EDO STATE AND THE REFERENCE STUDY BY COULIBALY et al., (2024) FROM BURKINA FASO.

According to Coulibaly et al. (2024), *Ocimum gratissimum* displays clear Thymol chemotype, which is a common and therapeutically significant profile. Their oil is dominated by monoterpenoids, with Thymol as the main compound (29.5%), followed by \gamma-Terpinene (20.5%) and p-Cymene (12.9%) (Coulibaly et al., 2024). The Oredo, Egor, and Ovia North East oils exhibit a different profile for *Ocimum gratissimum*. The primary compounds across all three samples are Decane (a saturated alkane) and Bis(2-ethylhexyl) phthalate (DEHP) (a phthalate ester). Thymol is present, but in a significantly lower proportion (5.12–7.14%) and is not the dominant constituent. This suggests a different chemotype, potentially influenced by environmental, genetic, or extraction factors, or a significant level of contamination. Decane and DEHP are aliphatic hydrocarbons and an industrial plasticizer/contaminant, respectively. These compounds are generally not considered the characteristic, therapeutically active major components of *Ocimum gratissimum* essential oil, which are typically phenolic monoterpenoids like Thymol or Eugenol, or other terpenoids (Coulibaly et al., 2024). Bis(2-ethylhexyl) phthalate (DEHP) is an industrial plasticizer frequently reported as a contaminant in essential oil analyses, often leaching from plastic containers or tubing used during extraction or storage. Its presence as a major compound (14.97–18.11%) strongly suggests contamination in the Oredo, Egor, and

Ovia North East samples. This significant contamination makes a direct, meaningful comparison of the "true" essential oil profile difficult. The concentration of Thymol is substantially lower in the Oredo (6.32% + 0.68% = 7.00% total), Egor (7.14% + 0.61% = 7.75% total), and Ovia North East (5.12% + 0.61% = 5.73% total) oils compared to the 29.5% reported by Coulibaly et al. (2024). This lower phenolic content would likely result in weaker antioxidant and antimicrobial properties for the Nigerian samples, as Thymol is a key compound responsible for these bioactivities.

### 5.3 Analysis of Major Constituents

The Gas Chromatography-Mass Spectrometry (GC-MS) analysis reveals that the essential oils from all three locations share a fundamentally similar composition but exhibit significant quantitative variations in their major components, defining distinct chemotypes influenced by the local environment.

#### 5.2.1 Non-Essential Oil Components

The two most abundant compounds across all samples are Decane (22.62%–28.28%) and Bis(2-ethylhexyl) phthalate (BEHP, 14.97%–18.11%).Decane is a straight-chain alkane,hydrocarbon. While alkanes can occur naturally in plant waxes and cuticles, its very high percentage suggests it may be a residue from the hexane or other non-polar solvent used during extraction, or a general non-volatile component carried over. Bis(2-ethylhexyl) phthalate (BEHP) is a plasticizer widely used in plastics. It is a common laboratory contaminant that often leaches from plastic equipment used during sample processing (e.g., tubing, stoppers). Given its high and consistent presence in all three samples, it is highly likely an artifact of the laboratory procedure rather than a natural constituent of the essential oil. Excluding these two likely non-essential oil components, the chemical profile is driven by the volatile $C_{10}$ and $C_{15}$ terpenes and phenylpropanoids.

### 5.2.2 Essential oil components

The true volatile chemotype of the *Ocimum gratissimum* essential oil is characterized by monoterpenes and phenolic monoterpenes (phenylpropanoids). Thymol (5.12%–7.14%) is a phenolic monoterpene and a key marker compound for *Ocimum gratissimum*. It is known for its strong antimicrobial and antifungal properties. Biosynthetically, it is derived from the p-menthane monoterpene pathway, which starts from Geranyl Pyrophosphate (GPP). o-Cymene (7.40%–12.01%) is a monoterpene and a biosynthetic precursor to Thymol. It is produced by the dehydrogenation of p-menthane structures. The relative levels of o-Cymene and Thymol are regulated by the activity of the enzyme c-cymene hydroxylase, which converts the former to the latter.

## 5.3 Chemotype Analysis

When focusing on the characteristic volatile components, the essential oil from all three locations exhibits a thymol/aromatic hydrocarbon chemotype. The key aromatic/terpenoid components in Oredo is o-Cymene (12.89%), Thymol (7.00%), and a significant Sesquiterpene (Naphthalene derivative) (6.10%), while Egor has Thymol (7.75%), o-Cymene (7.40%), and Aromatic Hydrocarbons (Benzene derivatives) and Ovia North-East has Thymol (5.12%), and higher levels of Aromatic Hydrocarbons (Benzene derivatives) (10%).

Despite the general dominance of non-metabolites, the Ocimum gratissimum oil from all three LGAs belongs to a Thymol-Rich Chemotype, although at lower-than-expected concentrations due to the high percentage of contaminants.

## 5.4 Effect of Built-up Area on Biosynthesis

The built-up nature of the collection sites is a key environmental variable that may be responsible for these chemotype shifts. The degree of urbanization and built-up area is often correlated with increased levels of abiotic stress, such as air pollution, heat island effects, soil contamination, and water stress.

### 5.4.1 Phenylpropanoid/Monoterpene Shift

The higher o-Cymene to Thymol ratio in the Oredo and Ovia North East samples compared to Egor suggests an inhibition or downregulation of the c-cymene hydroxylase enzyme in these environments. In stressed environments, the plant's metabolic resources may be diverted to other defense compounds or maintenance mechanisms, or the stress itself (e.g., pollutants, intense sunlight) may directly inhibit the final enzymatic step converting o-Cymene to Thymol. The accumulation of the precursor, o-Cymene, is a common result of such metabolic disruptions. Egor sample has lower o-Cymene to Thymol ratio, represent a site where the biosynthetic pathway to the pharmacologically active Thymol is more efficient or less inhibited by environmental pressures.

### 5.4.2 Presence of Squalene and Terpenoids

Squalene, a $C_{30}$ triterpene precursor to sterols, is highest in Ovia North East (1.92%) and present in Egor (0.80%), but not a major component in Oredo. The presence of Squalene is typically associated with general plant defense, membrane stability, and growth. Its higher concentration in the less stressed environment (Ovia North East) suggests that in the highly built-up areas (Oredo), the plant may downregulate triterpene synthesis, diverting resources primarily into the volatile C10 monoterpene pathway as a more immediate defense against local stressors (e.g., pests, microbes, heat).The high abundance of various alkyl-substituted benzenes (e.g., Benzene, 1-ethyl-3,5-dimethyl-; Benzene, 1,2,3-trimethyl-) and naphthalene derivatives (e.g., Naphthalene, decahydro-4a-methyl-1-methylene...) across all samples confirms the complex nature of the secondary metabolite profile, which is common in *Ocimum gratissimum*.

### 5.5 Effect of Built-up Microenvironment (LGA Comparison)

The comparison of the three LGAs suggests a potential, though subtle, effect of the microenvironment on the volatile profile. The essential oil harvested from Oredo is characterized by the highest concentration of o-Cymene and overall monoterpenes. This might suggest a stress response (e.g., pollution) leading to the accumulation of biosynthetic intermediates or a different ratio in the monoterpene synthesis pathway. Essential oil from Egor has the highest level of the desired active compound, Thymol, suggesting an environment conducive to the final step of monoterpene biosynthesis. Ovia North-East essential oil shows the lowest concentration of Thymol and o-Cymene, and a relatively high percentage of simple aromatic hydrocarbons (Benzene, 1-ethyl-3,5-dimethyl-, etc.) compared to the other two. The differences in the relative percentages of Thymol and its precursor, o-Cymene, across the LGAs suggest that the built-up microenvironment likely impacts the biosynthetic pathway of the essential oil compounds, thereby influencing the final chemotype.

### 5.6 FTIR COMPARATIVE ANALYSIS OF *Ocimum gratissimum* IN EDO STATE AND THE REFERENCE STUDY OF Raju *et al., (*2017).

FTIR spectroscopic analysis results from the samples, collected from Oredo, Egor, and Ovia North East, demonstrate a clear phytochemical difference when compared to the findings of Raju *et al.(*2017). Both sets of results confirm the fundamental organic composition of *Ocimum gratissimum* leaves, specifically the common presence of Alkyl groups (hydrocarbons). The C-H stretching and bending vibrations are consistent across both studies. Oredo and Ovia North East samples exhibited C-H stretching bands around 2963-2967cm$^{-1}$ and bending bands at 1461-1462cm$^{-1}$, which supports the Alkyl C-H stretching peak reported by Raju *et al*. (2017) at 2852cm$^{-1}$. This suggests that the basic lipid and volatile oil (terpene backbone) structures are preserved in the samples, regardless of the geographic source (Raju *et al.*, 2017).The most significant difference lies in the major functional groups identified, which points to a distinct chemotype variation on the samples compared to the material analyzed by Raju *et al*. (2017).

### 5.7 FTIR Confirmation of Functional Groups

The FTIR analysis provides complementary evidence for the presence of the compound classes identified by GC-MS.

Alkyl/Alkene Groups: The strong C-H stretches 2960cm$^{-1}$ and bending vibrations 1460cm$^{-1}$, 727cm$^{-1}$ methylene rocking a reconsistent with the high levels of alkanes (Decane, Undecane, etc.) and various terpenes (Caryophyllene, Squalene etc.) found in the oils.

Amine Groups: The presence of N-H stretch 3440cm$^{-1}$ and N-H bend 1643cm$^{-1}$ confirms the presence of nitrogen-containing compounds (amines/alkaloids) in all three oils, which are often minor but important components of plant extracts.

Aromatic/Phenolic Compounds: The GC-MS data shows significant amounts of o-Cymene and Thymol (both aromatic rings). While the specific C=C stretches for aromatic rings are less distinct due to the complex matrix, the high amount of alkyl-substituted benzenes confirms the dominance of aromatic structures.

## CONCLUSION

The essential oil of *Ocimum gratissimum* harvested from the three LGAs in Edo State possesses a Thymol-rich chemotype, but its medicinal potential is severely compromised by both significant laboratory contamination (DEHP) and a natural reduction in the key active component, Thymol, compared to international standards. The observed quantitative variations in the Thymol/o-Cymene ratio across the LGAs suggest that the built-up microenvironment is an influential factor in regulating the plant's secondary metabolite biosynthesis, potentially leading to a stress-induced accumulation of biosynthetic precursors. These findings underscore the need for strict quality control in essential oil analysis and highlight the sensitivity of *Ocimum gratissimum* chemotype to localized environmental stress.

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# APPENDIX I

## Materials

1. Fresh leaves of *Ocimum gratissimum*
2. Hexane (99% HPLC grade)
3. Amber vial bottles
4. Jar bottles

## Equipment

1. Analytical weighing balance
2. Electrical oven
3. Electrical blender
4. Soxhlet apparatus
5. Rotary evaporator
6. Gas Chromatography–Mass Spectrometry (GC–MS) machine
7. Fourier Transform Infrared Spectroscopy (FTIR) machine.

**APPENDIX II**

Plate 2: Grinding of *O. gratisimum* using Electric blender           Photo credit: Iyahen Blessing


Plate 3**:** Soxhlet apparatus setup                                    Photo credit: Iyahen Blessing

Plate 4: Rotary Evaporator Photo credit: Iyahen Blessing