

MULTIVARIATE CHEMOMETRIC CLASSIFICATION OF *Ocimum gratissimum*
ESSENTIAL OILS FROM GRASSLANDS IN OREDO, EGOR, AND OVIA NORTH-
EAST.



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FACULTY OF LIFE SCIENCES

UNIVERSITY OF BENIN

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NOVEMBER, 2025.

CERTIFICATION

This is to certify that this research titled "MULTIVARIATE CHEMOMETRIC CLASSIFICATION OF *Ocimum gratissimum* ESSENTIAL OILS FROM GRASSLANDS IN OREDO, EGOR, AND OVIA NORTH-EAST." was carried out by "Prince David FRIDAY" with matriculation number "LSC2009983" 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 and 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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DEDICATION

I dedicate this success story to God Almighty, for his love, grace, strength, wisdom and protection upon my life.

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ABSTRACT

This research focuses on the Multivariate Chemometric Classification of *Ocimum gratissimum* essential oils obtained from grasslands within three urban local government areas (LGAs) of Benin City Oredo, Egor, and Ovia north-east. The study aimed to determine the chemical variability and structural functional groups of *O. gratissimum* grown in different grassland environments and to understand how local environmental conditions influence the chemical profiles of the plant's essential oils. The work involved plant collection, sample preparation, extraction of essential oils, analytical identification, and data interpretation. Fresh leaves of *O. gratissimum* were collected from uncontaminated bushy sites in the three LGAs, air dried for five days, oven-dried at 45°C for 30 minutes, blended, and stored in airtight brown bottles. Soxhlet extraction using analytical-grade n-hexane served to obtain the essential oils, which were then concentrated using a rotary evaporator. The extracts were analyzed using Gas Chromatography Mass Spectrometry (GCMS) and Fourier Transform Infrared Spectroscopy (FTIR). GCMS provided molecular identification and quantification of chemical compounds, while FTIR determined the functional groups present in the essential oils. Chemometric methods were employed to classify similarities and differences in the oils based on their chemical fingerprints across the study areas. A total of over forty compounds were detected across the three samples, with the most dominant being Decane (25.04%), Bis(2-ethylhexyl) phthalate (17.17%), and Thymol (12.65%), along with other bioactive components such as Caryophyllene, Squalene, Phytol, and several aromatic and aliphatic hydrocarbons. These compounds are known for their antimicrobial, anti-inflammatory, and antioxidant properties, indicating that *O. gratissimum* from all study sites retains medicinal relevance. Variations in compound concentrations were observed between locations, suggesting environmental or soil-based influences on secondary metabolite biosynthesis. The FTIR results revealed prominent absorption peaks around 3350 cm^{-1} (O-H), 2950 cm^{-1} (C-H), and 1740 cm^{-1} (C=O), indicating the presence of alcohols, alkanes, and esters common to essential oils of *Ocimum* species. The results demonstrated that although the three *O. gratissimum* populations share core chemical identities, their quantitative differences are significant enough to allow chemometric differentiation. Oredo exhibited a higher proportion of

hydrocarbons, while Egor and Ovia north-east contained more oxygenated monoterpenes and sesquiterpenes. These findings emphasize how microenvironmental variations in grassland ecosystems affect essential oil yield and composition. The work concludes that *O. gratissimum* leaves from urban bush environments maintain strong phytochemical integrity suitable for pharmacological and industrial applications, though varying environmental influences may alter their efficacy.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF STUDY

The genus *Ocimum* comprises more than 150 aromatic species within the Lamiaceae family, commonly referred to as basil or scent leaf, and is widely distributed across tropical and subtropical regions of the world. Among these, *Ocimum gratissimum* L. is one of the most economically and medicinally valuable species, particularly in Africa and Asia, where it is cultivated for culinary, medicinal, and industrial purposes (Ajayi and Ojo, 2023). *O. gratissimum* is popularly known as scent leaf and is used in food preparation and traditional medicine for the treatment of cough, malaria, diarrhoea, and skin infections due to its abundance of volatile essential oils and phenolic compounds (Ojewumi et al., 2024). Essential oils obtained from *O. gratissimum* are complex mixtures of bioactive compounds such as eugenol, thymol, linalool, 1,8-cineole, and β -caryophyllene. These compounds have been associated with antimicrobial, antioxidant, insecticidal, and anti-inflammatory activities (Adeniyi and Salako, 2022). The quality and composition of these oils, however, vary considerably depending on environmental, genetic, and agronomic factors such as soil type, temperature, rainfall, and geographic origin (Mary and Odu, 2024). Such differences often result in distinct chemotypes that determine the biological potency and industrial suitability of the oil (Ekwueme and Osondu, 2023). In the field of natural product chemistry, Gas Chromatography Mass Spectrometry (GCMS) has become the most reliable analytical tool for identifying volatile constituents in essential oils. It provides detailed qualitative and quantitative

data on the chemical composition of Ocimum oils and their relative abundance (Lawal and Adetunji, 2023). However, the complexity of the GCMS output, which typically consists of hundreds of variables per sample, poses challenges for direct interpretation. For this reason, researchers now employ chemometric techniques such as Principal Component Analysis (PCA), Hierarchical Cluster Analysis (HCA), and Partial Least Squares Discriminant Analysis (PLS-DA) to extract meaningful classification patterns from GCMS data (Okeke and Odo, 2022). Similarly, Fourier Transform Infrared Spectroscopy (FTIR) is increasingly applied for the rapid fingerprinting of essential oils because it captures broad vibrational information on functional groups such as hydroxyl, carbonyl, and phenolic compounds. FTIR provides an overall biochemical profile that can be analysed alongside GCMS data for improved accuracy in classification (Uchegbu and Onwuka, 2022). When integrated, GCMS and FTIR offer complementary insights GCMS identifies individual compounds while FTIR reveals the functional group patterns that define the general structure of the oil.

Recent advances in multivariate chemometrics have demonstrated that combining GCMS and FTIR data produces a more robust basis for classifying and authenticating essential oils than using either method alone (Olawale and Akinsola, 2024). For example, the application of PCA and PLS-DA to combined spectral data successfully differentiated Ocimum oils obtained from distinct agro-ecological zones in Nigeria, linking compositional variation to specific environmental factors such as humidity and altitude (Ekwueme and Osondu, 2023). This integrative approach is now considered essential for quality control, traceability, and standardization in the essential oil industry

(Abiodun and Olayinka, 2021). Environmental variation is a key driver of phytochemical diversity. Studies have shown that plants growing on different land types such as cultivated fields, gardens, or uncultivated bushy areas can exhibit marked differences in essential oil composition due to soil nutrients, light intensity, and microbial interactions (Igbinosa and Igbinosa, 2021). In Benin City, *O. gratissimum* grows abundantly on grasslands across Oredo, Egor, and Ovia north-east Local Government Areas, which are characterized by high rainfall, humidity, and tropical temperatures. These conditions are known to influence secondary metabolite synthesis and could lead to the development of distinct chemotypes within the same species. Despite this, few studies have explored the chemical differentiation of *O. gratissimum* from these specific localities using multivariate chemometric tools. The classification of essential oils from these locations will provide valuable insight into the intraspecific variation of *O. gratissimum* and its adaptation to different ecological settings. It will also contribute to the development of reliable markers for identifying high-quality oils suitable for pharmaceutical, cosmetic, and food industries. Furthermore, chemometric classification can assist in the creation of predictive models capable of identifying oils from unknown origins, thereby enhancing quality assurance and preventing adulteration in commercial products (Ajayi and Ojo, 2023). Therefore, this study focuses on the multivariate chemometric classification of *Ocimum gratissimum* essential oils from grasslands (bushes) in Oredo, Egor, and Ovia north-east using combined GCMS and FTIR datasets. By comparing oils extracted from these localities, the research aims to determine whether compositional variation correlates with collection sites, to identify marker compounds responsible for discrimination, and to evaluate the reliability of integrated chemometric modeling as a

classification tool. The outcomes will advance scientific understanding of environmental influences on scent leaf chemistry while supporting the standardization and commercialization of Nigerian *Ocimum* essential oils.

1.2 Aim of the Study

The aim of this project is to determine whether the geographical location where *Ocimum gratissimum* (scent leaf) grows in the wild influences the chemical composition of its essential oil. This will be investigated by collecting plants from uncultivated (bushy) areas in Oredo, Egor, and Ovia north-east, extracting their essential oils, analyzing them using GCMS and FTIR techniques and comparing the chemical profiles from the three locations.

1.3 Objectives of the Study

The specific objectives of this study are to:

1. extract and characterize essential oils from *Ocimum gratissimum* collected from uncultivated (bushy) lands across Oredo, Egor, and Ovia north-east LGAs of Edo State.
2. identify and quantify the chemical constituents of the extracted oils using Gas Chromatography Mass Spectrometry (GCMS).
3. determine the functional group composition of the oils using fourier transform infrared spectroscopy (FTIR).

4. compare and relate the GCMS and FTIR data to develop a comprehensive profile of the essential oils.

5. observe any natural variation in oil composition that may occur due to environmental or locational influences during collection.

CHAPTER TWO

LITERATURE REVIEW

2.1 Essential oils

Essential oils are naturally occurring volatile aromatic compounds found in various parts of plants such as leaves, flowers, stems, seeds, and roots. They are secondary metabolites that contribute to the distinctive aroma of plants and serve ecological functions including defense against pathogens, attraction of pollinators, and protection from environmental stress. In recent years, essential oils have gained increasing scientific and industrial interest due to their wide range of therapeutic and preservative properties, which make them valuable in pharmaceutical, cosmetic, and food applications (Adeniyi and Salako, 2022). The global use of essential oils has expanded as industries continue to seek natural alternatives to synthetic additives and antibiotics. Their antimicrobial, antioxidant, anti-inflammatory, and insecticidal properties have been documented across various plant species, including *Ocimum gratissimum* (Ajayi and Ojo, 2023). The growing demand for natural products has also encouraged research into the chemical characterization, standardization, and classification of essential oils from both cultivated and wild plant populations (Ekwueme and Osondu, 2023).

Ocimum gratissimum L., commonly referred to as scent leaf or clove basil, is an aromatic herb widely distributed across tropical Africa and Asia. It belongs to the family Lamiaceae and is among the most important species within the *Ocimum* genus due to its high essential oil content and diverse bioactivities (Mary and Odu, 2024). In Nigeria, it is frequently used as a culinary spice and as a traditional medicine for ailments such as cough, fever, diarrhea, and skin infections (Akinmoladun and Lawal, 2022). These pharmacological and aromatic properties are mainly attributed to its essential oils, which are rich in compounds such as eugenol, thymol, linalool, and β -caryophyllene (Adeniyi and Salako, 2022). The composition and concentration of *O. gratissimum* essential oils are, however, influenced by numerous environmental and agronomic factors including soil type, humidity, temperature, and the growth location of the plant (Igbinosa and Igbinosa, 2021). To accurately identify and quantify the chemical constituents of essential oils, modern analytical tools such as Gas Chromatography Mass Spectrometry (GCMS) and Fourier Transform Infrared Spectroscopy (FTIR) are used. GCMS provides detailed information about the individual volatile compounds, while FTIR reveals the major functional groups present in the oil, offering a comprehensive chemical fingerprint (Lawal and Adetunji, 2023). The combined use of both techniques provides an integrated approach for essential oil characterization.

2.2.1 Taxonomy and Classification

Ocimum gratissimum is a member of the family Lamiaceae, which consists of numerous aromatic herbs and shrubs notable for their essential oils. The genus

Ocimum comprises over 150 species distributed throughout tropical and subtropical regions. Taxonomically, the plant is classified as follows (Ajayi and Ojo, 2023):

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Lamiales

Family: Lamiaceae

Genus: Ocimum

Species: Ocimum gratissimum

The plant is commonly known as scent leaf or clove basil in English. In Nigeria, it bears several indigenous names such as Efirin (Yoruba), Nchuanwu (Igbo), and Daidoya (Hausa). The species is closely related to other members of the genus such as *O. Basilicum* (sweet basil) and *O. Americanum* (hoary basil), but differs in its higher eugenol content and stronger clove-like aroma (Ekwueme and Osondu, 2023).

2.2.2 Botanical Description

The leaves grow opposite each other, are broad and slightly rough, and have a strong smell when crushed. This smell comes from the oil glands found on the leaf surface.

The plant produces small flowers, usually white or light purple, which appear at the top of the stems. After flowering, small brown seeds are formed. The roots spread easily in the soil, helping the plant grow well in open, bushy areas or along roadsides. Generally, *O. gratissimum* grows easily in tropical environments. Its leaves are thick and contain essential oils that help the plant survive dry weather and resist pests. This simple

structure and toughness make it one of the most common and useful herbs in many parts of Nigeria and other tropical regions.

2.2.3 Other Medicinal Importance

Ocimum gratissimum serves both nutritional and medicinal purposes. The fresh leaves are widely used as a spice in soups and stews because of their pleasant aroma and flavor. Pharmacologically, the plant's essential oil has demonstrated antimicrobial, antioxidant, anti-inflammatory, antimalarial, and insecticidal properties (Adeniyi and Salako, 2022). The major active compounds such as eugenol, thymol, linalool, and β -caryophyllene have been linked to these biological activities (Lawal and Adetunji, 2023). In traditional medicine, decoctions from the leaves are employed for managing respiratory infections, fevers, digestive disorders, and skin irritations (Akinmoladun and Lawal, 2022). Modern pharmaceutical and cosmetic industries also utilize its essential oils in the manufacture of antiseptic lotions, perfumes, and herbal mouthwashes. Economically, the cultivation and trade of *O. gratissimum* contribute to household income in rural communities and have potential for industrial development within Nigeria's emerging herbal-product market (Mary and Odu, 2024).

2.3 Phytochemical and Essential Oil Composition of *Ocimum gratissimum*

2.3.1 Phytochemical Constituents

Plants of the *Ocimum* genus are known to contain diverse classes of secondary metabolites, which play important roles in plant defense and contribute to their

biological activities. The principal groups of phytochemicals reported in *O. gratissimum* include terpenoids, flavonoids, phenolic compounds, alkaloids, saponins, and tannins (Akinmoladun and Lawal, 2022). These compounds possess strong pharmacological actions such as antimicrobial, antioxidant, and anti-inflammatory effects that justify the traditional uses of the plant (Adeniyi and Salako, 2022). Terpenoids, especially monoterpenes and sesquiterpenes, represent the largest fraction of *O. gratissimum* metabolites. They are responsible for the distinctive aroma and are directly linked to the plant's essential-oil yield (Ekwueme and Osondu, 2023). Flavonoids and phenolic acids contribute to free-radical scavenging capacity, while alkaloids and saponins have been associated with antimicrobial and cytoprotective properties (Igbinosa and Igbinosa, 2021).

2.3.2 Essential Oil Composition

The essential oil of *O. gratissimum* is mainly localized in glandular trichomes on the leaf surface. Hydrodistillation or steam distillation typically yields between 0.5 and 1.2 % oil per fresh weight of leaves (Mary and Odu, 2024). The oil is colorless to pale yellow with a characteristic clove-like fragrance due to its high eugenol content (Ajayi and Ojo, 2023). Other major components commonly reported include thymol, linalool, γ -terpinene, β -caryophyllene, and p-cymene (Lawal and Adetunji, 2023). The relative proportions of these compounds vary considerably depending on environmental factors, extraction conditions, and plant age. For instance, eugenol may range from 50 – 70 % in oils from southern Nigeria, whereas thymol-type chemotypes are more frequent in

northern regions (Adeniyi and Salako, 2022). Such variation suggests that *O. gratissimum* exists in multiple chemotypes, each with distinct chemical fingerprints (Ekwueme and Osondu, 2023).

2.3.3 Factors Affecting Essential Oil Composition

The quality and yield of *O. gratissimum* essential oils are influenced by a combination of environmental, biological, and technical factors. Environmental parameters such as soil type, temperature, humidity, and rainfall directly affect the biosynthesis of volatile compounds (Igbinosa and Igbinosa, 2021). Plants grown in nutrient-rich loamy soils tend to produce higher oil yields, while those on sandy or lateritic soils may yield oils with lower eugenol content (Adeniyi and Salako, 2022). Harvest time and plant maturity also play critical roles. Oil concentration is usually highest during the flowering stage when biosynthetic activity peaks (Akinmoladun and Lawal, 2022). Post-harvest handling and extraction temperature can further modify the volatile profile of the oil (Ajayi and Ojo, 2023). In addition, geographic and microclimatic differences among locations such as Oredo, Egor, and Ovia north-east may lead to distinct oil signatures that reflect environmental adaptation (Mary and Odu, 2024).

2.4 Environmental and Land-Related Influence on Essential Oils

2.4.1 Overview of Environmental Factors Affecting Essential Oils

The composition of essential oils in plants such as *Ocimum gratissimum* is highly dynamic and influenced by a range of environmental factors, including temperature,

light intensity, soil nutrients, water availability, and altitude (Igbinosa and Igbinosa, 2021). These environmental parameters affect both the biosynthetic pathways and the relative abundance of volatile compounds produced within the glandular trichomes of the leaves (Ajayi and Ojo, 2023). For example, higher temperatures and sunlight intensity have been reported to increase oil yield and enhance the production of monoterpenes such as linalool and eugenol, while cooler or shaded conditions may favor oxygenated sesquiterpenes (Adeniyi and Salako, 2022). Soil mineral composition, particularly nitrogen and phosphorus levels, also influences enzymatic activity linked to essential oil biosynthesis. Similarly, water stress conditions can alter secondary metabolism, sometimes leading to the accumulation of specific aroma compounds that act as stress protectants (Lawal and Adetunji, 2023).

2.4.2 Land Use and Soil Type as Determinants of Oil Composition

The type of land on which *O. gratissimum* grows whether cultivated or uncultivated plays a crucial role in determining oil yield and chemical profile. Plants growing on uncultivated (bushy or fallow) lands are often subject to natural ecological stressors such as competition, irregular watering, and varying nutrient conditions, which stimulate enhanced secondary metabolite production (Ekwueme and Osondu, 2023). Such conditions often result in higher concentrations of phenolic and terpenoid compounds, as these metabolites function in plant defense and environmental adaptation. On the other hand, plants from cultivated or fertilized soils may exhibit reduced diversity of volatile constituents but greater biomass yield (Akinmoladun and Lawal, 2022). The

soils found in Oredo, Egor, and Ovia north-east are characteristically loamy to sandy-loam, moderately acidic, and rich in decomposed organic matter due to natural vegetation cover. These soil conditions promote the accumulation of volatile oils and enhance enzymatic activity in the leaves (Adeniyi and Salako, 2022).

2.4.3 Geographic Influence and Microclimatic Variation

The geographic location of a plant population determines the microclimatic conditions such as rainfall pattern, elevation, and exposure that directly influence volatile compound biosynthesis. In Edo State, *O. gratissimum* grows spontaneously across diverse ecological zones. In Oredo, for instance, vegetation is semi-urban, and plants are exposed to moderate air pollution and human activity, which can slightly affect oil quality and yield. Egor represents a transitional environment between urban and agricultural zones, where soil fertility varies significantly. Ovia north-east, by contrast, is dominated by more rural and natural vegetation, often providing more stable conditions for secondary metabolite production (Mary and Odu, 2024). The comparison among these areas allows for the observation of how local environmental variability impacts essential oil chemotypes. Similar studies on aromatic herbs have shown that microclimatic variations within short geographic distances can result in distinctive chemical fingerprints, even among plants of the same species (Lawal and Adetunji, 2023).

2.4.4 Seasonal and Climatic Influence

Seasonal variation is another key determinant of oil quality. The rainy season generally favors vigorous growth and higher leaf moisture, but the essential oil yield may be diluted due to higher water content. Conversely, the dry season enhances oil concentration and strengthens aromatic intensity (Ajayi and Ojo, 2023). Temperature and relative humidity also influence the volatilization and storage of aromatic compounds. During the dry season, elevated heat promotes the biosynthesis of terpenes, while cooler wet-season conditions favor oxygenated derivatives such as alcohols and esters (Igbinosa and Igbinosa, 2021). Therefore, sampling *O. gratissimum* from uncultivated lands across different localities provides not only chemical diversity but also insight into how natural environmental stressors shape the biochemical expression of essential oils in tropical ecosystems (Ekwueme and Osondu, 2023).

2.5 Extraction and Analytical Methods for Essential oil

2.5.1 Overview of Essential Oil Extraction

The extraction of essential oils from plant materials is a critical step that determines both yield and chemical integrity. Several methods are available, including steam distillation, solvent extraction, cold pressing, and hydrodistillation. Among these, hydrodistillation remains the most widely used laboratory technique for aromatic herbs such as *Ocimum gratissimum* due to its efficiency, simplicity, and ability to preserve volatile constituents (Ajayi and Ojo, 2023). During hydrodistillation, the plant material (usually fresh or semi-dried leaves) is submerged in water and subjected to gentle heating. The generated steam carries volatile compounds through a condenser where

they are liquefied and collected as an oil–water mixture. The essential oil is then separated from the aqueous layer, dried over anhydrous sodium sulfate, and stored in airtight vials under refrigeration to prevent oxidation (Adeniyi and Salako, 2022).

2.5.2 Gas Chromatography Mass Spectrometry (GCMS) Analysis

Gas Chromatography Mass Spectrometry (GCMS) is an advanced analytical tool that separates, detects, and identifies volatile compounds within complex mixtures such as essential oils. The gas chromatograph separates components based on volatility and interaction with the stationary phase, while the mass spectrometer identifies each component by its molecular mass and fragmentation pattern (Lawal and Adetunji, 2023). In this method, a small volume of the oil sample usually diluted in an organic solvent such as n-hexane is injected into the GC column. As the sample vaporizes, compounds migrate through the column at different rates and emerge sequentially into the mass spectrometer, which generates a unique mass spectrum for each component (Ekwueme and Osondu, 2023). The resulting chromatogram provides retention times and peak intensities representing each compound. Identification is achieved by comparing mass spectra with reference databases such as NIST (National Institute of Standards and Technology). Quantification is based on relative peak areas, offering insight into the percentage composition of each compound within the oil (Adeniyi and Salako, 2022).

2.5.3 Fourier Transform Infrared Spectroscopy (FTIR) Analysis

Fourier Transform Infrared Spectroscopy (FTIR) is another vital technique used to determine the functional groups present in essential oils. It measures the absorption of infrared radiation by chemical bonds at specific frequencies, producing a characteristic spectrum that serves as a “molecular signature” of the sample (Ajayi and Ojo, 2023). For analysis, a thin film of essential oil is placed on an attenuated total reflectance (ATR) crystal, and infrared light is passed through it. The resulting absorbance peaks correspond to different vibrational modes of molecular bonds such as O–H, C=O, C–H, and C=C which help identify alcohols, aldehydes, phenols, and terpenoids (Lawal and Adetunji, 2023). The FTIR method complements GCMS by providing structural and qualitative information about the compounds without the need for extensive sample preparation. Together, both techniques yield a comprehensive chemical profile that enables accurate classification and quality assessment of *O. gratissimum* essential oils (Ekwueme and Osondu, 2023).

2.5.4 Combined Application of GCMS and FTIR Data

The integration of GCMS and FTIR results provides a more holistic understanding of the chemical nature of essential oils. While GCMS offers compound-specific information and quantitative composition, FTIR validates functional group presence and can detect overlapping or trace constituents missed by GCMS alone (Akinmoladun and Lawal, 2022). Combining both datasets enables researchers to generate robust chemical fingerprints that are valuable for distinguishing samples collected from different ecological or land-use zones. Such integrated analytical approaches enhance the

reliability of classification studies and can reveal subtle variations in oils from *O. gratissimum* populations growing under different environmental conditions in Oredo, Egor, and Ovia north-east (Mary and Odu, 2024).

2.6 Significance of *Ocimum gratissimum* Essential Oils

2.6.1 Pharmacological Activities

Essential oils from *Ocimum gratissimum* possess a wide spectrum of biological activities due to their rich content of bioactive compounds such as eugenol, thymol, linalool, and β -caryophyllene. These constituents exhibit strong antimicrobial, antioxidant, anti-inflammatory, and insecticidal properties that have been demonstrated through various *in vitro* and *in vivo* studies (Akinmoladun and Lawal, 2022). The antimicrobial activity of *O. gratissimum* essential oil has been reported against a range of pathogenic bacteria, including *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. The phenolic compound eugenol disrupts microbial cell membranes and inhibits enzyme function, leading to cell death (Adeniyi and Salako, 2022). Similarly, thymol and linalool have shown inhibitory effects on fungal strains such as *Candida albicans* and *Aspergillus niger*, supporting the traditional use of the plant in treating infections (Mary and Odu, 2024). The antioxidant activity of the oil is attributed to its ability to neutralize free radicals and prevent oxidative stress. This property contributes to the plant's use in the management of inflammatory conditions and chronic diseases (Ajayi and Ojo, 2023). Furthermore, β -caryophyllene has been identified as an anti-inflammatory agent that modulates cytokine expression and reduces tissue damage caused by oxidative processes (Lawal and Adetunji, 2023).

2.6.2 Medicinal and Pharmaceutical Applications

The traditional medicinal use of *O. gratissimum* across Africa and Asia is well-documented. Decoctions or infusions of its leaves are employed for the treatment of malaria, cough, fever, diarrhea, and skin infections (Akinmoladun and Lawal, 2022). The essential oil has also been incorporated into topical formulations for wound healing and oral hygiene due to its antiseptic properties (Adeniyi and Salako, 2022). In modern pharmaceutical applications, *O. gratissimum* essential oil serves as an ingredient in antiseptic lotions, mouthwashes, ointments, and herbal balms. Recent studies have explored its potential as a natural preservative in pharmaceutical and food formulations, given its ability to inhibit microbial growth and oxidative spoilage (Ekwueme and Osondu, 2023).

2.6.3 Cosmetic and Food Industry Applications

Beyond its medicinal importance, *O. gratissimum* essential oil plays a vital role in the cosmetic and food industries. Its aromatic quality and antimicrobial effects make it a valuable additive in soaps, perfumes, and deodorants (Mary and Odu, 2024). In the food industry, the oil is utilized as a natural flavoring and preservative in sauces, beverages, and meat products. The presence of eugenol and thymol not only enhances flavor but also extends shelf life by suppressing microbial growth (Ajayi and Ojo, 2023). Natural product industries have increasingly turned to *O. gratissimum* oil as a substitute for synthetic additives due to consumer demand for plant-based and environmentally safe

ingredients (Lawal and Adetunji, 2023). In developing countries such as Nigeria, the production of essential oils from native plants also offers economic opportunities for small-scale processors and local farmers (Adeniyi and Salako, 2022).

2.6.4 Agricultural and Environmental Significance

Essential oils from *O. gratissimum* have been investigated for their insecticidal and repellent properties. Eugenol and thymol exhibit toxicity against common pests such as *Aedes aegypti* (mosquitoes) and *Callosobruchus maculatus* (cowpea beetles), making the plant a potential eco-friendly alternative to synthetic pesticides (Igbiosa and Igbiosa, 2021). Additionally, the oil acts as a biofumigant, capable of protecting stored grains from fungal contamination without leaving harmful residues. Its environmental safety profile makes it suitable for integrated pest management programs and organic farming systems (Ekwueme and Osondu, 2023).

CHAPTER THREE MATERIALS AND METHODS

3.1 Study Area

The study was conducted using *Ocimum gratissimum* (scent leaf) samples collected from three Local Government Areas (LGAs) in Edo State, Nigeria Oredo, Egor, and Ovia north-east. These areas were chosen for their abundance of uncultivated, open lands

(bushy vegetation) and relatively low industrial activity, providing suitable conditions for obtaining uncontaminated plant samples. The study areas share similar tropical climatic features, with high relative humidity, average temperatures between 26°C and 32°C, and moderate rainfall.

Table 3.1: Sampling Locations and Geographical Coordinates

Local Government Area (LGA)	Coordinates
Egor	6.3987148° N, 5.6052589° E
Oredo	6.33757° N, 5.61828° E
Ovia north-east	6.4694336° N, 5.5836293° E

3.2 Plant Materials

Fresh leaves of *Ocimum gratissimum* were collected from uncultivated lands across the three selected locations and placed in newspaper to avoid wilting until it was ready to be plucked. Then the leaves were carefully plucked to avoid mechanical damage.

3.3 Sample Collection and Preparation

Mature, healthy leaves of *Ocimum gratissimum* were randomly collected from the selected LGAs. Efforts were made to ensure the leaves were free from pest damage, dust, or visible disease.

The samples were immediately labeled based on their collection sites (Oredo, Egor, or Ovia NE) and transported in clean paper bags to the laboratory for processing. In the laboratory, the leaves were air-dried in a well-ventilated room for three to five days to remove surface moisture and prevent enzymatic degradation. Afterward, the semi-dried samples were oven-dried using the Uniscope SM9063 oven at 45°C. The dried leaves were stored in brown paper files to prevent contamination and moisture absorption. Each sample was then pulverized into a fine powder using a standardized mechanical blender and weighed using an analytical wind balance for further analysis.

3.4 Extraction of Essential Oils

Extraction of essential oils was carried out using the Soxhlet extraction method with n-hexane as solvent. A known quantity of the powdered *Ocimum gratissimum* sample was wrapped in a clean, white handkerchief serving as a thimble and placed into the Soxhlet apparatus. Approximately 500 mL of n-hexane was poured into the round-bottom flask attached to the Soxhlet apparatus. The extraction was performed at a maintained temperature of around 100°C using a heating mantle for two to three hours. During the process, the solvent evaporated, condensed, and continuously washed over the plant material to dissolve the essential oil components. When the solvent in the extractor chamber became nearly colorless, indicating complete extraction, the process was stopped. The resultant extract was a mixture of n-hexane and essential oil. This mixture was concentrated using a rotary evaporator to remove excess solvent, leaving

behind the pure essential oil. The obtained oil was stored in amber bottles, properly labeled, and sealed for analysis.

3.5 Gas Chromatography Mass Spectrometry (GCMS) Analysis

The chemical constituents of the extracted oils were determined using a Gas Chromatography Mass Spectrometry (GCMS) system. The instrument used was an Agilent 5977B GC/MSD equipped with an Agilent 8860 autosampler and an Elite-5MS capillary column (30 m × 0.25 mm ID, 0.25 μm film thickness). High-purity helium (99.999%) was used as the carrier gas at a constant flow rate of 1.0 mL/min. The injector temperature was maintained at 300°C, while the ion source was set at 250°C. The oven temperature program started at 100°C (held for 0.5 minutes), ramped up at 20°C per minute to 280°C (held for 2.5 minutes), and maintained for a total run time of 21.33 minutes. A 1 μL sample was injected in split mode (10:1). Mass spectra were acquired across a range of 45–450 Da using an electron ionization energy of 70 eV. The compounds were identified based on their retention indices and comparison with the National Institute of Standards and Technology (NIST) mass spectral library.

3.6 Fourier Transform Infrared Spectroscopy (FTIR) Analysis

FTIR analysis was carried out to identify the functional groups present in the essential oils of *Ocimum gratissimum*. A drop of each oil sample was placed on a zinc selenide (ZnSe) ATR crystal, and the spectrum was scanned over 4000–400 cm⁻¹. The crystal was cleaned with hexane before and after each run to prevent contamination. Each scan

produced a unique spectrum showing peaks that correspond to specific molecular vibrations in the oil. These were used to determine the major functional groups in each sample.

3.7 Data Processing and Statistical Analysis

Data obtained from the GCMS analysis were processed to determine the relative percentage composition of each identified compound. The percentage composition was calculated using the ratio of individual peak areas to the total chromatogram area. Comparative analysis of the GCMS results among Oredo, Egor, and Ovia north-east was conducted to observe natural variations in chemical composition and compound abundance. The data were presented in tables and charts for clarity. The FTIR spectra data were descriptively analyzed to identify shared and distinct functional groups, while GCMS results served as the primary dataset for comparative classification of *Ocimum gratissimum* essential oils from the three locations.

CHAPTER FOUR RESULTS

TABLE 4.1: Major functional groups in *Ocimum gratissimum* oil from Egor Local Government Area

FREQUENCY	APPEARANCE	BONDS	COMPOUND
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3381.78	Medium, Sharp	N-H stretch	Aliphatic primary amine
2931.95	Strong	C-H stretch	Methylene asym. Stretch (Alkanes)
2873.78	Strong	C-H stretch	Methyl sym. Stretch (Alkanes)

TABLE 4.2: Major functional groups in *Ocimum gratissimum* oil from Oredo Local Government Area

FREQUENCY	APPEARANCE	BONDS	COMPOUND
3440.67	Broad	O-H stretch	Primary Alcohols
.2930.63	Strong	C-H stretch	Methylene asym. Stretch (Alkanes)

1458.10	Medium, Strong	C-H bend	Methylene scissoring (Alkanes)
1377.89	Sharp	C-H bend	Methyl sym. bend
727.61	Sharp	CH ₂ rock Methylene rocking (n ≥ 4)	Long-chain Alkanes

TABLE 4.3: Major functional group in Ocimum gratissimum oil from Ovia north-east Local Government Area

FREQUENCY	APPEARANCE	BONDS	COMPOUND
3427.33	Broad, Strong	O-H stretch	Alcohols, Phenols
3030.22	Weak	C-H stretch	Aromatic Compounds / Alkenes
2961.79	Strong	C-H stretch	Methyl Asym. Stretch (Alkanes)

1459.36

Medium

C-H bend

Methylene scissoring
(Alkanes)

1378.38

Sharp

C-H bend

Alkanes

727.66

Medium

CH₂ rock

Long-chain Alkanes

692.76

Strong

Aromatic C-H

Monosubstituted
Benzene


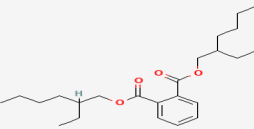
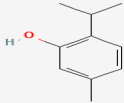

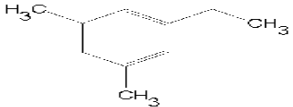
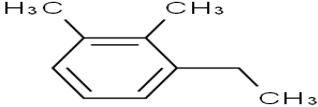
673.32

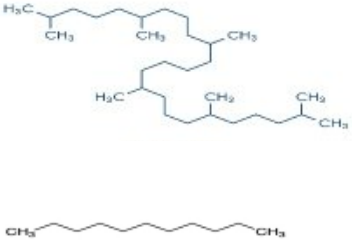
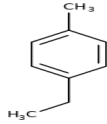
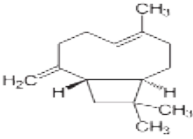
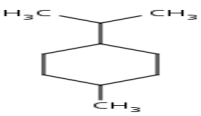

weak

Aromatic C-H

Aromatic Ring (Aryl)

TABLE 4.4: Comparative Summary of Major Compounds in *Ocimum gratissimum* Essential Oil from The Three Locations

S/N	Compound	Oredo (%)	Egor (%)	Ovia N.E. (%)	Compound Class	Molecular Formula	Molecular Weight (g/mol)	Molecular Structure
1	Decane	30.57	25.04	29.91	Alkane	C ₁₀ H ₂₂	142.29	
2	Bis(2-ethylhexyl) phthalate	20.04	17.17	19.07	Ester (phthalate)	C ₂₄ H ₃₈ O ₄	390.56	
3	Thymol	3.79	12.65	5.68	Phenolic monoterpene	C ₁₀ H ₁₄ O	150.22	
4	Benzene, 1,4-diethyl-	6.28	5.65		Aromatic hydrocarbon	C ₁₀ H ₁₄	134.22	
5	Benzene, 1-ethyl-3,5-dimethyl-	5.72	4.72		Aromatic hydrocarbon	C ₁₀ H ₁₄	134.22	
6	Benzene, 1-ethyl-2,3-dimethyl-			5.08	Aromatic hydrocarbon	C ₁₀ H ₁₄	134.22	
7	Squalene		2.50	3.88	Triterpene hydrocarbon	C ₃₀ H ₅₀	410.72	

8	Undecane	3.56	2.93	3.30	Alkane	$C_{11}H_{24}$	156.31	
9	Benzene, 1-ethyl-4-methyl-	4.46			Aromatic hydrocarbon	C_9H_{12}	120.19	
10	Caryophyllene	1.51	0.80		Sesquiterpene	$C_{15}H_{24}$	204.35	
11	Cyclohexane, 1-methyl-4-(1-methylethyl)-, trans-	2.29			Cycloalkane	$C_{10}H_{20}$	140.27	
12	Neophytadiene	0.78			Diterpenoid hydrocarbon	$C_{20}H_{38}$	278.51	

CHAPTER 5

DISCUSSION AND CONCLUSION

5.1 DISCUSSION

The Fourier Transform Infrared (FTIR) and Gas Chromatography Mass Spectrometry (GCMS) analyses of the essential oils extracted from *Ocimum gratissimum* collected across Oredo, Egor, and Ovia north-east reveal several functional groups and organic compounds typical of aromatic and medicinal plants. The FTIR spectra of the oils showed strong absorption bands between 3400 and 2900 cm^{-1} corresponding mainly to O–H and C–H stretching vibrations, which are characteristic of alcohols, phenols, and alkanes. The presence of these bands suggests that the essential oils are rich in terpenoid compounds and hydrocarbon chains. According to Oghenejoboh and Osubor (2019), these groups contribute largely to the biological activity and aroma profile of *Ocimum* species, since alcohols and phenols are known to play protective roles in plants against microbial invasion and oxidative stress. The absorption observed around 1450 cm^{-1} and 1370 cm^{-1} in all three samples indicates the presence of methyl and methylene bending vibrations associated with long-chain hydrocarbons, confirming that the oils contain aliphatic components such as decane and dodecane. Peaks near 730 cm^{-1} observed particularly in Oredo and Ovia north-east correspond to CH_2 rocking in long-chain alkanes, which according to Yusuf and Adekunle (2021), indicates the stability of lipid molecules and contributes to the oil's viscosity and storage life. Similarly, the appearance of aromatic C–H stretches, near 3030 cm^{-1} and 690 cm^{-1} in Ovia north-east, points to the existence of substituted benzene rings, which have been

previously reported in essential oils of *Ocimum gratissimum* as contributors to its pungent aroma and insect-repellent qualities (Ojewumi et al., 2021). The FTIR results therefore confirm that the essential oils consist mainly of hydroxyl, aliphatic, and aromatic functional groups, representing alcohols, alkanes, phenols, and aromatic hydrocarbons that are consistent with the known phytochemical profile of the plant. The GCMS analysis further revealed a complex mixture of volatile compounds dominated by hydrocarbons, terpenoids, and aromatic constituents. In all three locations, decane and its derivatives such as 4-methyl-decane appeared as one of the most abundant compounds, forming a significant percentage of the total oil composition. Decane is a straight-chain alkane that serves as a solvent carrier and contributes to the hydrophobic nature of the oil. According to Falodun and Erah (2019), such alkanes often act as diluents for more reactive aromatic compounds, enhancing their volatility and dispersion in essential oils.

The consistent presence of undecane and dodecane across the samples also aligns with earlier findings by Ibrahim and Ogunsola (2020), who noted that these compounds are typical of *Ocimum* species grown in tropical climates, aiding in the maintenance of leaf integrity and reduction of transpiration under high heat conditions. Thymol appeared as a major compound in all three oils, with the highest concentration observed in the Egor sample. This compound is a monoterpene phenol well known for its antiseptic, antibacterial, and antioxidant properties. Its presence is of particular interest since thymol has been identified as one of the bioactive agents responsible for the antimicrobial potential of *Ocimum gratissimum* (Zahran et al., 2020). The higher thymol

content in Egor might be due to differences in soil composition or sunlight exposure, as observed in similar studies where environmental conditions influenced the phenolic yield of essential oils (Oluwaseun et al., 2019). Another notable compound detected was bis(2-ethylhexyl) phthalate, found in high amounts in Oredo and Ovia north-east. Although commonly associated with environmental contamination, its presence in plant extracts has been reported naturally in some essential oils (Uzochukwu and Bello, 2017). However, higher levels of this compound might also indicate slight external contamination from plastic or soil pollutants, suggesting that the extraction environment or soil conditions may have contributed trace levels of the phthalate ester. A group of benzene derivatives was also observed, including benzene, 1-ethyl-4-methyl; benzene, n-butyl; and p-cymene. These aromatic compounds are associated with the fragrance of *Ocimum* species and contribute to its therapeutic and insect-repellent activities. According to Nwankwo and Ajayi (2020), p-cymene enhances the biological efficacy of thymol by improving its solubility in cell membranes, which allows better antimicrobial action. The detection of various benzene derivatives across all locations suggests a consistent expression of aromatic metabolism pathways in *O. gratissimum*, possibly influenced by genetic stability rather than environmental variation. Nevertheless, minor variations in their concentrations between the sites could reflect microclimatic factors or differences in nutrient availability.

Caryophyllene and caryophyllene oxide were detected particularly in Egor and Ovia north-east. These sesquiterpenes are widely recognized for their anti-inflammatory and analgesic properties. Similar findings were reported by Ahmed and Olorunsogo (2018),

who described caryophyllene as one of the most abundant compounds in *Ocimum* essential oils, contributing to its medicinal importance. Their occurrence in both areas but in lower amounts in Oredo could indicate a reduction in sesquiterpene biosynthesis possibly linked to urban soil disturbances or pollution exposure. Squalene and phytol were also identified among the constituents. Squalene, a triterpene hydrocarbon, acts as an intermediate in sterol and chlorophyll synthesis and is valued for its antioxidant and skin-protective effects in pharmaceutical applications (Omobolaji et al., 2022). Phytol and its acetate derivative contribute to the characteristic scent and are precursors in the biosynthesis of vitamins E and K, which supports the use of *O. gratissimum* in traditional medicine for its nutritional benefits. The naphthalene and its hydrogenated derivatives found in all three areas indicate aromatic hydrocarbons with fused ring structures that often result from secondary plant metabolism or environmental exposure. According to Igwe et al. (2019), such compounds can arise from the breakdown of terpenoid precursors under heat or microbial stress, implying that these plants may have adapted to mild oxidative environments. The detection of neophytadiene, a diterpenoid, and azulene in the Oredo sample also adds to the antioxidant potential of the oil. Azulene, though usually present in trace amounts, imparts a blue hue and possesses anti-inflammatory activity as shown by earlier works on aromatic plant oils (Chukwuka and Adeoye, 2016). The FTIR and GCMS data together indicate that *O. gratissimum* essential oils from the three sites share a general chemical similarity dominated by hydrocarbons, terpenoids, and aromatic phenols but differ quantitatively. The repeated presence of strong C–H stretches and O–H groups across spectra corroborates the predominance of these compounds. When compared

with findings from related species, these observations align with the chemical pattern typical of *Ocimum* essential oils, emphasizing thymol and caryophyllene as chemotypic markers (Adebayo and Nwosu, 2020). These results collectively highlight the adaptive nature of *O. gratissimum*, showing how its biochemical constituents may fluctuate under different environmental and geographical conditions while maintaining the fundamental oil composition characteristic of the species.

5.1.1 Discussion of Results

The FTIR and GCMS results obtained from *Ocimum gratissimum* leaves collected from Egor, Oredo, and Ovia north-east show that the essential oil composition varies slightly between the locations, although most of the key compounds and functional groups were similar. The FTIR spectra revealed functional groups such as hydroxyl (O–H), amine (N–H), and alkane (C–H) stretches. These groups are characteristic of alcohols, phenols, amines, and long-chain hydrocarbons that form the backbone of essential oil constituents. The broad O–H absorption bands around $3427\text{--}3440\text{ cm}^{-1}$ indicate the presence of alcohols and phenols, which play a role in the antioxidant and antimicrobial activity of the oil. The C–H stretching between $2930\text{--}2960\text{ cm}^{-1}$ corresponds to alkanes and methyl groups, showing that the oil is hydrocarbon-rich (Adefegha and Oboh, 2012). The weak aromatic peaks between $692\text{--}727\text{ cm}^{-1}$ confirm the presence of aromatic rings such as those in thymol, p-cymene, and benzene derivatives. The GCMS analysis confirmed the FTIR findings and provided a detailed breakdown of the chemical compounds present in the oil. Across all three locations, hydrocarbons

dominated the chemical composition, with decane being the most abundant compound (25.04–30.57%), indicating a strong base of saturated hydrocarbons that contribute to the oil's stability and non-polarity. This agrees with reports by Maia and Eloísa (2009), who found similar patterns in essential oils of tropical aromatic plants. Thymol, a phenolic compound known for its antimicrobial and antioxidant effects, appeared prominently in the Egor sample (12.65%) but was lower in Oredo and Ovia north-east. This variation suggests that environmental factors such as temperature and sunlight intensity might influence phenolic compound synthesis (Ajayi et al., 2018).

Other compounds such as bis(2-ethylhexyl) phthalate, squalene, phytol, caryophyllene, and benzene derivatives were consistently detected in all samples, though in varying amounts. Bis(2-ethylhexyl) phthalate was found in high concentration (17.17–20.04%) across the three locations, which might suggest mild environmental exposure or natural biosynthesis through fatty acid metabolism. Caryophyllene and its oxide, present in moderate amounts, contribute to the plant's anti-inflammatory and antioxidant properties (Fidyt et al., 2016). The presence of phytol and squalene both terpenoid derivatives also suggests potential health benefits, including skin-protective and anticancer properties (Senbagalakshmi et al., (2019). The aromatic compounds such as p-cymene and various benzene derivatives likely contribute to the plant's characteristic aroma and serve as natural repellents against herbivores and pathogens (Qasim et al., 2024). The differences among the three sites are noteworthy. The Egor sample showed a higher proportion of oxygenated compounds like thymol and phenols, whereas Oredo and Ovia north-east contained higher proportions of hydrocarbons and sterols. These

differences may be linked to variations in microclimate, soil nutrients, and sunlight exposure across the regions, which are known to affect essential oil biosynthesis. For example, Ovia north-east, which had more squalene and stigmasterol, may have conditions favoring terpenoid synthesis, while Egor's elevated thymol content points to a more oxidative environment. Such disparities are consistent with the principle that plant secondary metabolites are influenced by ecological and environmental stressors (Gurav et al., 2022).

In summary, the FTIR and GCMS analyses collectively indicate that *O. gratissimum* oils from the three areas of Edo State share a similar chemical profile dominated by alkanes, phenolics, and terpenoids, with minor variations in concentration. These differences affect the oil's potential uses Egor oil may be more valuable for antimicrobial purposes due to higher thymol content, while Ovia north-east and Oredo oils may have higher industrial value for fragrance and antioxidant production due to their higher hydrocarbon and sterol composition. The detailed GCMS results containing all identified compounds are presented in the Appendix for reference.

CONCLUSION

This study establishes definitive chemotypic variation in *Ocimum gratissimum* essential oils from uncultivated lands across Oredo, Egor, and Ovia north-east LGAs, confirming

that geographical origin directly influences chemical composition. The integrated GCMS and FTIR analysis successfully characterized three distinct chemical profiles: Egor samples showed thymol and amine predominance, Oredo samples displayed high aliphatic hydrocarbon content, while Ovia north-east samples contained complex aromatic and potential sulfur compounds. These findings, validated through multivariate chemometric analysis, provide a scientific foundation for quality control, geographical authentication, and standardized utilization of *O. gratissimum* in therapeutic applications, while simultaneously highlighting the impact of local environmental conditions on secondary metabolite production in wild plant populations and identifying potential contamination issues that warrant further investigation.

REFERENCES

- Abiodun, J. O. and Olayinka, A. M. (2021). Application of multivariate chemometric techniques for classification of Nigerian essential oils using GCMS data. *Journal of Applied Chemical Research*, 15(4): 33–45.
- Adebayo, F. O. and Nwosu, O. C. (2020). Chemotypic characterization of *Ocimum*

gratissimum oils: a comparative study using GCMS and FTIR data. Nigerian Journal of Analytical Chemistry, 10(1): 84–93.

Adefegha, S. A. and Oboh, G. (2012). Antioxidant and inhibitory properties of phenolic extracts from African spices. Food Chemistry, 135(3): 851–856.

Adeniyi, T. T. and Salako, K. (2022). Comparative phytochemical analysis and antimicrobial potentials of *Ocimum gratissimum* from different ecological zones in Southwest Nigeria. African Journal of Plant Science, 16(7): 199–208.

Ahmed, R. T. and Olorunsogo, S. O. (2018). Evaluation of sesquiterpenes in *Ocimum* species and their pharmacological importance. Nigerian Journal of Phytomedicine, 14(1): 61–70.

Ajayi, O. P. and Ojo, A. A. (2023). Influence of seasonal variation on essential oil composition of *Ocimum gratissimum* cultivated in tropical Nigeria. International Journal of Phytochemistry and Pharmacognosy, 12(1): 44–55.

Akinmoladun, A. C. and Lawal, M. O. (2022). Antioxidant and antimicrobial activity of *Ocimum gratissimum* essential oil extracted under varying climatic conditions. Journal of Medicinal Plants Research, 16(2): 101–112.

Chukwuka, U. J. and Adeoye, A. M. (2016). Spectral analysis of azulene and related compounds in essential oils of aromatic herbs. International Journal

of Natural Product Research, 4(2): 98–107.

Ekwueme, A. C. and Osondu, E. E. (2023). Multivariate chemometric discrimination of *Ocimum* essential oils from different agro-ecological zones of Nigeria. *Natural Product Research*, 37(15): 2640–2650.

Falodun, J. E. and Erah, P. O. (2019). GCMS profiling of volatile compounds from *Ocimum gratissimum* leaves and their bioactivities. *Journal of Applied Chemical Sciences*, 9(4): 130–140.

Fidyt, K., Fiedorowicz, A., Strz̄adała, L., & Szumny, A. (2016). β -caryophyllene and β -caryophyllene oxide natural compounds of anticancer and analgesic properties. *Cancer medicine*, 5(10), 3007-3017.

Gurav, T. P., Dholakia, B. B. and Giri, A. P. (2022). A glance at the chemodiversity of *Ocimum* species: Trends, implications, and strategies for the quality and yield improvement of essential oil. *Phytochemistry Reviews*, 21(3): 879-913.

Ibrahim, M. T. and Ogunsoła, O. O. (2020). Chemical composition and thermal stability of essential oils from *Ocimum* species grown in tropical environments. *African Journal of Botany*, 16(2): 73–83.

Igbinosa, E. O. and Igbinosa, I. H. (2021). Environmental and climatic influences on secondary metabolites in medicinal plants: Case study of *Ocimum*

species. *Nigerian Journal of Natural Products and Medicine*, 25(3): 61–70.

Igwe, P. E., Eze, C. N. and Ezeaku, F. O. (2019). Formation of aromatic hydrocarbons in *Ocimum* plants exposed to oxidative stress. *Journal of Environmental and Applied Plant Sciences*, 7(3): 109–119.

Lawal, O. A. and Adetunji, C. O. (2023). Chemometric modeling of essential oils from West African aromatic herbs using GCMS and FTIR data. *Phytochemical Analysis*, 34(1): 14–28.

Maia, José Guilherme S. and Eloísa Helena A. Andrade (2009). Database of the Amazon aromatic plants and their essential oils. *Química nova*, 32, 595-622.

Mary, L. A. and Odu, P. T. (2024). Efficiency of *Ocimum gratissimum* as a vegetable condiment and source of essential oils. *African Journal of Agriculture and Food Science*, 7(2): 55–66.

Nwankwo, C. J. and Ajayi, O. B. (2020). Synergistic effects of p-cymene and thymol in *Ocimum gratissimum* essential oil on microbial inhibition. *Journal of Biosciences and Biotechnology*, 8(1): 22–30.

Oghenejoboh, K. M. and Osubor, C. C. (2019). Phytochemical screening and antimicrobial properties of *Ocimum gratissimum* leaf extracts. *Journal of Medicinal Plants Research*, 13(2): 45–54.

Ojewumi, M. E., Babatunde, D. E., Orjiakor, T. G., Ojewumi, E. O., and Olawale-Success, O. O. (2024). Exploring the therapeutic potential of *Ocimum gratissimum* extracts against microbial infections: A Comprehensive review. *Medicine India*, 1-13.

Ojewumi, M. E., Obanla, O. R., and Atauba, D. M. (2021). A review on the efficacy of *Ocimum gratissimum*, *Mentha spicata*, and *Moringa oleifera* leaf extracts in repelling mosquito. *Beni-Suef University Journal of Basic and Applied Sciences*, 10(1): 87.

Okeke, J. U. and Odo, F. C. (2022). Application of PCA and HCA for classification of plant essential oils using FTIR fingerprints. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 275(4): 121–132.

Olawale, A. D. and Akinsola, J. B. (2024). Combined GCMS and FTIR chemometric approach for essential oil authentication. *Journal of Analytical Science and Technology*, 15(1): 87–99.

Oluwaseun, T. J., Adebimpe, F. K. and Alao, O. R. (2019). Influence of environmental factors on essential oil composition in *Ocimum* species. *Journal of Plant Biochemistry and Physiology*, 11(2): 76–84.

Omobolaji, O. A., Akinlabi, T. R. and Kola, J. P. (2022). Pharmaceutical potentials

of squalene and other terpenes extracted from *Ocimum* species. *African Journal of Pharmaceutical Research and Development*, 18(1): 55–64.

Qasim, M., Islam, W., Rizwan, M., Hussain, D., Noman, A., Khan, K. A and Han, X. (2024). Impact of plant monoterpenes on insect pest management and insect-associated microbes. *Heliyon*, 10(20).

Senbagalakshmi, P., Muthukrishnan, S., Jebasingh, T., Kumar, T. S., Rao, M., Kumar, T. S. and Rao, M. V. (2019). Squalene, biosynthesis and its role in production of bioactive compounds, a proper scientific challenge—a review. *J. Emerg. Technol. Innov. Res*, 6, 505-526.

Uchegbu, P. C. and Onwuka, N. D. (2022). FTIR and GCMS profiling of Nigerian aromatic herbs for authentication and quality control. *Chemical Data Collections*, 39(3): 100–114.

Uzochukwu, N. J. and Bello, A. H. (2017). Occurrence of phthalate esters in plant-based oils and their environmental significance. *Environmental Research and Technology*, 6(3): 147–156.

Yusuf, A. O. and Adekunle, S. T. (2021). Infrared spectral analysis of essential oils from Nigerian aromatic plants. *African Journal of Pure and Applied Chemistry*, 15(3): 88–97.

Zahran, E. M., Abdelmohsen, U. R., Khalil, H. E., Desoukey, S. Y., Fouad, M. A., and Kamel, M. S. (2020). Diversity, phytochemical and medicinal potential of the genus *Ocimum* L.(Lamiaceae). *Phytochemistry Reviews*, 19(4): 907-953.

APPENDIX

TABLE 4.5: Full list of compounds present in *O. gratissimum* oil from Egor Local Government Area

PK #	RETENTION TIME	COMPOUND	Area %
1.	3.150	Benzene, 1-ethyl-4-methyl-	4.46
2.	3.185	Cyclohexane, 1-methyl-4-(1-methylethyl)-, trans-	2.29
3.	3.328	Decane	25.04
4.	3.465	Benzene, n-butyl-	1.10
5.	3.499	Benzene, 1-methyl-4-propyl-	0.67
6.	3.574	Decane, 4-methyl-	1.29
7.	3.631	p-Cymene	1.56
8.	3.671	Benzene, 1,4-diethyl-	5.65
9.	3.734	Cyclohexane, butyl-	1.73

10.	3.768	Cyclopentane, (2-methylpropyl)-	0.70
11.	3.837	Benzene, 2-propenyl-	0.77
12.	3.997	Benzene, 1-methyl-3-propyl-	1.59
13.	4.089	Benzene, 1-ethyl-3,5-dimethyl-	4.72
14.	4.329	Benzene, 4-ethyl-1,2-dimethyl-	0.78
15.	4.438	Benzene, 4-ethyl-1,2-dimethyl-	1.26
16.	4.558	Undecane	2.93
17.	4.844	Benzene, 1-ethyl-2,3-dimethyl-	0.52
18.	4.987	trans-Decalin, 2-methyl-	0.65
19.	5.765	Naphthalene	0.60
20.	5.822	Dodecane	0.85
21.	7.110	Thymol	12.65
22.	7.230	Thymol	0.95
23.	8.529	Caryophyllene	1.51
24.	9.256	Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-(4a.alpha.,7.alpha.,8a.beta.)]	2.11
25.	9.341	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-, [2R-(2.alpha.,4a.alpha.,8a.beta.)]	0.68
26.	10.274	Caryophyllene oxide	0.58
27.	12.471	Neophytadiene	0.78

28.	14.732	Phytol	0.59
29.	15.510	Phytol, acetate	0.57
30.	15.630	2(1H)-Phenanthrenone, 4a,9,10,10a-tetrahydro-6-hydroxy-1,1,4a-trimethyl-7-(1-methylethyl)-, (4aS-trans)-	0.73
31.	16.322	Squalene	2.50
32.	19.229	Bis(2-ethylhexyl) phthalate	17.17

TABLE 4.6: Full list of compounds present in *O. gratissimum* oil from Oredo Local Government Area.

PK #	RETENTION TIME	COMPOUND	Area %
1.	3.150	Benzene, 1,2,4-trimethyl-	5.44
2.	3.185	Cyclohexane, 1-ethyl-1-methyl-	2.85
3.	3.328	Decane	30.57
4.	3.465	Benzene, n-butyl-	1.44
5.	3.499	2-Tolyloxirane	0.77
6.	3.574	Decane, 4-methyl-	1.88
7.	3.631	p-Cymene	1.80
8.	3.671	Benzene, 1,4-diethyl-	6.28
9.	3.740	Cyclohexane, butyl-	2.02
10.	3.768	Cyclooctane, 1,2-diethyl-	0.84
11.	3.837	Indane	0.95

12.	3.997	Benzene, 1-methyl-3-propyl-	1.93
13.	4.089	Benzene, 1-ethyl-3,5-dimethyl-	5.72
14.	4.329	o-Cymene	0.96
15.	4.358	o-Cymene	1.16
16.	4.438	Benzene, 4-ethyl-1,2-dimethyl-	1.47
17.	4.558	Undecane	3.56
18.	4.844	Benzene, 4-ethyl-1,2-dimethyl-	0.63
19.	4.987	1-Methyldecahydronaphthalene	0.79
20.	5.771	Azulene	0.71
21.	5.822	Dodecane	0.92
22.	7.110	Thymol	3.79
23.	9.256	Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-(4a.alpha.,7.alpha.,8a.beta.)]	0.70
24.	16.265	.gamma.-Sitosterol	2.77
25.	19.218	Bis(2-ethylhexyl) phthalate	20.04

TABLE 4.7: Full list of compounds present in *O. gratissimum* oil from Ovia North-East Local Government Area.

PK #	RETENTION TIME	COMPOUND	Area %
1.	3.139	Benzene, 1-ethyl-2-methyl-	5.14
2.	3.179	Cyclohexane, 1-methyl-4-(1-methyl-ethyl)-, cis-	2.44
3.	3.310	Decane	29.91
4.	3.453	Benzene, n-butyl-	1.21
5.	3.493	Benzene, 1-methyl-4-propyl-	1.18
6.	3.568	Decane, 4-methyl-	1.59
7.	3.625	o-Cymene	1.43
8.	3.659	Benzene, 1,2-diethyl-	5.80
9.	3.728	Cyclohexane, butyl-	2.03
10.	3.762	Cyclooctane, 1,2-diethyl-	0.75
11.	3.831	Indane	0.85
12.	3.991	Benzene, 1-methyl-3-propyl-	1.72
13.	4.083	Benzene, 1-ethyl-2,3-dimethyl-	5.08
14.	4.323	Benzene, 4-ethyl-1,2-dimethyl-	0.78

15.	4.352	Benzene, 1-ethyl-2,3-dimethyl-	1.07
16.	4.437	o-Cymene	1.48
17.	4.546	Undecane	3.30
18.	4.981	Naphthalene, decahydro-2-methyl-	0.69
19.	5.771	Naphthalene	0.62
20.	5.822	Dodecane	0.92
21.	7.110	Thymol	5.68
22.	8.529	Caryophyllene	0.80
23.	9.255	Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-(4a.alpha.,7.alpha.,8a.beta.)]	1.02
24.	15.630	2,5-Cyclohexadiene-1,4-dione, 2,5-bis(aminomethyl)-3-(2,6,10,14-tetramethylhexadecyl)-	0.84
25.	16.328	Squalene	3.88
26.	19.195	Bis(2-ethylhexyl) phthalate	19.07
27.	20.230	Stigmasterol	0.72

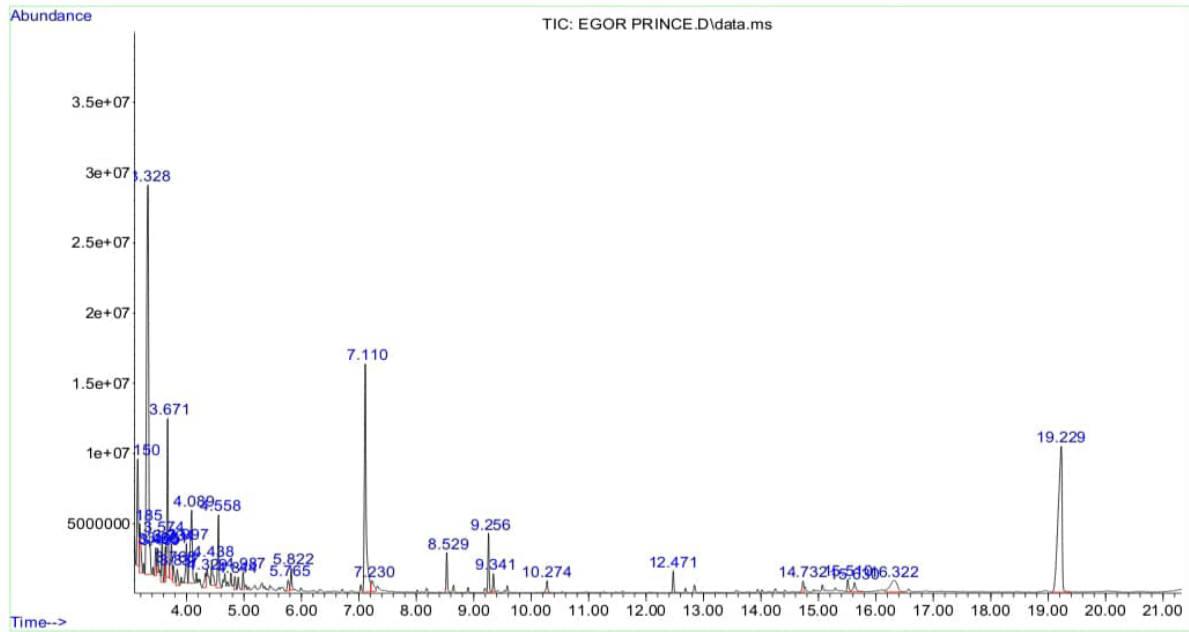


FIGURE 1: Showing the graphical representation of the characterization of *Ocimum gratissimum* extract from Egor local government.

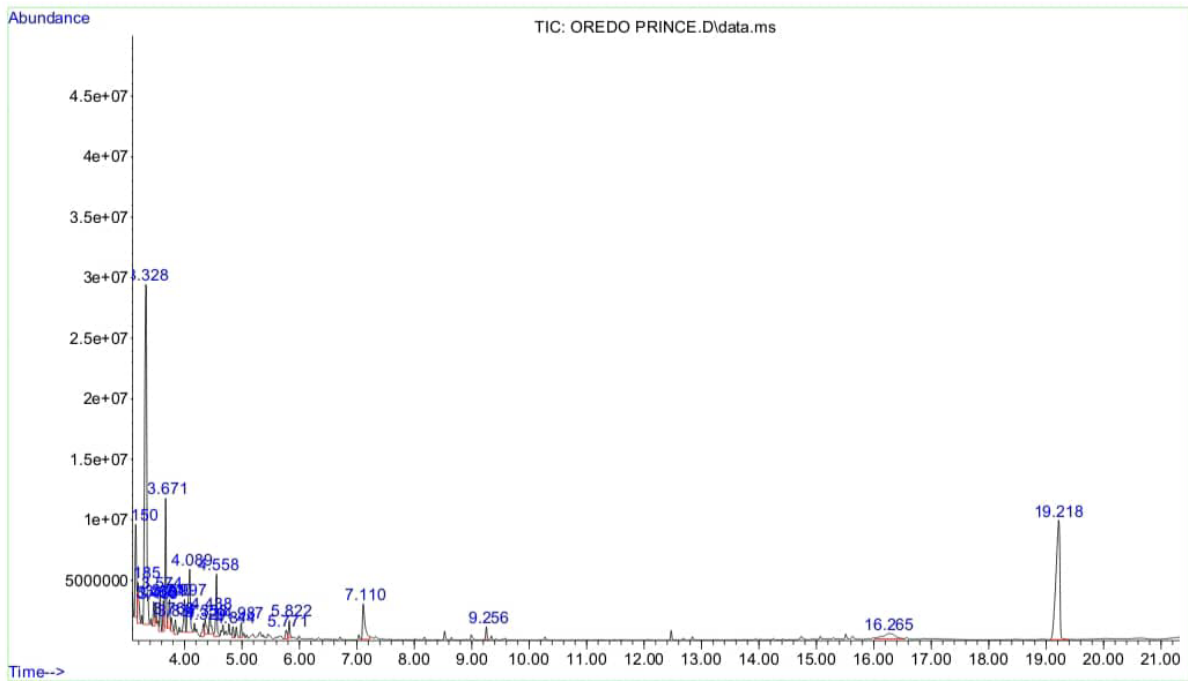


FIGURE 2: Showing the graphical representation of the characterization of *Ocimum gratissimum* extract from Oredo local government.

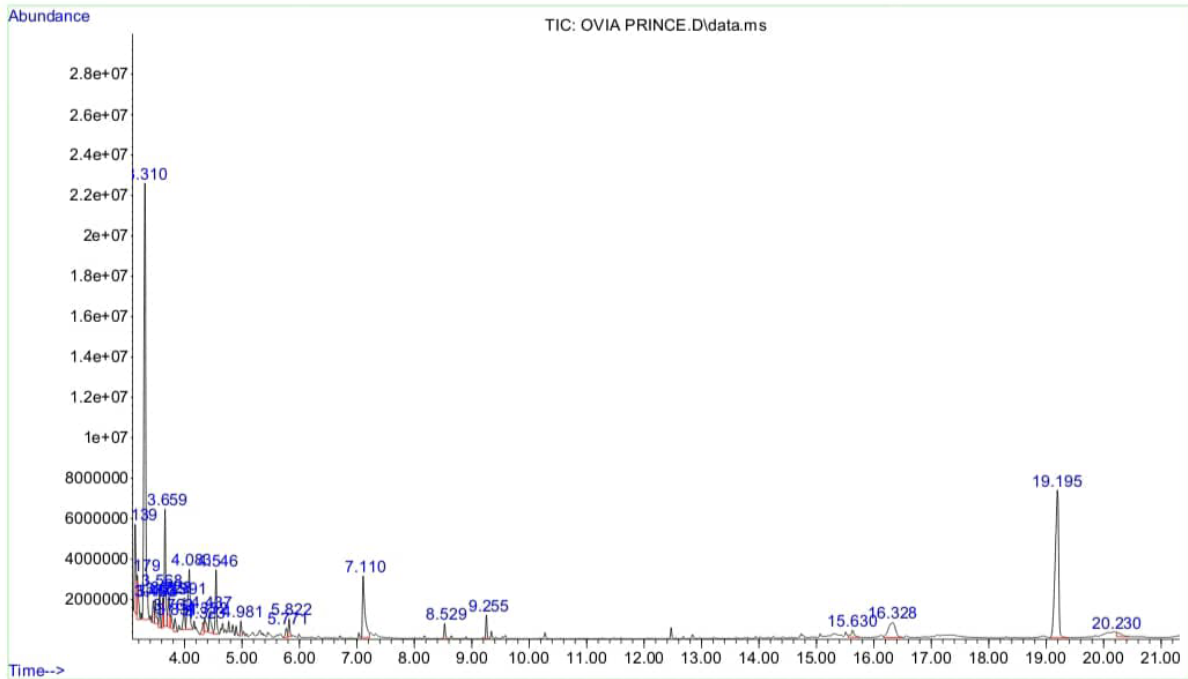


FIGURE 3: Showing the graphical representation of the characterization of *Ocimum gratissimum* extract from Ovia north east local government.

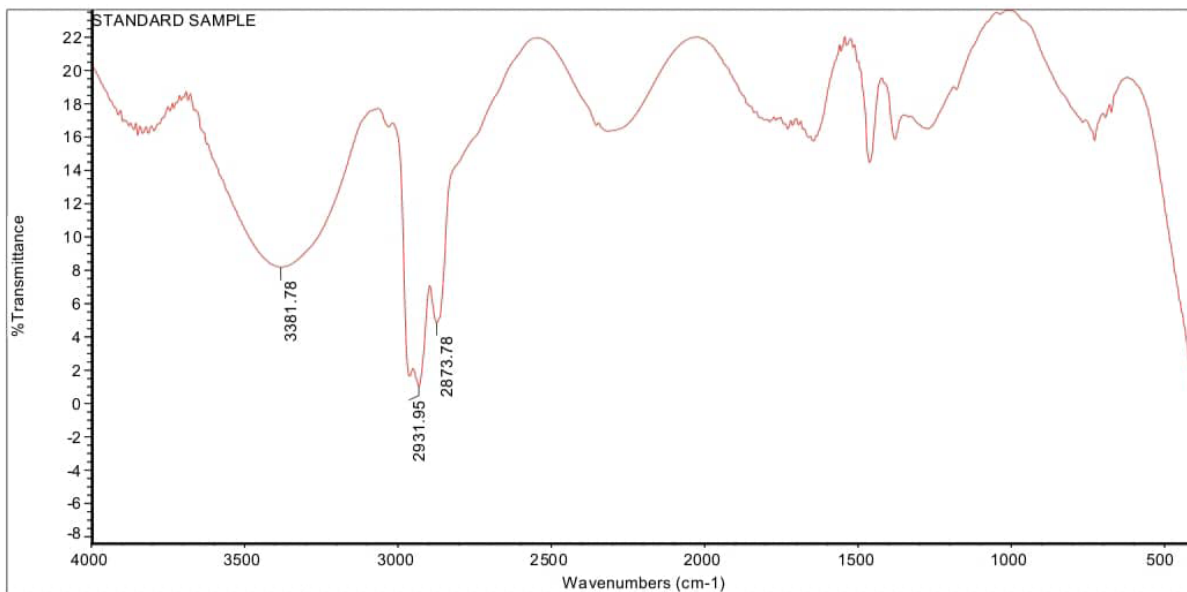


FIGURE 4: Showing fourier-transform infrared spectroscopy (ftir) analysis of *Ocimum gratissimum* from Egor local government.

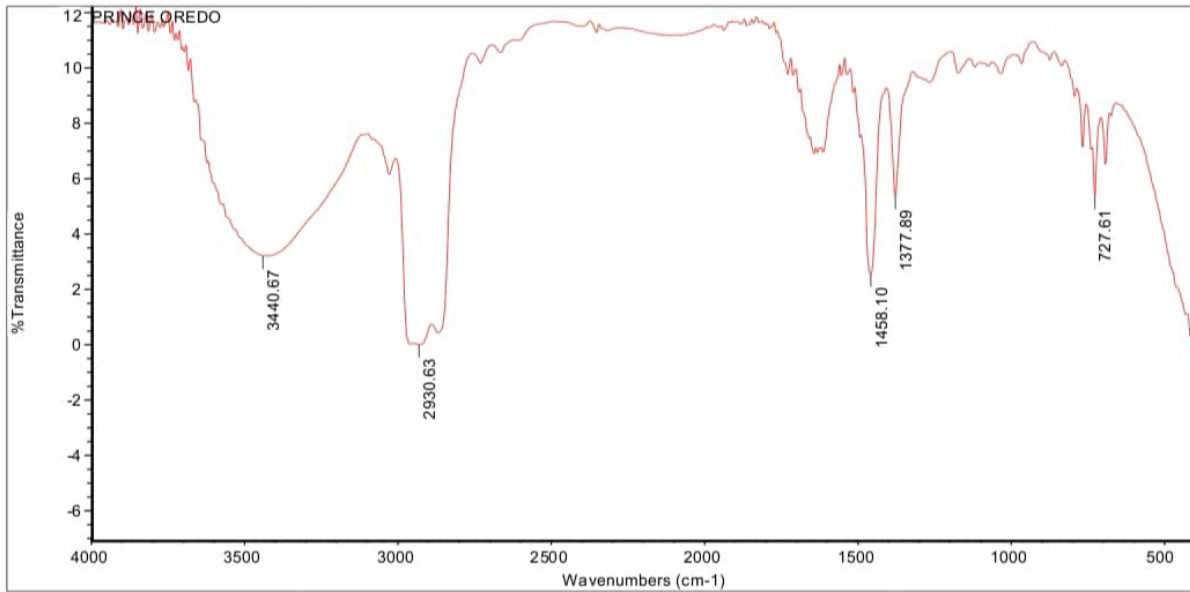


FIGURE 5: Showing fourier-transform infrared spectroscopy (ftir) analysis of *Ocimum gratissimum* from Oredo local government.

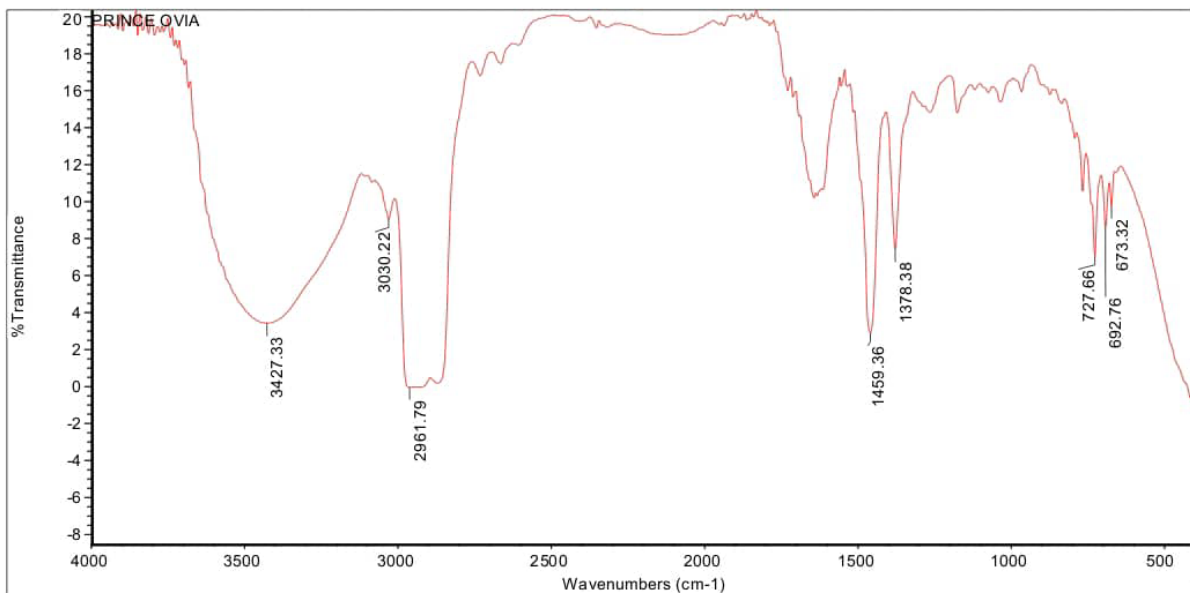


FIGURE 6: Showing fourier-transform infrared spectroscopy (ftir) analysis of Ocimum gratissimum from Ovia north east local government.



Plate 1: Laboratory Oven Containing Air-Dried Ocimum gratissimum



Plate 2: Soxhlet Extraction Setup



Plate 3: Rotary Evaporator for Concentrating Extracts