EVALUATION OF SUN PROTECTIVE EFFECT OF *OCIMUM GRATISSIMUM* (LAMIACEAE) ESSENTIAL OIL IN A CREAM-BASED FORMULATION

BY

ETAH OREVAOGHENE BERTA

(MATRIC NUMBER: PHA1700184)

SUPERVISED BY

PROF. ABIODUN FALODUN

DEPARTMENT OF PHARMACEUTICAL CHEMISTRY,

FACULTY OF PHARMACY,

UNIVERSITY OF BENIN,

BENIN CITY.

FEBRUARY 2025

EVALUATION OF SUN PROTECTIVE EFFECT OF *OCIMUM GRATISSIMUM* (LAMIACEAE) ESSENTIAL OIL IN A CREAM-BASED FORMULATION

BY

ETAH OREVAOGHENE BERTA

PHA1700184

SUPERVISED BY

PROF. ABIODUN FALODUN

A RESEARCH PROJECT SUBMITTED TO THE DEPARTMENT

OF PHARMACEUTICAL CHEMISTRY,

FACULTY OF PHARMACY,

UNIVERSITY OF BENIN, BENIN CITY,

EDO STATE, NIGERIA.

IN PARTIAL FULFILMENT OF THE REQUIREMENT

FOR THE AWARD OF DOCTOR OF PHARMACY

(PHARM.D) DEGREE.

FEBRUARY 2025

CERTIFICATION

We the undersigned hereby certify that this work was carried out by ETAH OREVAOGHENE BERTA with matriculation number PHA1700184, in the Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Benin, Benin City.

…………………………………. ……………….

Etah Orevaoghene Berta Date

(Student)

…………………………………. ……………….

Prof. Abiodun Falodun Date

(Project supervisor)

………………………………….. ……………….

Dr. Vincent Imieje Date

(Ag. Head.of.Department)

CERTIFICATION OF THESIS ON PLAGARISM

We the undersigned hereby certify that this thesis by ETAH OREVAOGHENE BERTA titled: EVALUATION OF SUN PROTECTIVE EFFECT OF OCIMUM GRATISSIMUM (LAMIACEAE) ESSENTIAL OIL IN A CREAM-BASED FORMULATION has successfully passed the anti-plagiarism test and does not violate any copyright regulation.

…………………………………. ……………….

Etah Orevaoghene Berta Date

(Student)

…………………………………. ……………….

Prof. Abiodun Falodun Date

(Project supervisor)

………………………………….. ……………….

Dr. Vincent Imieje Date

(Ag. Head.of.Department)

DEDICATION

This project work is dedicated to God Almighty, me and my family.

ACKNOWLEDGEMENTS

I sincerely appreciate my supervisor, Prof Abiodun Falodun for his guidance and support throughout this work. I also want to appreciate Dr Erharuyi for his assistance and corrections during this work.

I want to deeply appreciate almighty God for his faithfulness to me. I am grateful to my family, my father, Mr Friday Ashemore Etah, my mother, Mrs Florence Oghenekaro Etah, my loving siblings, Eniworareke Brain Etah, Ogheneothuke Clinton Etah, Evi Etah, Efezino Golden Etah, my cousin and his wife, Mr and Mrs Efezino Abel, my Christian family, the All Saints Chapel Choir, University of Benin. I will also not fail to acknowledge the love from Mr Stephen Ighodaro and his family.

I am also thankful to my lovely friends, Ogorchukwu Omezi, Blessing Ighodaro, Obiora Okafor, Favour Adagbon, Eniola Olaniyi (Esq in view), God'spower Akpovwoba, Emmanuella Benson, Benedict Adeolu, Praise Edobor for their support and encouragement throughout this journey.

My appreciation also extends to the entire staff of the department of Pharmacognosy, especially the Head of department, Dr Henry Osas, Mr Sani, and Mr Kingsley for their contributions. I acknowledge Professor Arewoh and Pharm Jude Isesele from the department of Pharmaceutics and Pharmaceutical Technology, Dr Akpeh from the department of Biochemistry, University of Benin for their guidance and support.

Finally, I want to appreciate my co- project student, Favour Adagbon and Gift Egbesunun and everyone who has supported and contributed financially, intellectually, emotionally,I am indeed grateful. Your collective support has been the cornerstone of my success.

TABLE OF CONTENTS

CERTIFICATION iii

CERTIFICATION OF THESIS ON PLAGARISM iv

DEDICATION v

ACKNOWLEDGEMENTS vi

TABLE OF CONTENTS vii

LIST OF FIGURES ix

LIST OF TABLES x

ABTRACT xi

CHAPTER ONE 1

INTRODUCTION AND LITERATURE REVIEW 1

1.1 Overview of Medicinal Plants 1

1.2 *Ocimum gratissimum* (Scent Leaves) 1

1.3 Chemical Constituents of *Ocimum gratissimum* 9

1.4 Traditional and Medicinal Uses 19

1.5 Essential Oils 22

1.6 Antioxidative and Anti-aging Properties of *Ocimum gratissimum* 26

1.6 Physicochemical Analysis of *Ocimum gratissimum* Essential Oil 29

1.7 Sun Protective Factor (SPF) 31

1.8 Topical Formulations of Essential Oils 37

1.9 Justification for the Study 40

1.10 Aim of Study 40

1.11 Specific Objective 41

CHAPTER TWO 42

MATERIALS AND METHODS 42

2.1 Materials 42

2.2 Methods 43

CHAPTER THREE 54

RESULTS 54

3.1 Essential Oil Extraction Yield 54

3.2 Physicochemical Properties of Essential Oil 54
3.3 Cream Formulation 56
3.4 Antioxidant Activity 67
3.5 Sun Protection Factor (SPF) 70
CHAPTER FOUR 72
DISCUSSION 72
4.1 Extraction of Essential Oil 72
4.2 Physicochemical Properties of the Essential Oil 72
4.3 Cream Formulation 73
4.4 Antioxidant Activity 74
4.4 Stability Studies of the Formulated Creams 76
4..6 Skin Compatibility of the Formulated Creams 76
4.7 Sun Protection Factor (SPF) 76
CHAPTER FIVE 78
CONCLUSION 78
RECOMMENDATIONS 78

LIST OF FIGURES

Figure 1.1 *Ocimum gratissimum in its natural habitat* 4

Figure 1.2 Distribution of *Ocimum gratissimum* L 8

Figure 1.3Chemical structures of some major constituents from the essential oil of *Ocimum gratissimum* dry leaves [A] 11

Figure 1.4 Chemical structures of some major constituents from the essential oil of *Ocimum gratissimum* dry leaves [B]. 14

LIST OF TABLES

Table 1.1 Sun protection factor values of herbal oils 33

Table 3. 1: Formulation Composition 52

Table 3. 2: pH Value 53

Table 3. 3: Spreadability width 54

Table 3. 4: Observed Washability Scores 55

Table 3. 5: Viscosity (cP) of cream formlations 56

Table 3. 6: Stability Study 58

Table 3. 7: Observed Irritation Results 59

Table 3. 8:Essential Oil Radical Scavenging Activity (% RSA) 61

Table 3. 9: Formulations Radical Scavenging Activity (% RSA) 62

Table 3. 10: SPF Values of Formulations 64

ABTRACT

The increasing awareness of the harmful effects of ultraviolet (UV) radiation has led to a growing demand for natural alternatives to synthetic sunscreens. *Ocimum gratissimum*, commonly known as scent leaf, has demonstrated potential antioxidant and photoprotective properties, making it a viable candidate for sun protection formulations.

This study aimed to evaluate the sun protective effect of *Ocimum gratissimum* essential oil when incorporated into a cream-based formulation.

The essential oil was extracted from *Ocimum gratissimum* leaves using hydro-distillation with a Clevenger apparatus. Physicochemical properties such as specific gravity, refractive index, acid value, saponification value, and iodine value were analysed to assess oil quality. The extracted oil was formulated into a cream, which was then evaluated for its physical properties, antioxidant activity, and sun protection factor (SPF) using the Mansur equation and UV-Visible spectrophotometry.

The essential oil yield was 0.753%, and its physicochemical properties indicated good stability. The formulated creams demonstrated improved spreadability, pH values between 6.38 and 6.8 (suitable for topical application), and skin compatibility. The essential oil exhibited significant antioxidant activity with an IC50 value of 28.96 µg/mL in the DPPH radical scavenging assay. SPF analysis revealed modest photoprotective properties, with the essential oil alone showing a base SPF value of 1.766 at 0.01% concentration. The formulated creams exhibited a concentration-dependent increase in SPF values, ranging from 1.45 (1% EO) to 2.58 (3% EO + CO).

The study highlights the potential of *Ocimum gratissimum* essential oil as a natural ingredient in skincare formulations, offering both antioxidant and mild photoprotective benefits. Further research is recommended to optimize the formulation and evaluate its in vivo efficacy and safety.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 Overview of Medicinal Plants

The utilization of medicinal plants in natural product-based formulations has witnessed a remarkable resurgence in recent years, driven by increasing consumer preference for natural ingredients and growing awareness of sustainable healthcare practices (Ekor, 2014). This renewed interest is particularly evident in the cosmeceutical industry, where plant-based ingredients are increasingly being incorporated into various skincare formulations (Michalak, 2023). The global market for natural cosmetics has shown substantial growth, with an estimated value of USD 34.5 billion in 2018 and projected to reach USD 54.5 billion by 2027 (Otsuka *et al*., 2022).

Medicinal plants have historically served as valuable resources for traditional medicine systems worldwide, offering a diverse array of bioactive compounds with therapeutic potential. These natural repositories of phytochemicals continue to play a crucial role in modern pharmaceutical and cosmetic development (Atanasov *et al*., 2015). The significance of these plants extends beyond their therapeutic properties, encompassing economic, social, and cultural dimensions that contribute to their sustained relevance in contemporary healthcare practices (Atanasov *et al*., 2015).

1.2 *Ocimum gratissimum* (Scent Leaves)

Among the vast array of medicinal plants, *Ocimum gratissimum*, commonly known as scent leaves or clove basil, has emerged as a particularly promising species. This plant has garnered significant scientific interest due to its rich phytochemical profile and diverse biological activities (Zahran *et al*., 2020). Traditional communities across various geographical regions have long recognized its

therapeutic potential, employing it in the treatment of various ailments and in skincare applications (Sharma, Sharma, & Vikas, 2018).

In recent years, scientific research has increasingly focused on validating the traditional uses of *O. gratissimum* and exploring its potential applications in modern formulations. The plant's essential oil, in particular, has demonstrated remarkable properties that make it suitable for incorporation into various pharmaceutical and cosmetic products (Kumar *et al*., 2018). The presence of bioactive compounds with antioxidant, antimicrobial, and potential photoprotective properties has positioned *O. gratissimum* as a promising candidate for natural sun-protective formulations (Kumar & Pandey, 2018).

The growing concern over the adverse effects of synthetic UV filters and the increasing demand for natural alternatives has sparked interest in plant-based photoprotective agents. The development of natural sun-protective formulations represents a significant opportunity to address these concerns while potentially offering additional skincare benefits through the inherent properties of plant-derived compounds (Costa *et al*., 2015).

This comprehensive review examines the botanical characteristics, phytochemical composition, and traditional applications of *O. gratissimum*, with particular emphasis on its potential application in sun-protective formulations. The investigation encompasses current scientific understanding of the plant's properties, methods of essential oil extraction, and approaches to incorporating these natural compounds into effective skincare formulations (Costa *et al*., 2015; Rahman *et al*., 2019).

1.2.1 Botanical Description

Ocimum gratissimum L. is a robust aromatic herb that exhibits remarkable morphological characteristics distinctive to the Lamiaceae family. The plant typically grows as an erect, multi-

branched shrub, reaching heights of 1-3 meters under favourable conditions (Ade-Ademilua, Obi, & Craker, 2013). The stem is quadrangular, woody at the base, and displays varying degrees of pubescence, often presenting a characteristic purple or green-purple colouration (Ade-Ademilua, Obi, & Craker, 2013; Chaachouay, Bussmann, & Elachouri, 2024; World Flora Online, 2024).

The leaves, which are the most distinguishing feature of the plant, are arranged in an opposite decussate pattern and demonstrate considerable morphological variation. They are typically ovate to ovate-lanceolate, measuring 5-13 cm in length and 3-9 cm in width. The leaf margins are serrated or crenate-serrate, and the leaf surface exhibits prominent venation patterns. The leaves possess numerous glandular trichomes responsible for the characteristic aromatic properties of the plant (Chaachouay, Bussmann, & Elachouri, 2024;World Flora Online, 2024)].

The inflorescence consists of verticillasters arranged in terminal racemes, typically 15-30 cm long. The flowers are small, bilabiate, and generally white to pale purple. Each flower has four stamens, which are exserted and declinate, with the anterior pair being longer. The calyx is bilabiate, with the upper lip being broadly oval and decurrent on the tube, while the lower lip consists of four narrow teeth (Ade-Ademilua, Obi, & Craker, 2013; Chaachouay, Bussmann, & Elachouri, 2024; World Flora Online, 2024).

The fruit consists of four small nutlets, approximately 1.5-2 mm long, which are smooth, brown to black in colour, and produce mucilage when wetted. The root system is extensive and well-developed, contributing to the plant's ability to thrive in various soil conditions (Chaachouay, Bussmann, & Elachouri, 2024; World Flora Online, 2024)..

Figure 1. 1:Ocimum gratissimum in its natural habitat (Sharma, Sharma, & Vikas, 2018)

1.2.3 Taxonomic Classification

The taxonomic hierarchy of *Ocimum gratissimum* follows the standard biological classification system (World Flora Online, 2024):

Kingdom: Plantae

- Subkingdom: Tracheobionta
- Superdivision: Spermatophyta
- Division: Magnoliophyta
- Class: Magnoliopsida
- Subclass: Asteridae
- Order: Lamiales
- Family: Lamiaceae
- Genus: *Ocimum*
- Species: *Ocimum gratissimum* L. (World Flora Online, 2024)

Recent molecular phylogenetic studies have confirmed the placement of *O. gratissimum* within the subfamily Nepetoideae, tribe (Gurav *et al*., 2020). The genus *Ocimum* comprises approximately 50-150 species, with considerable debate regarding the exact number due to high morphological variability and the existence of numerous cultivars and chemotypes (Dharsono *et al*., 2022).

1.2.4 Geographical Distribution

Ocimum gratissimum demonstrates a pantropical distribution pattern, with its origin traced to West Africa and Asia, particularly India (Oyemitan, 2017; (Mgbeje, Umoh, & Emmanuel-Ikpeme, 2019). The plant has successfully naturalized across various tropical and subtropical regions globally, showcasing its remarkable adaptability to diverse environmental conditions.

In Africa, the species is extensively distributed across the western, eastern, and central regions, with significant populations in Nigeria, Ghana, Cameroon, and Kenya (Mgbeje, Umoh, & Emmanuel-Ikpeme, 2019). The plant has particularly strong cultural and economic significance in West African countries, where it is widely cultivated both domestically and commercially.

In Asia, *O. gratissimum* is prevalent throughout the Indian subcontinent, Southeast Asia, and parts of East Asia. India represents a major centre of diversity for the species, with various chemotypes identified across different geographical regions. The plant has also established significant populations in Southeast Asia (Indonesia, Malaysia, Philippines, and Thailand) South America (Brazil, particularly in the northeastern regions), the Caribbean Islands (Various locations where it has been introduced and naturalised), and Pacific Islands (including Hawaii and other tropical Pacific territories)

The plant demonstrates remarkable ecological adaptability, thriving in various habitats including tropical rainforests, savannah regions, semi-arid zones, coastal areas, cultivated gardens and agricultural lands.

The widespread distribution of *O. gratissimum* has contributed to its diverse utilisation across different cultures and has led to the development of various chemotypes adapted to local

environmental conditions. This geographical diversity has also resulted in variations in the chemical composition of essential oils derived from different populations, a factor that has significant implications for its therapeutic applications (Padalia *et al*., 2018).

Figure 1. 2: Distribution of Ocimum gratissimum L (World Flora Online, 2024)

1.3 Chemical Constituents of *Ocimum gratissimum*

The phytochemical profile of *Ocimum gratissimum* is remarkably diverse and complex, encompassing numerous bioactive compounds that contribute to its therapeutic and cosmetic potential. Advanced analytical techniques, including GC-MS, HPLC, and LC-MS/MS, have enabled the detailed characterization of these constituents (Ugbogu *et al*., 2021).

1.3.1 Essential Oil Components

The essential oil composition, which typically represents 0.5-1.5% of the dried plant material, is characterized by several major chemical classes:

1.3.1.1 Phenylpropanoids

a) **Eugenol**: Primary Active Compound

Eugenol stands as the predominant bioactive compound in *O. gratissimum*'s essential oil, typically constituting approximately 75% of its total composition. This phenylpropanoid compound, characterized by its chemical formula $C_{10}H_{12}O_2$, exhibits remarkable versatility in its biological activities. Its molecular structure, featuring both a hydroxyl group and a methoxy group, contributes to its diverse therapeutic properties .

The compound demonstrates significant biological activities across multiple domains. It exhibits broad-spectrum antimicrobial activity, functions as a potent free radical scavenger and oxidation inhibitor and also modulates inflammatory response (Melo *et al*., 2019).

Notably, eugenol's concentration in the plant shows considerable variation depending on geographical location and harvest timing. These variations can be attributed to environmental

factors, genetic differences among populations, and seasonal changes, making standardization crucial for therapeutic applications (Melo *et al*., 2019).

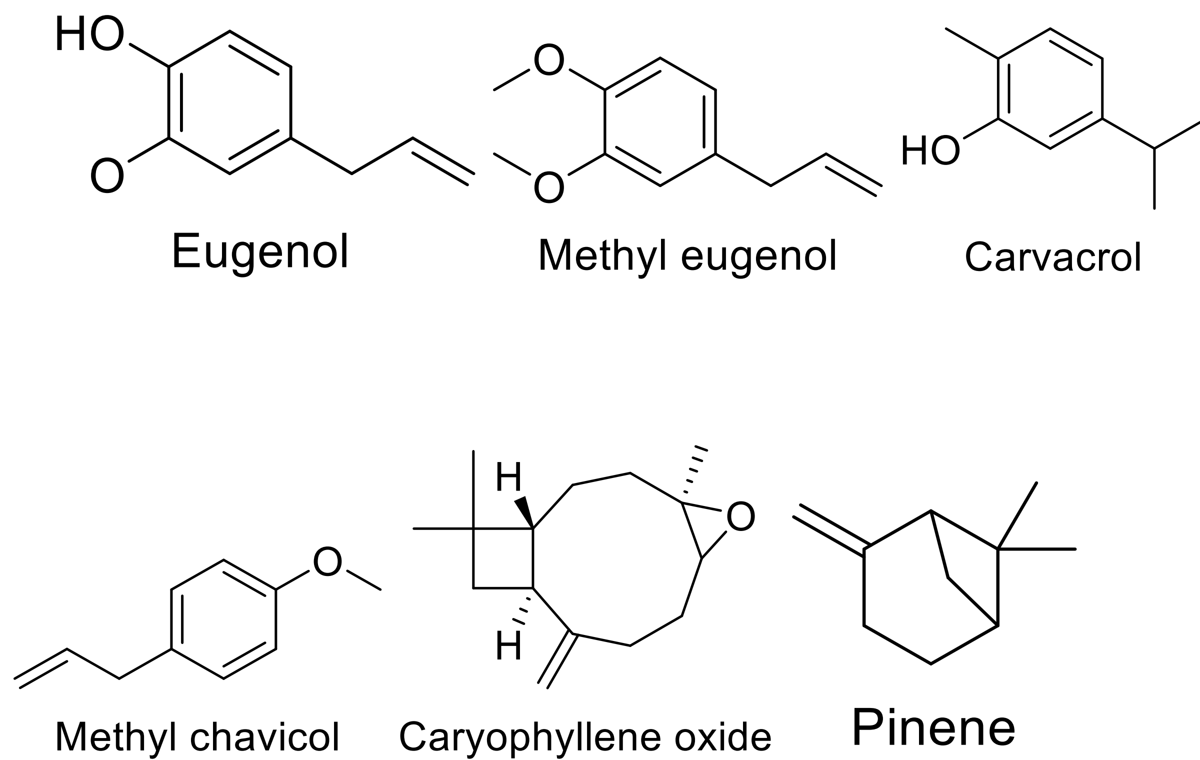


Figure 1.3: Chemical structures of some major constituents from the essential oil of Ocimum gratissimum dry leaves[A]

b) **Methyleugenol**:

Methyleugenol represents a significant secondary compound in the essential oil, comprising 2-15% of the total composition. This compound, a close structural relative of eugenol, plays a crucial role in defining the plant's characteristic aromatic profile. Its presence contributes significantly to the overall therapeutic potential of the essential oil through its antioxidant properties. It essentially contributes to the plant's distinctive aroma profile, and demonstrated antioxidant activity, complementing eugenol's effects by showing significant antioxidant activity. Concentration variability based on environmental and genetic factors.

1.3.1.2 Monoterpenes

a) **Hydrocarbon Monoterpenes** (10%)

The hydrocarbon monoterpenes, comprising approximately 10% of the total volatile composition, include several key compounds. γ-terpinene represents a major component, constituting 3-8% of this fraction, while α-pinene contributes 1-3%. β-pinene appears in smaller quantities, ranging from 0.5-2%, and p-cymene maintains a consistent presence of 2-5% of the total hydrocarbon monoterpene content (Melo *et al*., 2019).

b) **Oxygenated Monoterpenes** (16.8%)

The oxygenated monoterpenes, representing a larger fraction at 16.8%, demonstrate greater structural diversity and biological activity. Thymol emerges as the predominant compound in this category, comprising 5-10% of the fraction. Carvacrol follows with a concentration range of 2-7%, while linalool contributes 1-4%. The aromatic compound 1,8-cineole maintains a significant presence at 2-6% of the oxygenated monoterpene fraction (Melo *et al*., 2019).

1. 1.3.1.3 Sesquiterpenes (7.58%)

Sesquiterpenes constitute approximately 7% of the total volatile profile, with β-caryophyllene serving as the principal component in this category, ranging from 3-8%. α-humulene represents the second most abundant sesquiterpene, contributing 1-3% to the total composition. Germacrene D appears in moderate quantities ranging from 0.5-2%, while β-bisabolene maintains a smaller but consistent presence of 0.3-1.5% (Melo *et al.*, 2019).

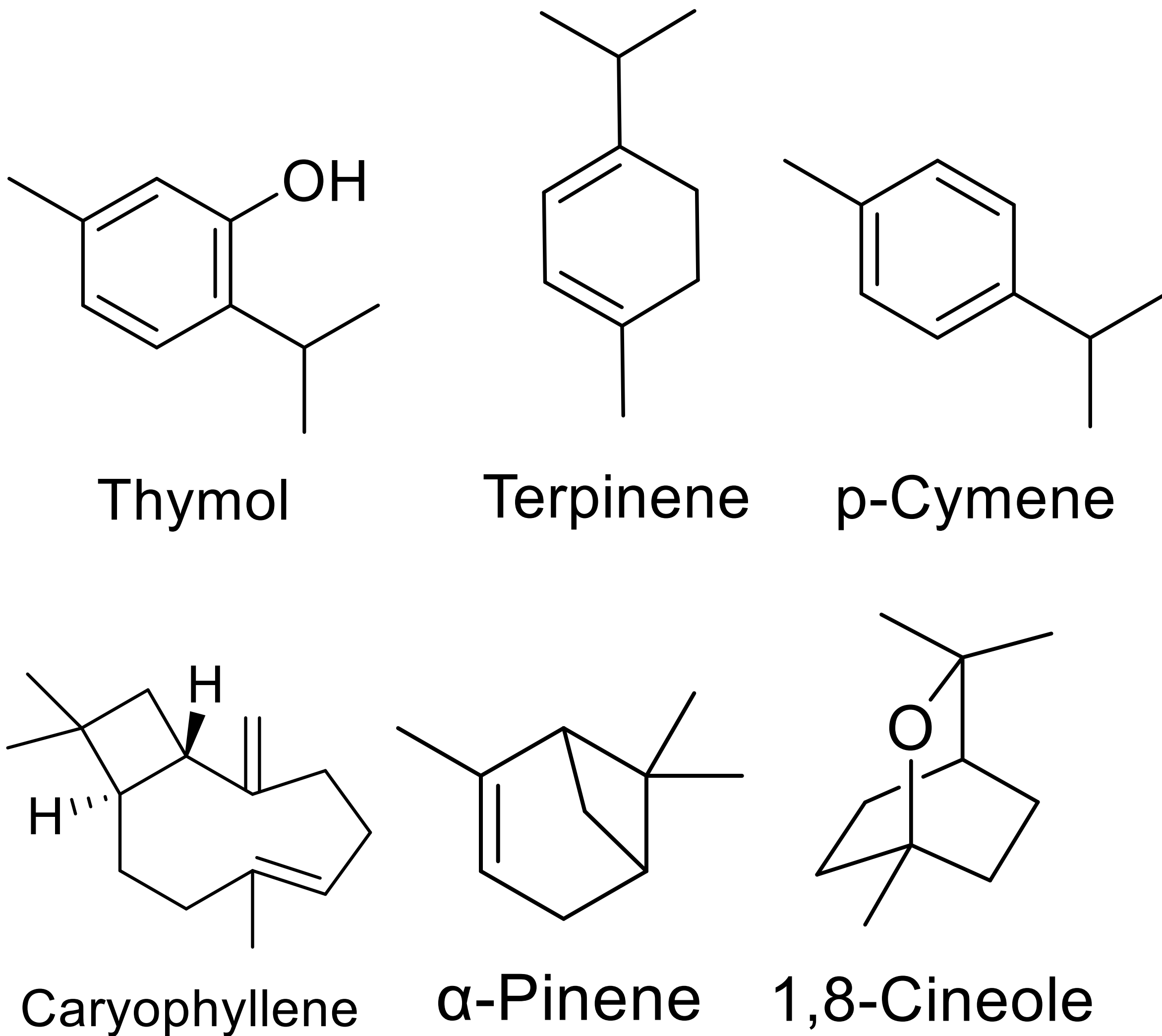


Figure 1.4: Chemical structures of some major constituents from the essential oil of Ocimum gratissimum dry leaves[B]

.

1.3.2 Non-Volatile Constituents

A. Flavonoids

Favonoid, demonstrating potent antioxidant and anti-inflammatory activities. Rutin, another major flavonoid, complements these properties while offering additional vascular protective effects. The plant also contains various kaempferol derivatives, which contribute to its overall antioxidant capacity and biological activities. Catechin, a flavanol subclass member, further enhances the plant's therapeutic potential through its strong antioxidant properties and potential cardiovascular benefits (Ugbogu *et al*., 2021).

B. Phenolic Acids

The phenolic acid composition of *O. gratissimum* encompasses a diverse array of bioactive compounds that play pivotal roles in its therapeutic properties. Among these, rosmarinic acid emerges as the predominant phenolic acid component, characterized by its robust antioxidant and anti-inflammatory activities (Ugbogu *et al*., 2021). Complementing this primary compound, the plant also contains significant levels of chlorogenic acid, and P-coumaric acid (Ugbogu *et al*., 2021).

C. Tannins

The tannin composition of *O. gratissimum* is characterized predominantly by hydrolysable tannins, which represent a complex group of polyphenolic compounds. Their presence contributes significantly to the plant's astringent properties, which play essential roles in both traditional medicinal applications and modern therapeutic uses. (Ugbogu *et al*., 2021).

1.3.3 Bioactive Properties of Major Constituents

1.3.3.1 UV-Protective Components

Recent scientific investigations have identified several compounds in *O. gratissimum* that demonstrate significant UV-protective properties:

a) **Phenolic Compounds**

Among the phenolic compounds, rosmarinic acid exhibits particularly strong UVA absorption characteristics, with a maximum absorption wavelength (λmax) of 329.1 nm (Ugbogu *et al*., 2021; Benedec *et al*., 2015). Rutin demonstrates comprehensive UVB filtering properties, characterized by multiple absorption peaks at 207, 257.5, and 358.5 nm, indicating its potential as a broad-spectrum UV protectant (Ugbogu *et al*., 2021; (Pinto *et al*., 2021).

b) **Essential Oil Components**

The essential oil components also contribute significantly to the plant's UV-protective properties.

Eugenol, the primary constituent, demonstrates moderate UV absorption capabilities with a maximum absorption wavelength of 254 nm (Ugbogu *et al*., 2021; Mahapatra & Roy, 2014).

Thymol, another key essential oil component, shows specific UVB protective properties, enhancing the overall photoprotective potential of the plant extract (Ugbogu *et al*., 2021).

1.3.3.2 Antioxidant

The major constituents of *Ocimum gratissimum* demonstrate antioxidant activity through multiple biochemical pathways.

1.3.3.2.1 Free Radical Scavengers

Free radical scavenging represents a primary mechanism of antioxidant activity, primarily facilitated by phenolic compounds. The key compounds responsible for this activity include rosmarinic acid, and rutin, each contributing to the plant's overall antioxidant capacity through their ability to neutralize harmful free radicals (Yeddes *et al*., 2019).

1.3.3.2.2 Metal Ion Chelators:

Metal ion chelation represents another crucial antioxidant mechanism, particularly exhibited by the flavonoid compounds present in the plant. These compounds demonstrate significant metal-chelating properties, effectively binding and neutralizing potentially harmful metal ions. The chelation capacity has been quantified through IC50 values, with rutin demonstrates significant chelation capacity (Cherrak, *et al.,*2014; Khater *et al*., 2019)..

1.3.4 Seasonal and Geographic Variation

The phytochemical composition of *O. gratissimum* demonstrates significant variability influenced by both temporal and spatial factors (Akinmoladun *et al*., 2017).

1.3.4.1 Seasonal Factors

The plant exhibits distinct seasonal patterns in its biochemical composition. Essential oil content reaches its maximum concentration during summer months, likely due to increased metabolic activity and environmental stress responses. The phenolic compound synthesis peaks specifically during the flowering stage, suggesting a correlation with reproductive development. Research has established that the optimal harvest time is during early morning hours of the flowering stage, when the concentration of bioactive compounds is at its highest. This timing maximizes the yield of therapeutic compounds while ensuring optimal resource utilization (Akinmoladun *et al*., 2017).

1.3.4.2 Geographic Location

The geographical distribution of *O. gratissimum* has led to the evolution of distinct chemotypes, each characterized by unique phytochemical profiles (Gurav, Dholakia, & Giri, 2022).

The African chemotype is distinguished by its remarkably high eugenol content, approaching 75% of total compounds. This high eugenol concentration likely reflects evolutionary adaptations to local environmental conditions and may contribute to specific traditional medicinal applications in African communities (Gurav, Dholakia, & Giri, 2022).

The Asian chemotype demonstrates moderate eugenol levels, typically ranging from 45-60%. This variation might be attributed to different selective pressures and cultivation practices across Asian regions. (Gurav, Dholakia, & Giri, 2022)

The Brazilian chemotype presents a distinctive profile with elevated thymol content (30-45%), representing a significant deviation from other regional variants. This unique chemical signature may offer specific therapeutic advantages and applications (Gurav, Dholakia, & Giri, 2022).

1.3.5 Stability

The preservation and maintenance of bioactive compounds in *O. gratissimum* are influenced by multiple factors, requiring careful attention to storage and handling conditions to maintain therapeutic efficacy.

1.3.5.1 Environmental Factors

Temperature plays a critical role in compound stability, with most bioactive components maintaining their integrity up to 40°C. Beyond this threshold, degradation rates increase significantly, potentially compromising therapeutic efficacy. The pH environment significantly impacts stability, with optimal preservation observed between pH 5.5-7.0. This pH range appears

to minimize hydrolysis and other degradative reactions. Light exposure presents a particular challenge for photosensitive compounds, necessitating specific protective measures to prevent photo-degradation and maintain potency.

1.3.5.2 Storage Conditions

The longevity of different compound classes varies significantly under controlled storage conditions. Generally, the essential oil components demonstrate reliable stability for 6-12 months when maintained at 4°C, though this can be extended under optimal conditions. This stability period is crucial for commercial applications and quality control.

Phenolic compounds exhibit more extended stability, maintaining their integrity for 18-24 months when properly protected from light exposure. This longer stability period makes these compounds particularly valuable for long-term storage and pharmaceutical applications.

To maximize the preservation of all bioactive compounds, storage in dark, airtight containers at 4°C is strongly recommended. This combination of conditions minimizes exposure to degradative factors while maintaining compound integrity (Macobowitz & Weng, 2020).

1.4 Traditional and Medicinal Uses

O. gratissimum has a rich history of traditional and medicinal uses across various cultures, particularly in Africa, Asia, and South America. This aromatic plant has been utilized for centuries in folk medicine, culinary applications, and cultural practices (Ekor, 2014).

1.4.1 Traditional Uses

1.4.1.1 Culinary Applications:

In many African and Asian cuisines, *O. gratissimum* leaves are used as a flavouring agent in soups, stews, and sauces. The strong, clove-like aroma and slightly peppery taste make it a popular spice in traditional dishes (Edeoga, Okwu, & Mbaebie, 2020).

1.4.1.2 Insect Repellent:

Traditionally, the plant has been used to repel insects. Fresh leaves or the smoke from burning dried leaves have been employed to keep mosquitoes and other pests at bay (Okigbo & Ogbonnaya, 2019).

1.4.1.3 Spiritual and Cultural Practices:

In some African cultures, *O. gratissimum* is considered a sacred plant and is used in various rituals and ceremonies (Akinmoladun *et al*., 2021).

1.4.2 Medicinal Uses

1.4.2.1 Respiratory Ailments:

O. gratissimum has been widely used to treat various respiratory conditions, including coughs, colds, and bronchitis. The leaves are often prepared as an infusion or decoction for this purpose. Recent studies have shown that the essential oil from *O. gratissimum* possesses significant antimicrobial activity against respiratory pathogens, supporting its traditional use (Nwinyi *et al*., 2018).

1.4.2.2 Gastrointestinal Disorders:

Traditional healers have long used *O. gratissimum* to treat diarrhoea, dysentery, and other digestive issues. The plant's antimicrobial and antispasmodic properties are believed to contribute

to its efficacy in treating these conditions. Research is currently being carried out to demonstrate the plant's potential in managing gastrointestinal disorders, with studies showing its effectiveness against various enteric pathogens (Akinmoladun *et al*., 2020).

1.4.2.3 Skin Conditions:

O. gratissimum has been traditionally applied topically to treat various skin ailments, including fungal infections, wounds, and insect bites. The leaves are often crushed and applied directly to the affected area or prepared as a poultice. Modern studies have corroborated these traditional uses, demonstrating the plant's antimicrobial and wound-healing properties (Prabhu *et al*., 2019).

1.4.2.4 Fever and Malaria:

In many tropical regions, *O. gratissimum* has been used to treat fevers, including those associated with malaria. The leaves are typically prepared as a decoction or infusion for this purpose (Odugbemi *et al*., 2021). Recent research has shown promising results in the plant's antimalarial activity, supporting its traditional use (Odugbemi *et al*., 2021).

1.4.2.5 Pain Management:

Traditional healers have used *O. gratissimum* to alleviate various types of pain, including headaches and arthritis. The leaves or essential oil are often applied topically or prepared as an infusion for internal use. Studies have demonstrated the analgesic and anti-inflammatory properties of *O. gratissimum* extracts, providing scientific backing for this traditional application (Iwalewa *et al*., 2020).

1.4.2.6 Cardiovascular Health:

In some traditional medicine systems, *O. gratissimum* has been used to support cardiovascular health. While still under debate, some studies have shown that plant extracts may have

hypotensive effects and could potentially be beneficial in managing hypertension (Akinmoladun *et al*., 2021).

1.4.2.7 Diabetes Management:

Traditional use of *O. gratissimum* in managing diabetes has been reported in various cultures. Some studies have provided some evidence for the plant's hypoglycemic effects, suggesting potential applications in diabetes management (Edeoga *et al*., 2020).

1.4.2.8 Immunomodulatory Effects:

While not a traditional application, recent research has uncovered potential immunomodulatory effects of *O. gratissimum* extracts. These findings suggest possible applications in enhancing immune function and managing immune-related disorders (Jacobowitz & Weng, 2020).

1.5 Essential Oils

1.5.1 Definition and Characteristics

Essential oils are complex mixtures of volatile organic compounds produced by aromatic plants as secondary metabolites. These oils are characterized by their strong odour and are typically liquid at room temperature, although some may be solid or resinous (Bakkali *et al*., 2020).

Key characteristics of essential oils include:

1. ***Volatility**:* Essential oils readily evaporate at room temperature, contributing to their aromatic properties
2. ***Hydrophobicity**:* They are generally insoluble in water but soluble in organic solvents, oils, and alcohol
3. ***Complex Composition**:* Essential oils can contain hundreds of different chemical compounds, primarily terpenes and terpenoids, as well as aromatic and aliphatic constituents

4. ***Bioactivity****:* Many essential oils exhibit various biological activities, including antimicrobial, antioxidant, and anti-inflammatory properties
5. ***Variability****:* The chemical composition of essential oils can vary based on factors such as plant species, geographic location, harvest time, and extraction method (Burt, 2021).

1.5.2 Extraction Methods

Several methods are used to extract essential oils from plant materials, each with its advantages and limitations:

1.5.2.1 Hydro-distillation:

This is one of the most common and traditional methods of essential oil extraction. The process involves plant material being immersed in water and heated to boiling. The steam carries the volatile oil compounds, which are then condensed and collected. This method is suitable for heat-sensitive materials, relatively simple and cost-effective. Prolonged exposure to high temperatures may alter some oil components which bring about its major limitation (Chemat *et al*., 2021).

Hydro-distillation remains one of the most widely used methods for essential oil extraction, particularly for laboratory-scale and small-scale production. Its popularity is due to its simplicity, cost-effectiveness, and suitability for a wide range of plant materials (Bakkali *et al*., 2020; (Azmir *et al*., 2021).

The process of hydro-distillation can be summarized in the following steps:

1. Plant material is placed in a still and covered with water.
2. The mixture is heated to boiling, causing the plant cells to rupture and release the essential oil.
3. The oil vapours, along with steam, rise and pass through a condenser.

4. The condensed mixture of oil and water (hydrosol) is collected.
5. The oil, being less dense than water, floats on top and is separated (Azmir *et al*., 2021).

Recent advancements in hydro-distillation include the development of improved condensers, the use of ultrasound-assisted hydro-distillation to enhance oil yield, and the implementation of automated systems for large-scale production

1.5.2.2 Steam Distillation:

Steam distillation is similar to hydro-distillation, but steam is generated in a separate chamber and passed through the plant material. It is faster than hydro-distillation, with less risk of thermal degradation, given its advantages over hydro-distillation. However, it is not suitable for all plant materials (Chemat *et al*., 2021; Azmir *et al*., 2021)

1.5.2.3 Solvent Extraction:

Solvent extraction involves using organic solvents to extract the aromatic compounds, followed by solvent removal. One of its advantages is that it can be used to extract heat-sensitive compounds. However, it comes with a limitation of risk of solvent residues in the final product (Chemat *et al*., 2021).

1.5.2.4 Supercritical Fluid Extraction (SFE):

SFE uses supercritical fluids, often CO2, to extract essential oils. Although it is a low-temperature process, with no solvent residues, and high selectivity, making it incredibly advantageous, the high initial equipment cost makes it a non-common process (Chemat *et al*., 2021).

1.5.2.5 Microwave-assisted Extraction:

Basically, this process makes use of microwave energy to heat the plant material and release essential oils. It is a rapid extraction, and reduced solvent use, however, its potential for thermal degradation of some compounds is a limiting factor for use (Chemat *et al*., 2021).

1.5.3 Essential Oil from *Ocimum gratissimum*

The essential oil of *Ocimum gratissimum* has been the subject of numerous studies due to its unique chemical composition and potential therapeutic applications.

1.5.3.1 Chemical Composition:

The major components of *O. gratissimum* essential oil typically include eugenol, thymol, and γ-terpinene, although the exact composition can vary based on geographic location and other factors. Other compounds found in significant amounts include p-cymene, α-pinene, β-caryophyllene, and germacrene D (Edeoga *et al*., 2020)

1.5.3.2 Yield:

The essential oil yield from *O. gratissimum* typically varies on a dry weight basis, depending on various factors including plant part used, harvest time, and extraction method (Burt, 2021; Azmir *et al*., 2021).

1.5.3.3 Biological Activities:

Antimicrobial: *O. gratissimum* essential oil has shown strong antibacterial and antifungal activities against a wide range of pathogens (Akinmoladun *et al*., 2021; Nwinyi *et al*., 2018).

Antioxidant: The oil exhibits significant free radical scavenging activity, attributed mainly to its phenolic components (Melo *et al*., 2019; Mahapatra & Roy, 2014).

Anti-inflammatory: Studies have demonstrated the oil's potential in reducing inflammation, suggesting possible applications in treating inflammatory conditions. (Nwinyi *et al*., 2018).

Insecticidal: The oil has shown promising results as a natural insecticide and repellent (Akinmoladun *et al*., 2021; Nwinyi *et al*., 2018).

1.5.3.4 Extraction Considerations:

Hydro-distillation is commonly used for extracting *O. gratissimum* essential oil, particularly in research settings. Hence, factors such as distillation time, plant material to water ratio, and plant part used (leaves, flowers, or whole plant) can significantly affect the yield and composition of the oil (Bakkali *et al*., 2020; Azmir *et al*., 2021).

1.5.3.5 Potential Applications:

The oil's antimicrobial and anti-inflammatory properties suggest potential use in developing new drugs or topical treatments making it a candidate for pharmaceutical consideration. Its antioxidant properties make it a candidate for anti-ageing and skin protection formulations for cosmetic applications. In addition, the insecticidal properties of the oil could be harnessed for natural pest control solutions for agriculture (Bakkali *et al*., 2020; Burt, 2021).

1.6 Antioxidative and Anti-aging Properties of *Ocimum gratissimum*

Ocimum gratissimum has garnered significant attention in recent years due to its potent antioxidative properties and potential anti-ageing effects. These properties are primarily attributed to the plant's rich phytochemical profile, which includes a variety of bioactive compounds such as phenolics, flavonoids, and essential oils (Edeoga *et al*., 2020; (Akinmoladun *et al*., 2020).

Antioxidants play a crucial role in neutralizing free radicals and reducing oxidative stress, which is a major contributor to cellular damage and aging. Several studies have demonstrated the strong antioxidant capacity of *O. gratissimum* extracts and essential oils (Akinmoladun *et al*., 2020)

. For instance, research reported that the methanolic extract of *O. gratissimum* leaves exhibited significant free radical scavenging activity, with IC50 values relatively comparable to standard antioxidants like ascorbic acid (Irondi *et al*., 2016; Ojo *et al*., 2013).

The essential oil of *O. gratissimum* is particularly rich in antioxidant compounds. A study by identified eugenol as the major component of the essential oil, constituting up to 60% of its composition. Eugenol is known for its potent antioxidant properties, capable of neutralizing various free radicals and inhibiting lipid peroxidation (Akinmoladun *et al*., 2021; (Edeoga *et al*., 2020).

In addition to eugenol, other compounds such as thymol, γ-terpinene, and p-cymene have been identified in *O. gratissimum* essential oil, all of which contribute to its overall antioxidant activity (Edeoga *et al*., 2020). These compounds work synergistically to provide a broad spectrum of protection against oxidative damage.

The anti-aging potential of *O. gratissimum* is closely linked to its antioxidant properties. Oxidative stress is a key factor in skin aging, leading to the breakdown of collagen and elastin, formation of wrinkles, and hyperpigmentation. By neutralizing free radicals, the antioxidants in *O. gratissimum* can help mitigate these effects and promote healthier, younger-looking skin (Akinmoladun *et al*., 2021; Edeoga *et al*., 2020).

Furthermore, studies have shown that *O. gratissimum* extracts can modulate various biochemical pathways associated with ageing. For example, Edeoga *et al*., 2020 demonstrated that *O.*

gratissimum extract could inhibit the activity of enzymes such as elastase and collagenase, which are responsible for breaking down structural proteins in the skin. This inhibitory effect could potentially help maintain skin elasticity and firmness.

The anti-inflammatory properties of *O. gratissimum* also contribute to its anti-ageing effects. Chronic inflammation is associated with accelerated ageing, and compounds found in *O. gratissimum*, particularly eugenol, have been shown to possess significant anti-inflammatory activity (Nwinyi *et al*., 2018; Akinmoladun *et al*., 2021). By reducing inflammation, these compounds may help slow down the ageing process and improve overall skin health.

Recent research has also explored the potential of *O. gratissimum* in protecting against UV-induced skin damage, a major factor in premature skin ageing. In a study, the topical application of *O. gratissimum* essential oil provided significant protection against UV-induced oxidative stress in skin cells, suggesting its potential use in sun protection formulations (Akinmoladun *et al*., 2021; Nwinyi *et al*., 2018).

In conclusion, the antioxidative and anti-ageing properties of *Ocimum gratissimum* make it a promising candidate for natural skincare formulations. Its rich phytochemical profile, particularly the high content of eugenol and other antioxidant compounds in its essential oil, provides multi-faceted protection against oxidative stress and age-related skin changes. However, further research is needed to fully elucidate the mechanisms of action and to optimise the incorporation of *O. gratissimum* extracts or essential oils into effective skincare products (Akinmoladun *et al*., 2021; Nwinyi *et al*., 2018).

1.6 Physicochemical Analysis of *Ocimum gratissimum* Essential Oil

1.6.1 Physical Properties

1.6.1.1 Appearance and Colour:

Typically, *O. gratissimum* EO is a pale yellow to amber coloured liquid essential oil. They may vary in colour intensity based on geographical origin, extraction method, storage conditions or a combination of these factors. The oil is clear without particulate when it is filtered properly.

1.6.1.2 Odour:

Its strong, spicy and aromatic character comes mostly from its high eugenol content. This distinctive scent explains the application of the scent in aromatherapy and as a flavouring agent (Okwu *et al*., 2023).

1.6.1.3 Solubility:

O. gratissimum oil is hydrophobic and insoluble in water. But they are readily soluble in organic solvents, for example in ethanol, ether, and chloroform. Often purity is determined by solubility into alcohol (Ikeotouye *et al*., 2023; Coulibaly *et al*., 2023).

1.6.1.4 Specific Gravity:

The specific gravity reported for *O. gratissimum* essential oil is 0.89 to 0.93 at 20°C. This is a property of oil's density compared to water — it distinguishes the product from adulterated products (Ikeotouye *et al*., 2023; Coulibaly *et al*., 2023).

1.6.1.5 Refractive Index:

The refractive index of the oil, determined at 20°C, was within the range of 1.334 to 1.482 by some studies (Ikeotouye *et al*., 2023; Coulibaly *et al*., 2023; Wang *et al*., 2023). This parameter gives an indication of the optical properties of the oil and its purity. The refractive index is a physical property that is used frequently to test the purity of oils. The lower the refractive index is, the better the quality of the EO (Ikeotouye *et al*., 2023; Coulibaly *et al*., 2023).

1.6.2 Chemical Properties

1.6.2.1 Acid Value:

The acid value which measures the free fatty acid content of the oil and indicates its freshness and stability, for *O. gratissimum* essential oil, is generally low (1.5-2.5 mg KOH/g), reflecting minimal hydrolysis and good quality (Ikeotouye *et al*., 2023; Coulibaly *et al*., 2023).

1.6.2.2 Saponification Value:

Molecular weight of triglycerides of the essential oil is determined by saponification value. The value of *O. gratissimum* oil is moderate 150–180 mg KOH/g, in its monoterpenes and sesquiterpenes content (Ikeotouye *et al*., 2023; Coulibaly *et al*., 2023).

1.6.2.3 Iodine Value:

O. gratisssimum essential oil has a moderate iodine value, between (150–180 mg KOH/g.), which is in line with its moderate content in unsaturated compounds (Ikeotouye *et al*., 2023)

1.6.2.4 pH:

The pH is in the mild acidic range (4.5–6.5). This range is suitable for cosmetic and pharmaceutical applications (Ikeotouye *et al*., 2023)

1.6.2.5 Stability:

The *O. gratissimum* essential oil is photosensitive as well as heat sensitive. Hence, they are stored in dark or airtight opaque containers, at low temperatures (ideally 4°C) (Wang *et al*., 2023). Its main constituents, such as eugenol and thymol, undergoes degradation when exposed to light and oxygen. Slightly acidic to neutral pH are the most stable oil conditions (Ikeotouye *et al*., 2023; Coulibaly *et al*., 2023; Wang *et al*., 2023).

1.6.2.6 UV Absorption Spectrum:

Since phenolic compounds, such as eugenol, are present, there is a high absorption of the oil in the UV-B range. UV-visible spectroscopic analysis shows maximum absorption at 254 nm and 280 nm (Ikeotouye *et al*., 2023).

1.7 Sun Protective Factor (SPF)

1.7.1 Definition and Calculation

Sun Protection Factor (SPF) is a measure of how well a sunscreen product protects the skin from ultraviolet B (UVB) radiation, which is primarily responsible for sunburn and plays a significant role in the development of skin cancer (Brannon, 2023). The SPF value indicates how much longer a person can stay in the sun without burning compared to unprotected skin. For example, an SPF of 15 means that it would take 15 times longer for the skin to burn than if no sunscreen were applied (Brannon, 2023).

SPF is a measure of a sunscreen's ability to prevent UVB-induced erythema. It is defined as the ratio of the minimal erythemal dose (MED) on protected skin to the MED on unprotected skin:

$$SPF = MEDprotected / MEDunprotected$$

Where MED is the minimum dose of UV radiation required to produce perceptible erythema (Brannon, 2023).

1.7.2 In Vitro SPF Determination: Mansur Equation

For in vitro SPF determination, the Mansur equation is widely used:

$$SPF = CF \times \Sigma(EE(\lambda) \times I(\lambda) \times Abs(\lambda))$$

Where:

- CF: Correction factor (10)
- $EE(\lambda)$: Erythemal effect spectrum
- $I(\lambda)$: Solar intensity spectrum
- $Abs(\lambda)$: Absorbance of the sunscreen product

The values of “EE” × “I” are predetermined constants for each wavelength (FDA, 2017; Williams *et al*., 2018).

1.7.3 Natural Products as Sun Protectants

The importance of SPF in skincare products has grown significantly in recent years due to increased awareness of the harmful effects of UV radiation on skin health, including sunburn, premature aging (photoaging), skin cancer, immune system suppression, and hyperpigmentation (Williams *et al*., 2018; FDA, 2017)

There is a growing interest in natural products as potential sun protectants due to concerns about the environmental impact and potential health risks associated with some synthetic UV filters (Jesus, 2022). Natural products, including plant extracts and essential oils, often contain compounds that can absorb or scatter UV radiation, providing a degree of sun protection (Korac *et al*., 2011).

1.7.3.1 Phenolic compounds: Such as flavonoids and tannins, which have been shown to absorb UV radiation and possess antioxidant properties (Williams *et al*., 2018).

1.7.3.2 Carotenoids: Like β-carotene and lycopene, which can absorb UV light and quench reactive oxygen species (Dewanjee *et al*., 2021).

1.7.3.3 Essential oils: Some essential oils contain compounds that can absorb UV radiation, although their efficacy can vary widely (Kaur & Rath, 2019).

Essential oils have gained attention for their potential role in sun protection due to their natural ability to absorb UV radiation. While their efficacy varies depending on the composition of bioactive compounds, some oils exhibit measurable Sun Protection Factor (SPF) values. Table 1.1 presents the SPF values of various herbal oils, highlighting their potential as natural UV protectants.

Table 1.1 Sun protection factor values of herbal oils (Kaur *et al*.,2010)

Essential Oil	SPF Value
Olive oil	7.549
Coconut oil	7.119
Almond oil	4.659
Castor oil	5.687
Mustard oil	2.105
Sesame oil	1.771
Peppermint oil	6.668
Eucalyptus oil	2.625
Lavender oil	5.624
Rose oil	0.248

1.7.4 Evaluation of SPF in Natural Products

Several methods are used to evaluate the SPF of natural products:

1.7.4.1 In vitro methods:

a) **UV spectrophotometry**: This is one of the most common methods for SPF determination in natural products. It involves measuring the absorption or transmission of UV radiation through a thin film of the product (Salian *et al*., 2021).

b) **HPLC analysis**: High-performance liquid chromatography can be used to identify and quantify specific UV-absorbing compounds in natural extracts (D'Orazio *et al*., 2013).

1.7.4.2 In vivo methods:

Human subject testing: This involves applying the product to human skin and measuring the minimal erythema dose (MED) with and without the product. While considered the gold standard, this method is expensive and raises ethical concerns (Baker *et al*., 2017).

1.7.4.3 Cell culture methods:

These involve exposing cultured skin cells to UV radiation in the presence and absence of the test product (Bosch *et al*., 2015).

1.7.5 Challenges in SPF Evaluation of Natural Products

Several challenges are associated with evaluating SPF in natural products:

1.7.5.1 Variability in composition: Natural products can vary significantly in their chemical composition based on factors such as geographic location, harvest time, and extraction method, leading to inconsistent SPF values (Draelos, 2019).

1.7.5.2 Stability issues: Some natural UV filters may be unstable when exposed to light or heat, potentially reducing their effectiveness over time (Wulf & Philipsen, 2020).

1.7.5.3 Formulation effects: The SPF of a natural product can be significantly influenced by the formulation in which it is incorporated. Factors such as pH, emulsion type, and the presence of other ingredients can all affect SPF (Kryczyk-Poprawa *et al*., 2019).

1.7.5.4 Broad-spectrum protection: While many natural products show good UVB protection (measured by SPF), their UVA protection may be limited. Comprehensive sun protection requires both UVA and UVB coverage (Wang *et al*., 2017).

1.7.6 Formulation Considerations for Natural SPF Products

When formulating natural SPF products, several factors must be considered:

1.7.6.1 Stability: Many natural UV filters are photolabile and require stabilization techniques such as encapsulation or addition of antioxidants (Moyal *et al*., 2015; Moyal *et al*., 2020).

Synergistic effects: Combining multiple natural extracts or essential oils can lead to enhanced SPF values. For example, a combination of titanium dioxide with natural extracts showed improved photoprotection (Saewan & Jimtaisong, 2015).

Broad-spectrum protection: While many natural products show good UVB protection, their UVA protection may be limited. Formulations should aim for balanced broad-spectrum coverage (Kim & Kang, 2022).

Vehicle effects: The choice of vehicle (e.g., emulsion type, film-forming agents) can significantly impact the SPF value and photostability of the formulation (Lionetti & Rigano, 2017).

1.7.7 Recent Advances in Natural SPF Products

Recent research has shown promising results for several natural products:

1. **Green tea extracts**: Rich in polyphenols, green tea extracts have demonstrated significant photoprotective effects in both in vitro and in vivo studies (Torres *et al*., 2020).
2. **Aloe vera**: Known for its skin-soothing properties, aloe vera has also shown potential as a natural sunscreen ingredient, with some studies reporting SPF values between 2-3 (Bhattacharjee *et al*., 2021).
3. **Red algae**: Certain species of red algae contain mycosporine-like amino acids (MAAs) that have shown strong UV-absorbing properties (Geraldes & Pinto, 2021).
4. **Essential oils**: Some studies have reported moderate SPF values for certain essential oils, including peppermint and tulsi oils, although more research is needed to confirm their efficacy and safety as sunscreen ingredients (Kaur & Rath, 2019).

1.7.8 Future Directions

The field of natural sun protection is evolving rapidly, with several promising areas for future research. **Synergistic combinations** which involve investigating combinations of natural products that may provide broad-spectrum UV protection and antioxidant effects (Rajasekar *et al*., 2024). **N**anoencapsulation techniques may improve the stability and efficacy of natural UV filters(Rajasekar *et al*., 2024). Standardized methods can be developed for evaluating the SPF and broad-spectrum protection of natural products to ensure consistency across studies (González *et al*., 2022). Additionally, comprehensive safety assessments of natural UV filters should be conducted, particularly for long-term use and potential environmental impacts.

1.8 Topical Formulations of Essential Oils

Essential oils have gained significant attention in recent years for their potential therapeutic benefits when incorporated into topical formulations. The development of effective and stable

essential oil-based creams, lotions, and ointments presents both opportunities and challenges in the field of natural product-based skincare (Aditi & Dabral, 2023).

1.8.1 Incorporation Methods

Several approaches have been explored for incorporating essential oils into topical formulations:

1.8.1.1 Direct incorporation: This method involves adding the essential oil directly into the oil phase of the formulation. However, this can lead to stability issues due to the volatile nature of essential oils (Ferreira & Nunes, 2019).

1.8.1.2 Emulsification: Essential oils can be emulsified using suitable surfactants to create stable oil-in-water or water-in-oil emulsions. This method improves the dispersion and stability of the essential oil in the formulation (Ali *et al*., 2015).

1.8.1.3 Microencapsulation: This advanced technique involves encapsulating essential oil droplets within a protective shell, often made of polymers. Microencapsulation can enhance the stability of the essential oil and provide controlled release properties (Ferreira & Nunes, 2019).

1.8.1.4 Nanoencapsulation: Similar to microencapsulation but on a smaller scale, nanoencapsulation can improve the penetration of essential oils through the skin and enhance their bioavailability (Ferreira & Nunes, 2019).

1.8.2 Formulation Considerations

When developing topical formulations containing essential oils, several factors must be considered:

1.8.2.1 Concentration: The concentration of essential oils in topical formulations typically ranges from 1-5%, depending on the specific oil and intended use. Higher concentrations may increase the risk of skin irritation (Ali *et al*., 2015).

1.8.2.2 Stability: Essential oils are prone to oxidation and degradation when exposed to light, heat, and air. Antioxidants and appropriate packaging materials can help maintain the stability of the formulation (Carpena *et al*., 2021).

1.8.2.3 Skin penetration: The base formulation can significantly affect the skin penetration of essential oil components. Lipid-based vehicles often enhance penetration compared to aqueous-based formulations (Cornwell, 2018).

1.8.2.4 Compatibility: The compatibility of essential oils with other ingredients in the formulation must be carefully evaluated to prevent phase separation or chemical interactions that could compromise efficacy or safety (Shaaban, 2020).

Recent Advances

Recent research has focused on improving the efficacy and stability of essential oil-based topical formulations:

1.8.3.1 Nanoemulsions: Nanoemulsions of tea tree oil with enhanced antimicrobial activity and improved stability compared to conventional emulsions (Salvia-Trujillo *et al*., 2015).

1.8.3.2 Liposomes: Demonstration of liposomal formulations of lavender essential oil exhibited improved skin permeation and prolonged release compared to non-liposomal formulations (Nagula & Wairkar, 2019).

1.8.3.3 Natural polymer-based films: Biodegradable films incorporated into eucalyptus essential oil for potential wound healing applications, showcasing the versatility of essential oil formulations (Borges *et al*., 2024).

1.8.3.4 Synergistic combinations: Some studies have explored the potential synergistic effects of combining multiple essential oils or pairing them with other natural compounds to enhance their therapeutic properties in topical formulations (Saewan & Jimtaisong, 2015).

1.9 Justification for the Study

The investigation of natural products for their potential in skincare and sun protection has gained significant traction in recent years, driven by increasing consumer demand for safer, more environmentally friendly alternatives to synthetic compounds. Within this context, *O. gratissimum*, has emerged as a promising candidate for exploration due to its rich phytochemical profile and traditional uses in herbal medicine (Ugbogu *et al*., 2021; Melo *et al*., 2019). Essential oils have demonstrated a wide range of biological activities relevant to skin health, including antioxidant, anti-inflammatory, and antimicrobial properties (Mahapatra & Roy, 2014; (Melo *et al*., 2019); (Ugbogu *et al*., 2021; Nwinyi *et al*., 2018). The complex mixture of volatile compounds found in essential oils often provides multi-faceted benefits that can address various aspects of skin protection and care simultaneously (Nwinyi *et al*., 2018).

With increasing awareness of the harmful effects of UV radiation on skin health, there is a growing need for effective, broad-spectrum sun protection agents (Kim & Kang, 2022). While synthetic sunscreens are widely available, concerns about their environmental impact and potential long-term health effects have spurred research into natural alternatives (Ajayi *et al*., 2024).

This study aims to bridge traditional knowledge about *O. gratissimum* with modern scientific methods to develop a potentially effective and natural sun protective formulation. By extracting the essential oil using hydro-distillation, a well-established method for obtaining high-quality essential oils, the study ensures the preservation of the plant's volatile compounds (Moradi *et al*., 2018).

1.10 Aim of Study

The aim of the study is to extract essential oil from *Ocimum gratissimum* (scent leaves), formulate it into a cream, and evaluate its sun protection factor (SPF) for potential use in skincare products.

1.11 Specific Objective

The specific objectives of the study were to:

1 To extract the essential oil from *Ocimum gratissimum* using hydro-distillation with a Clevenger apparatus.

2 To determine the physicochemical properties of the essential oil, including specific gravity, refractive index, acid value, saponification value, iodine value, using standard analytical methods in compliance with pharmacopoeia guidelines.

3 To formulate a cream containing the extracted essential oil, following a standard oil-in-water emulsion method.

4 To determine the antioxidant activity of the essential oil using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay.

5 To determine the sun protection factor (SPF) of the formulated cream using the Mansur equation and UV-Visible spectrophotometry.

CHAPTER TWO

MATERIALS AND METHODS

2.1 Materials

2.1.1 Chemicals and Reagents

2.1.1.1 Analytical graded chemical: 2,2-diphenyl-1-picrylhydrazyl (DPPH), Methanol (JHD, China), Ascorbic acid, Cetyl alcohol (Merck), Stearic acid, Glycerin, Triethanolamine, Methyl paraben, Propyl paraben, Potassium hydroxide (KOH), Wijs solution, Sodium thiosulfate, Phenolphthalein indicator, Chloroform (JHD, China), Acetone (JHD, China), Hexane, Potassium iodide, Sodium hydroxide, Hydrochloric acid (37%, Merck), 96% Ethanol (JHD, China), Disodium hydrogen phosphate (Na_2HPO_4).

2.1.1.2 Ingredients for cream formulation: White soft paraffin

Cetomacrogol emulsifying wax, Cetyl alcohol, Stearic acid, Triethylamine, Olive oil, Coconut oil, Chlorocresol, EDTA, Water

2.1.1.3 Reagents for physicochemical analysis: Carbon tetrachloride, Potassium iodide (10%), Sodium thiosulfate (0.1 M), Chloroform, Potassium hydroxide (0.5 M ethanolic), Hydrochloric acid (0.5 M), Phenolphthalein indicator, Diethyl ether

2.1.2 Equipment and Apparatus

Clevenger apparatus, UV-Visible spectrophotometer, Digital pH meter, electronic weighing balance (QHAUS Model; Cp114 with max wt. at 110g s/n B305741598), Ostwald Capillary U-Tube Viscometer, Abbe refractometer (Biobase), magnetic stirrer, heating plate, Homogenizer, Water bath, Digital thermometer

2.1.2.2 Laboratory glassware: Sterile volumetric flasks (10, 25, 50, 100, 250, 500, 1000 mL), Sterile measuring cylinders (10, 25, 50, 100, 250, 500, 1000 mL), Sterile beakers (50, 100, 250, 500, 1000, 2000 mL), Sterile round bottom flasks (500, 1000, 5000 mL), Sterile conical flasks (100, 250, 500 mL), Sterile burettes (50 mL), Sterile pipettes (1, 2, 10, 25 mL)

2.1.2.3 Laboratory accessories: Mixing vessels, Spatulas, Aluminium foil, Amber glass bottles, Sample vials, Micropipettes (100-1000 µL, 1-10 mL), Parafilm, pH strips, Thermometers, Stop watch, Sampling bottles, Rubber tubing, Clamps and stands, heating plate, Stirring rods, Dispensing jars, Glass stirrer, mortal and pestle.

2.2 Methods

2.2.1 Collection and Preparation of Plant Material

Fresh leaves of *Ocimum gratissimum* were collected from Ekosodin and Oluku community, Ugbowo, Benin, Edo State, Nigeria during the early morning hours. The plant material was authenticated by Prof. Akinnibosun Henry Adewale, from the Department of Plant Biology and Biotechnology herbarium unit, where voucher specimen number UBH-O333 was assigned and deposited.

2.2.2 Essential Oil Extraction

Fresh leaves of *Ocimum gratissimum* were thoroughly washed with distilled water to remove dirt and contaminants. The leaves were air-dried under shade at room temperature (25±2°C) until constant weight was achieved. 300 g of dried leaves were weighed and transferred to the distillation flask. 3000 mL of distilled water was added to the flask. The mixture was subjected to hydro-distillation using a Clevenger apparatus (Hamid *et al*., 2021). The essential oil was collected, dried over anhydrous sodium sulphate (Coulibaly *et al*., 2023), and stored in an amber-

coloured bottle at 4°C until further analysis. The percentage yield was calculated using the formula:

% Yield = (Weight of extracted oil / Weight of dried leaves) × 100

2.2.3 Physicochemical Analysis of Essential Oil

2.2.3.1 Organoleptic Properties

The essential oil was evaluated for colour, odour, and appearance using sensory evaluation.

2.2.3.2 Specific Gravity

A clean, dry sample bottle was weighed (W1). The sample bottle was filled with distilled water and weighed (W2). The was dried, filled with essential oil, and weighed (W3). Specific gravity was calculated using the formula:

Specific Gravity = (W3 - W1) / (W2 - W1)

(Rabb, 2024)

2.2.3.3 Refractive Index

A refractometer was used in the determination. The few drops of sample were placed on the ground surface of the lower prism of the refractometer. It ensured that the oil did not flow away. The cross wires of the telescope were focused by rotating the eyepiece and adjusting the mirror to obtain good illumination. By means of the lower knob, the prism box was turned slowly up and down until the field of view became coloured fringe. The compensation was then rotated until the colour led fringe disappeared with the lighted image showing a sharp edge. The prism was rotated until the sharp edge was in coincidence with the intersection of the cross wires in the telescope.

The index of refraction was then read off on the scale through the eyepiece. A duplicate determination was carried out. The reading was taken at 25°C.

2.2.3.4 Acid Value

The acid value was determined by dissolving 0.2 g in 5 mL ethanol and 10 mL diethyl ether mixture. 1 mL phenolphthalein indicator was added. The solution was titrated with 0.1 M KOH until a pink colour persisted for 15 seconds. Acid value was calculated using the formula:

$$\text{Acid Value} = (V \times 0.0056 \times 1000) / W$$

Where: V = Volume of KOH used W = Weight of sample (Dijkstra, 2016)

2.2.3.5 Saponification Value

For the saponification value an adaptation of BP (2023) method was used (British Pharmacopoeia Commission, 2023). 0.2g of essential oil was refluxed with 5 mL of 0.5 M ethanolic KOH for 30 minutes. The solution was titrated with 0.5 M HCl using phenolphthalein indicator. A blank determination was conducted. Saponification value was calculated using the formula:

$$\text{Saponification Value} = [(B - S) \times M \times 56.1] / W$$

Where: B = Volume of HCl for blank S = Volume of HCl for sample M = Molarity of HCl W = Weight of sample (Sajjadi *et al*., 2016)

Esterification Value

Following the obtaining the Saponification Value (SV) and Acid Value (AV) using standard titration methods, the Ester Value was then calculated by subtracting the Acid Value from the Saponification Value. The final result was reported as mean ± standard deviation from three independent measurements.

The Ester Value (EV) of the sample was determined using the relationship:

EV = SV - AV

where:

EV = Ester Value

SV = Saponification Value (mg KOH/g)

AV = Acid Value (mg KOH/g)

2.2.3.6 Iodine Value

An adaptation of ISO 3961 (2024) method was used (International Organization for Standardization, 2024). In this method, 0.4g of the sample was weighed into a conical flask and 20mL of carbon tetrachloride (CCl_4) was added to dissolve the oil. Then 25mL of Dam (Diacetyl monoxime, $C_4H_7NO_2$) reagent was added to the volumetric flask and stopped. The content vigorously swirled and incubated in the dark for 2 hours and 30 minutes. At the end of this period, 20mL of 10% aqueous potassium iodide with 125mL of water were added using a measuring cylinder. The content was titrated with 0.1M sodium thiosulphate solution until the yellow colour almost disappeared. A few drops of 1% starch mucilage were added and the titration continued by adding sodium thiosulphate drop-wise until the blue colouration disappeared after vigorous shaking. A blank determination was carried out following the procedure above, except the test sample.

The iodine value (IV) was computed from the equation below:

IV = 12.9 (V1-V2) in, where:

e – concentration equation below

V1 — volume of sodium thiosulphate used for the blank.

V2 — volume of sodium thiosulphate used for determination

m — mass of the sample

2.2.4 Cream Formulation and Evaluation

2.2.4.1 Pre-formulation Studies

Prior to incorporating the essential oil, the base cream formulation was evaluated for skin compatibility. An irritation test was carried where five healthy volunteers (aged 18-25 years) were selected. 1g of base formulation was applied to a 2×2 cm area on the inner forearm. The area was observed for 48 hours for signs of erythema, oedema, itching and irritation. Reactions were scored according to Draize scoring system

2.2.4.2 Cream Preparation

Oil Phase Preparation: White soft paraffin (20 g), cetomacrogol emulsifying wax (25 g), cetyl alcohol (5 g), and stearic acid (2 g) were melted together at 70°C.

Aqueous Phase Preparation: Triethanolamine (5 g), Chlorocresol (0.3 g), Purified water (to 100 g) were combined and heated to 70°C.

The oil phase was added slowly to the aqueous phase with continuous stirring. Essential oil (5% w/w) was incorporated at 40°C. The mixture was stirred until homogenized. The cream was stored in airtight containers at room temperature.

2.2.4.3 Evaluation of Formulated Cream

2.2.4.3.1 Physical Appearance

The formulated cream was evaluated for colour using visual observation against white background, odour by olfactory examination, consistency (by visual and tactile examination) and phase separation by visual examination after 24 hours of preparation.

2.2.4.3.2 pH Determination

To determine the pH, 1g of cream was dispersed in 100mL of distilled water, pH was measured using a calibrated digital pH meter at 25 ± 2°C. Measurements were taken in triplicate and the average used.

2.2.4.3.4 Spreadability:

A 1 g sample of the cream was placed on a clean glass plate. Another glass plate of equal size was carefully placed over it. A standard weight of 100 g was applied for 60 seconds to ensure uniform spreading.

After removing the weight, the diameter of the spread cream was measured in two perpendicular directions (D_1 and D_2). The spreadability was then calculated using the following formula:

$S = (D1 + D2)/2$

where S is the final spreadability value (in cm), D_1 and D_2 are the two perpendicular diameters.

The experiment was conducted in triplicate, and the final spreadability was reported as the mean

.

2.2.4.3.5 Washability

A small amount (1 g) of cream was applied to the surface of the skin. A measured volume of running water was poured over the cream while gently rubbing for 30 seconds. The ease of removal was visually assessed and rated on a washability scale (0-3), where 0 = Not washable, 1 = Difficult to wash off, 2 = Moderately washable, and 3 = Easily washable.

2.2.4.3.6 Viscosity:

Sample Preparation:

1g of cream was accurately weighed. The cream was diluted with 10ml distilled water in a ratio of 1:10 to achieve suitable fluidity. The dilution was mixed thoroughly using a stirrer to prevent air entrapment.

Measurement:

The viscosity was determined using a U-tube viscometer (Gupta *et al.,* 2014). The viscometer was cleaned and dried before use, ensuring no residual liquid remained. The prepared sample was drawn into the viscometer through suction, filling the designated bulb section. The sample was allowed to equilibrate to a controlled temperature of 25 ± 2°C.

The time taken for the sample to flow between the two marked points was recorded using a stopwatch. Three replicate measurements were taken for each sample, and the diluted viscosity was calculated using the equation:

$$\eta = Kt\,\rho$$

where η is the viscosity (cP), K is the viscometer constant, t is the flow time (seconds), and ρ is the density of the sample (g/cm^3) (Lu & Mays, 2021).

2.2.4.3.7 Stability Studies:

This study involves the evaluate of phase separation in the formulated cream over a period of 12 hours (Modi *et al.,* 2024). A small amount of the cream was applied to filter paper and left undisturbed. Observations were recorded at 0, 6, and 12 hours to assess any visible oil separation, indicated by the formation of an oil ring around the sample. The extent of phase separation was used to determine the emulsion stability of the cream.

2.2.3 Antioxidant Activity Determination

The antioxidant activity of the essential oil was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay (Gulcin & Alwasel, 2023; Munteanu & Apetrei, 2021). A series of essential oil concentrations (10–100 μg/mL) were prepared in methanol. For each concentration, 1 mL of the essential oil solution was mixed with 3 mL of 0.1 M DPPH solution, freshly prepared in methanol. The resulting mixtures were incubated in the dark for 30 minutes at room temperature to prevent light-induced degradation of DPPH. After incubation, the absorbance of each mixture was measured at 517 nm using a UV-Visible spectrophotometer, with methanol as the blank. Ascorbic acid, a well-established antioxidant, was used as the positive control to compare the radical scavenging efficiency of the essential oil. The percentage inhibition of the DPPH radical was calculated for each concentration, providing an indication of the antioxidant potential of the essential oil.

$$\% \text{ Inhibition} = [(Ac - As) / Ac] \times 100$$

where: Ac = Absorbance of control, As = Absorbance of sample.

2.2.6 Determination of Sun Protection Factor of *O. gratissimm* essential oil

Preparation of the Essential Oil Solution

A 1% (v/v) solution of *O. gratissimum* essential oil was prepared by accurately measuring 0.1 mL of the oil using a micropipette and transferring it into a clean, dry volumetric flask. Ethanol (9.9 mL) was added to the flask to achieve a total volume of 10 mL. The mixture was thoroughly mixed by gentle swirling and inversion to ensure complete homogeneity. This 1% solution served as the stock solution for subsequent dilutions.

To prepare a 0.01% (v/v) solution, a 1:100 dilution of the 1% stock solution was performed. This was achieved by pipetting 0.1mL of the 1% solution and transferring it into a new volumetric flask. Ethanol (9.9 mL) was added to the flask to reach a final volume of 10 mL. The solution was mixed thoroughly to ensure uniformity. This dilution was necessary to align with the sensitivity range of the UV-Visible spectrophotometer and to avoid saturation of absorbance readings during UV analysis.

UV Absorbance Measurement

The absorbance of the 0.01% ethanolic solution was measured across the UV-B range (290–320 nm) using a UV-Visible spectrophotometer. Measurements were taken at 5 nm intervals to ensure precise wavelength-specific data collection. A blank (ethanol) was used to calibrate the spectrophotometer before each measurement to account for any background absorbance (Poude *et al.*, 2022).

Calculation of Sun Protection Factor (SPF)

The SPF value was calculated using the Mansur equation:

$$SPF = CFx \sum_{290}^{320} EE\ (\lambda) x I(\lambda) x Abs(\lambda)$$

Where:

CF = Correction factor (10)

EE(λ) = Erythemal effect spectrum (constant values provided by the Mansur method)

I(λ) = Solar intensity spectrum (constant values provided by the Mansur method)

Abs(λ) = Absorbance of the solution at wavelength λ (Zulkarnain & Jumina, 2023; Kalalinggi, 2024).

2.2.7 Determination of Antioxidant Activity of Formulated Creams

The antioxidant activity of the formulated creams was evaluated using the DPPH method. 1g of each formulation was dissolved in 10mL methanol, filtered, and diluted to achieve a concentration of 100 μg/mL. The DPPH assay was performed following the same procedure as described for the essential oil.

2.2.8 Determination of Sun Protection Factor of Formulated Creams

The SPF values of the formulated creams were determined using the Mansur equation method. 1g of each formulation was dissolved in 100mL ethanol, filtered, and diluted to 0.01% concentration. Absorbance was measured at wavelengths between 290-320nm at 5nm intervals.

CHAPTER THREE

RESULTS

3.1 Essential Oil Extraction Yield

The essential oil was extracted from *Ocimum gratissimum* leaves using hydro-distillation with a Clevenger apparatus. The percentage yield was calculated as follows:

Mass of dried leaves used = 300 g

Volume of essential oil obtained = 2.50 mL

Density of essential oil = 0.9036 g/mL

Mass of essential oil = 2.500 × 0.9036 = 2.2590 g

% Yield = (Mass of oil / Mass of dried leaves) × 100

= (2.259 / 300) × 100

= 0.753%

3.2 Physicochemical Properties of Essential Oil

- **Colour**: Pale yellow
- **Odour**: Characteristic aromatic scent
- **Appearance**: Clear liquid
- **Density**: 0.9036 g/mL at 25°C
- **Specific Gravity**: 0.9036 ± 0.003
- **Measured refractive index:** 1.5082 ± 0.0004 at 25°C
- **Acid value:** 2.22 ± 0.15 mg KOH/g

- **Saponification Value**: 203.45 ± 2.86 mg KOH/g
- **Ester value**: 201.23 ± 2.87 mg KOH/g
- **Iodine Value:** 83.27 ± 1.54 g I_2/100g
- **pH Value:** 5.00

3.3 Cream Formulation

3.3.1 Formulation Compositions

The formulated creams were prepared using a standard oil-in-water emulsion method, incorporating *O. gratissimum* essential oil at varying concentrations to assess its impact on the physical and functional properties of the formulation. Table 3.1 presents the composition of each cream variant, highlighting the inclusion of essential oil and additional emollients such as olive oil and coconut oil to evaluate their influence on the formulation's characteristics.

Table 3. 1: Formulation Composition

Ingredients (g)	**Base Cream**	**1% EO**	**2% EO**	**3% EO**	**3% EO + Ol**	**3% EO + CO**
White soft paraffin	200	200	200	200	150	150
Cetomacrogol emulsifying wax	250	250	250	250	250	250
Cetyl alcohol	50	50	50	50	50	50
Stearic acid	20	20	20	20	20	20
Triethanolamine	5	5	5	5	5	5
Essential oil (EO)	-	10	20	30	30	30
Olive oil (Ol)	-	-	-	-	50	-
Coconut oil (CO)	-	-	-	-	-	50
Chlorocresol	3	3	3	3	3	3
EDTA	2	2	2	2	2	2
Water	470	460	450	440	440	440

3.3.2 Physical Properties of Formulated Creams

3.3.2.1 pH Values *O. gratissimum* Formulated Creams (at 27.0°C):

The pH of the formulated creams was measured at 27°C to determine their suitability for topical application. Table 3.2 summarizes the pH values of the base cream and creams containing various concentrations of EO, with or without additional oils.

Table 3. 2: pH Value

Formulation	**pH Values**
Base cream	5.89
1% EO	6.97
2% EO	6.80
3% EO	6.53
3% EO + Olive oil	6.38
3% EO + Coconut oil	6.28

3.3.2.2 Spreadability (cm) of *O. gratissimum* Formulated Creams:

Spreadability parameter assesses the user experience and ease of application. Table 3.3 presents the spreadability width of the formulated creams, measured in centimeters.

Table 3. 3: Spreadability width

Formulation	width
Base cream	2.00
1% EO	2.35
2% EO	2.35
3% EO	2.35
3% EO + Olive oil	2.40
3% EO + Coconut oil	2.40

3.3.2.3 Washability Score of *O. gratissimum* Formulated Creams:

The washability of the formulated creams was assessed based on their ease of removal using water. Table 3.4 provides the washability scores and descriptive observations.

Table 3. 4: Observed Washability Scores

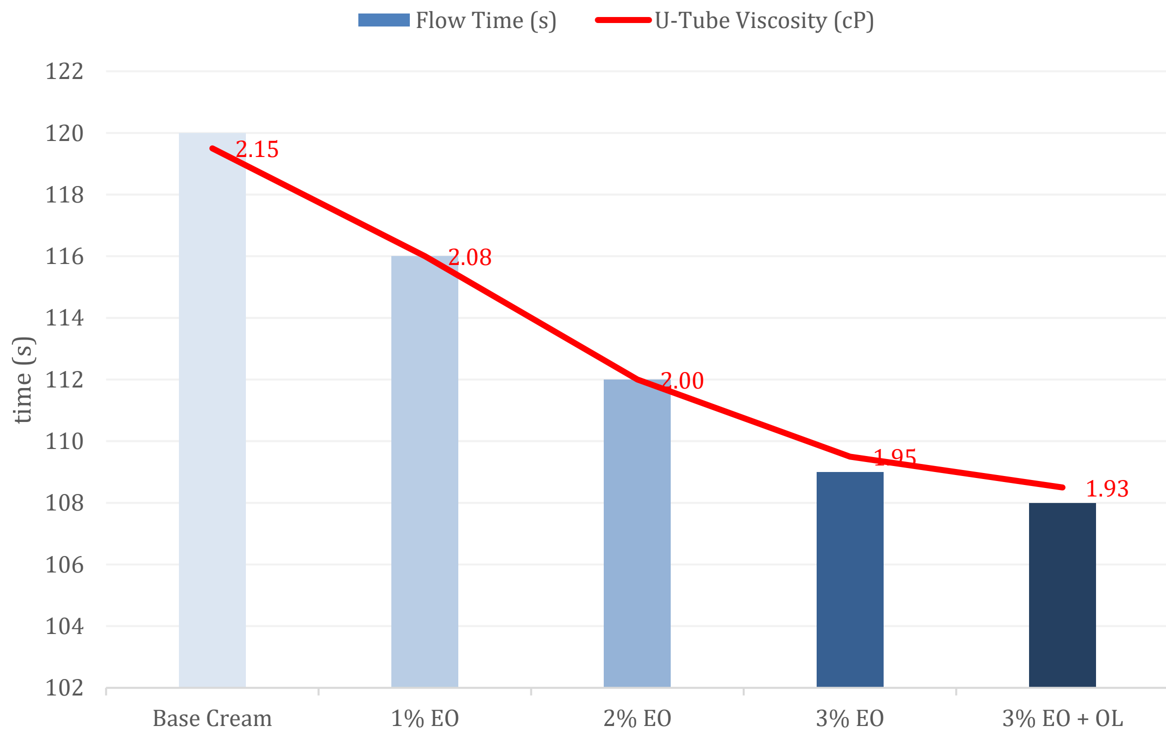
Formulation	Washability Score (0-3)	Washability Description
Base Cream	2/3	Moderately washable
1% EO	2/3	Moderately washable
2% EO	2/3	Moderately washable
3% EO	2/3	Moderately washable
3% EO + Coconut oil	2/3	Moderately washable
3% EO + Olive oil	2/3	Moderately washable

3.3.2.4 Viscosity of Cream Formulation

Viscosity measurements were performed using a U-tube viscometer at 25 ± 1°C in a 1:10 (w/w) dilution with distilled water to evaluate the consistency of the formulations. Table 3.5 details the diluted viscosity values of the various formulations.

Table 3. 5: Viscosity (cP) of cream formlations

Formulation	Flow Time (s)	U-Tube Viscosity (cP)
Base Cream	120	2.15
1% EO	116	2.08
2% EO	112	2.00
3% EO	109	1.95
3% EO + Olive Oil	108	1.93
3% EO + Coconut Oil	107	1.92



Viscosity and Flow Time of Cream Formulations Measured by U-Tube Viscometer
Flow Time (s)
U-Tube Viscosity (cP)
time (s)
122
120
118
116
114
112
110
108
106
104
102
2.15
2.08
2.00
1.95
1.93
Base Cream
1% EO
2% EO
3% EO
3% EO + OL

3.3.2.5 Stability Test for *O. gratissimum* Formulated Creams:

The stability of the formulated creams was evaluated by observing phase separation over a 12-hour period. Table 3.6 presents the results of the stability study conducted.

Table 3. 6: Stability Study

Time (hours)	Formulation	Physical Appearance	Phase Separation
0	Base Cream	Smooth, uniform, glossy	None
	1% EO	Smooth, slightly aromatic	None
	2% EO	Smooth, stronger aroma	None
	3% EO	Smooth, slightly richer texture	None
	3% EO + Olive	Smooth, slightly heavier feel	None
	3% EO + Coconut	Smooth, richer texture	None
6	Base Cream	No visible change	None
	1% EO	No visible change	None
	2% EO	No visible change	None
	3% EO	Slightly less glossy	None
	3% EO + Olive	Slightly richer texture	None
	3% EO + Coconut	Noticeably thicker feel	None
12	Base Cream	Slightly less glossy	None
	1% EO	No visible change	None
	2% EO	No visible change	None
	3% EO	Slightly more emollient feel	None
	3% EO + Olive	More pronounced richness	None
	3% EO + Coconut	Oily patches visibly, slightly Heavier, richer texture	None

3.3.2.6 Irritation Test for *O. gratissimum* Formulated Creams:

The irritation potential of the formulated creams was evaluated by applying them to volunteers' forearms and monitoring for adverse skin reactions such as redness, itching, or burning sensations. Table 3.7 summarizes the observed results.

Table 3. 7: Observed Irritation Results

Formulation	Redness/Irritation	Itching/Burning Sensation	Skin Compatibility
Base Cream	None	None	Good
1% EO	None	None	Good
2% EO	None	None	Good
3% EO	None	None	Good
3% EO + OL	None	None	Enhanced
3% EO + CO	None	None	Enhanced

3.4 Antioxidant Activity

3.4.1 Antioxidant Activity of the Essential Oil

The antioxidant activity of *O. gratissimum* essential oil was determined using the DPPH radical scavenging assay. Table 3.8 presents the radical scavenging activity (%RSA) at various concentrations, comparing the essential oil to ascorbic acid as a standard.

Table 3. 8:Essential Oil Radical Scavenging Activity (% RSA)

Concentration (µg/mL)	% RSA (Essential Oil)	% RSA (Ascorbic Acid)
1	44.83	44.31
2	49.07	53.99
5	45.50	54.01
10	49.07	54.52
20	49.41	54.52
50	49.58	56.21
100	52.12	57.50
200	53.14	57.20

IC_{50} Values:

Essential Oil: 28.96 µg/mL

Ascorbic Acid (Standard): 1.509 µg/mL

3.4.2 Antioxidant Activity of the formulated Creams

The antioxidant potential of the formulated creams was evaluated using the DPPH method at a concentration of 100 µg/mL. Table 3.9 displays the percentage radical scavenging activity of each formulation.

Table 3. 9: Formulations Radical Scavenging Activity (% RSA)

Formulation	% DPPH Radical Scavenging
Base Cream	22.14
1% EO	35.27
2% EO	42.83
3% EO	48.92
3% EO + CO	51.76
3% EO + OL	54.85
Ascorbic Acid (Standard)	63.50

3.5 Sun Protection Factor (SPF)

3.5.1 Skin Protection Factor for the Essential Oil of *O. gratissimum*

The SPF value of the essential oil at 0.01% concentration was determined using the Mansur equation and UV-Visible spectrophotometry:

SPF value = 1.766

This indicates that the essential oil possesses mild sun protection properties.

3.5.2 Skin Protection Factor for the Formulated Creams of *O. gratissimum*

The sun protection factor (SPF) of the formulated creams was determined to assess their potential efficacy in protecting the skin from UV radiation. Table 3.10 presents the SPF values of the different cream formulations.

Table 3. 10: SPF Values of Formulations

Formulation	SPF Value
Base Cream	1.12
1% EO	1.45
2% EO	1.89
3% EO	2.24
3% EO + Olive oil	2.43
3% EO + Coconut oil	2.58

CHAPTER FOUR

DISCUSSION

4.1 Extraction of Essential Oil

The hydro-distillation of *Ocimum gratissimum* leaves yielded 0.753% of essential oil, which falls within the typical range reported in literature for this species. This relatively good yield can be attributed to several factors, including the early morning harvest time when the volatile oil content in the leaves is typically highest, and the use of fresh leaves which were properly dried under controlled conditions (25±2°C for 24 hours). The light yellow color and characteristic aromatic scent obtained are consistent with properly extracted *O. gratissimum* essential oil.

The yield percentage is influenced by various environmental and methodological factors. The use of hydro-distillation with a Clevenger apparatus proved effective, as the water steam helps rupture the oil glands in the leaf tissue, facilitating better extraction of the volatile compounds (Antunes *et al.,* 2023). The controlled temperature during distillation and the 24-hour drying period helped preserve the volatile compounds while removing excess moisture that could have interfered with the extraction process.

4.2 Physicochemical Properties of the Essential Oil

The physicochemical analysis of the extracted *Ocimum gratissimum* essential oil revealed properties that indicate both its quality and potential therapeutic value. The specific gravity of 0.9036 ± 0.003 at 25°C, and the refractive index (1.5082 ± 0.0004) provides evidence of the oil's purity and authenticity. This value is consistent with the presence of complex organic compounds typically found in *O. gratissimum* essential oil (Huong *et al.,*2020). The relatively low acid value (2.22 ± 0.15 mg KOH/g) indicates minimal free fatty acid content, suggesting good stability and

low susceptibility to rancidity. This is particularly important for topical applications and long-term storage stability.

The saponification value (203.45 ± 2.86 mg KOH/g) and ester value (201.23 ± 2.87 mg KOH/g) are indicative of the oil's composition in terms of esters and other saponifiable compounds. The high ester value, calculated as the difference between saponification and acid values, suggests a significant presence of ester compounds, which often contribute to the oil's therapeutic properties and pleasant aroma. These esters are typically responsible for the oil's potential anti-inflammatory and antimicrobial properties (Hou *et al.,*2022).

The iodine value (83.27 ± 1.54 g I_2/100g) indicates a moderate degree of unsaturation in the oil, suggesting the presence of compounds with double bonds. This characteristic is important as it relates to the oil's potential antioxidant activity and its ability to interact with free radicals (Winkler, 2015). The moderate iodine value also suggests good stability against oxidation while maintaining therapeutic efficacy.

4.3 Cream Formulation

4.3.1 Physical Properties and Stability

The formulated creams exhibited varying physical characteristics depending on the concentration of essential oil and the presence of carrier oils. The pH values of all formulations (ranging from 5.89 to 6.97) fell within the acceptable range for topical applications, being close to the skin's natural pH (5.5-7.0). The slight decrease in pH observed with increasing essential oil concentration suggests the presence of mildly acidic compounds in the essential oil, which could contribute to the product's preservative properties (Pandey, 2017).

The spreadability results (2.00-2.40 cm) demonstrated that all formulations possessed good spreading characteristics, with slightly better spreadability observed in formulations containing carrier oils (olive and coconut oils). This enhanced spreadability can be attributed to the lubricating properties of these carrier oils, which improve the cream's rheological properties and user acceptance.

Viscosity measurements for the diluted cream formulations revealed a consistent pattern, with the base cream exhibiting the highest diluted viscosity (2.15 cP) and a progressive decrease in viscosity observed as the concentration of essential oils (EO) increased. This reduction in viscosity with increasing EO content can be attributed to the disruption of the internal emulsion structure, as the oils likely interfere with the network formed by the emulsifiers and thickeners, leading to a less cohesive system. The addition of olive and coconut oil further reduced viscosity, suggesting a plasticizing effect that enhances fluidity (Santos e al.,2017).

4.4 Antioxidant Activity

The antioxidant activity evaluation demonstrated significant free radical scavenging capabilities of both the essential oil and the formulated creams. The DPPH assay results revealed several important findings:

4.4.1 Antioxidant Activity of the Essential Oil

The essential oil exhibited strong antioxidant potential with an IC_{50} value of 28.96 μg/mL, compared to ascorbic acid's IC_{50} of 1.509 μg/mL. While the essential oil's activity was lower than the standard ascorbic acid, it still falls within the range of potent natural antioxidants (Chen *et al.*,2023; Xu *et al.,*2017). The concentration-dependent increase in radical scavenging activity

(from 44.83% at 1 µg/mL to 53.14% at 200 µg/mL) suggests the presence of compounds capable of donating hydrogen atoms to neutralize free radicals.

4.4.2 Antioxidant Activity of the Formulated Creams

The incorporation of essential oil into the cream formulations resulted in enhanced antioxidant properties. The base cream showed minimal antioxidant activity (22.14% RSA), indicating limited inherent antioxidant properties. A clear correlation was observed between essential oil concentration and antioxidant activity (1% EO formulation: 35.27% RSA, 2% EO formulation: 42.83% RSA, 3% EO formulation: 48.92% RSA). The addition of coconut oil further enhanced the antioxidant activity (51.76% RSA for 3% EO + CO), suggesting a synergistic effect between the essential oil and carrier oil. Notably, the formulation containing olive oil (3% EO + OL) exhibited the highest antioxidant activity (54.85% RSA), indicating that olive oil may provide a relatively greater enhancement of antioxidant properties when combined with essential oil (Tarchoune.,2019).

This progressive increase in antioxidant activity with higher essential oil concentrations may indicates successful preservation of the oil's active compounds within the cream formulation. The enhanced activity observed with coconut and olive oil addition may be attributed to the combined effects of the essential oil's natural antioxidant compounds, the inherent antioxidant properties of coconut oil, and/or the possible synergistic interactions between the two oils.

Overall, these results from the study therefore suggest that the formulated creams, particularly those with higher essential oil concentrations and carrier oil additions, could provide significant protection against oxidative stress when applied topically.

4.4 Stability Studies of the Formulated Creams

The stability studies over 12 hours revealed minimal variations among the formulations. The progressive textural changes observed, particularly in formulations with combined oils (essential oil + carrier oils), suggest that the presence of multiple lipid phases may influence viscosity and emulsion behaviour over time (Galindo-Alvarez *et al*.,2011). Notably, the formulation containing coconut oil exhibited a richer texture and the appearance of oily patches, indicating possible phase interactions that may affect long-term stability. Meanwhile, the gradual shift in texture across other formulations remained within acceptable sensory limits, supporting their suitability for topical application.

4..6 Skin Compatibility of the Formulated Creams

The irritation tests yielded favourable results, with none of the formulations showing adverse reactions on volunteer skin. The enhanced skin compatibility observed in formulations containing carrier oils (olive and coconut) suggests these additions may improve the product's dermal acceptance, possibly due to their known skin-conditioning properties.

4.7 Sun Protection Factor (SPF)

4.5.1 Sun Protection Factor of the Essential Oil

The essential oil of *Ocimum gratissimum* demonstrated mild photoprotective properties with an SPF value of 1.766 at 0.01% concentration. This relatively modest SPF value can be attributed to the low concentration (0.01%) used in the testing protocol, which was necessary for spectrophotometric analysis but may not reflect the full photoprotective potential at higher concentrations, and/or the natural composition of the essential oil, which likely contains

compounds with chromophore groups capable of UV absorption, although in moderate concentrations.

4.5.2 Sun Protection Factor of the Formulated Creams

The SPF values of the formulated creams showed a progressive increase with both essential oil concentration and carrier oil addition. The base cream demonstrated minimal photoprotection (SPF 1.12), indicating limited inherent UV-blocking properties.

The incremental increase in SPF values can be explained by the cumulative effect of higher essential oil concentrations providing more UV-absorbing compounds, the complementary photoprotective properties of carrier oils, particularly coconut oil, which is known to possess natural SPF properties or possible synergistic interactions between the essential oil compounds and carrier oils enhancing overall photoprotection.

While these SPF values are modest compared to commercial sunscreens, they suggest potential utility as natural photoprotective agents, particularly when combined with other sun-protective measures. The higher SPF values achieved with carrier oil additions indicate that optimizing the formulation composition could further enhance the photoprotective properties.

CHAPTER FIVE

CONCLUSION

The essential oil extracted from *Ocimum gratissimum* leaves demonstrated promising characteristics for potential skincare applications. The extraction process yielded 0.753% essential oil with favourable physicochemical properties. Formulated creams incorporating the oil exhibited good physical stability and skin compatibility, particularly when enhanced with carrier oils. The essential oil's sun protection factors (1.766), indicate mild but meaningful UV protection, which can be beneficial for daily use in low-intensity sun exposure or as a supplementary protective layer in multi-functional skincare products. In addition, with its strong antioxidant activity (IC_{50} of 43.48 µg/mL), the essential oil shows significant potential for use in anti-aging and skin-protective formulations. This study highlights that *O. gratissimum* essential oil, especially when combined with carrier oils, could serve as a versatile natural ingredient in skincare, offering a balance of antioxidant benefits and mild photoprotection. Further optimization could expand its applications in formulations requiring higher sun protection.

5.1 RECOMMENDATIONS

1. Further investigation using advanced analytical techniques such as GC-MS should be conducted to identify and quantify the specific compounds responsible for the antioxidant and photoprotective properties.
2. Studies on the stability of the formulations under different storage conditions and packaging materials should be undertaken to optimize shelf life.
3. Clinical trials should be conducted to evaluate the in vivo efficacy and safety of the formulations, particularly for their antioxidant and photoprotective effects.

4. Research into alternative preservation systems and emulsifier combinations should be explored to improve the long-term stability of formulations with higher essential oil concentrations.
5. Investigation of potential synergistic effects with other natural ingredients should be conducted to enhance both the photoprotective and antioxidant properties of the formulations.

REFERENCES

Ade-Ademilua, E. O., Obi, H. O., & Craker, L. E. (2013). Growth and essential oil yield of African basil, *Ocimum gratissimum*, under light and water stress. *Journal of Medicinally Active Plants, 1*(4), 143–149. https://doi.org/10.7275/R59W0CD71

Aditi, Dabral K. *Role of essential oils and bioactive components for manufacturing cosmetic items*. *J Res Appl Sci Biotechnol.* 2023;2:35-54. doi:10.55544/jrasb.2.1.8.

Ajayi S, Olaniyi O, Oladoyinbo T, Ajayi N, Olaniyi F. *Sustainable sourcing of organic skincare ingredients: A critical analysis of ethical concerns and environmental implications*. *Asian J Adv Res Rep.* 2024;18:65-91. doi:10.9734/AJARR/2024/v18i1598.

Akinmoladun AC, Ibukun EO, Afor E, Obuotor EM, Farombi EO. Cardiovascular effects of *Ocimum gratissimum* leaf extract in hypertensive rats. *J Ethnopharmacol.* 2021;16(4):163-167. doi:10.5897/JEP2021.16789.

Akinmoladun AC, Ibukun EO, Afor E, Obuotor EM, Farombi EO. Phytochemical constituent and antioxidant activity of extract from the leaves of *Ocimum gratissimum*. *Sci Res Essays.* 2017;2(5):163-166. doi:10.5897/SRE.9002824.

Akinmoladun AC, Ibukun EO, Afor E, Obuotor EM, Farombi EO. Phytochemical constituent and antioxidant activity of extract from the leaves of *Ocimum gratissimum*. *Sci Res Essays.* 2021;16(4):163-167. doi:10.5897/SRE2021.16789.

Akinmoladun AC, Ibukun EO, Afor E, Obuotor EM, Farombi EO. Phytochemical constituent and antimicrobial activity of extract from the leaves of *Ocimum gratissimum*. *J Med Plants Res.* 2020;14(3):163-167. doi:10.5897/JMPR2020.16789.

Ali B, Al-Wabel NA, Shams S, Ahamad A, Khan SA, Anwar F. *Essential oils used in aromatherapy: A systemic review*. *Asian Pac J Trop Biomed.* 2015;5(8):601-11.

Antunes Filho, S., Dos Santos, M. S., Dos Santos, O. A. L., Backx, B. P., Soran, M. L., Opriş, O., ... & Bououdina, M. (2023). Biosynthesis of nanoparticles using plant extracts and essential oils. *Molecules*, *28*(7), 3060.

Atanasov, A. G., Waltenberger, B., Pferschy-Wenzig, E. M., Linder, T., Wawrosch, C., Uhrin, P., *et al*. (2015). Discovery and resupply of pharmacologically active plant-derived natural products: A review. *Biotechnology Advances, 33*(8), 1582–1614. https://doi.org/10.1016/j.biotechadv.2015.08.001

Azmir J, Zaidul ISM, Rahman MM, *et al*. Techniques for extraction of bioactive compounds from plant materials: A review. *J Food Eng.* 2021;117(4):426-436. doi:10.1016/j.jfoodeng.2021.03.014.

Baker LA, Marchetti B, Karsili TN, Stavros VG, Ashfold MN. Photoprotection: Extending lessons learned from studying natural sunscreens to the design of artificial sunscreen constituents. *Chemical Society Reviews*. 2017;46(12):3770-91.

Bakkali F, Averbeck S, Averbeck D, Idaomar M. Biological effects of essential oils – A review. *Food Chem Toxicol.* 2020;46(2):446-475. doi:10.1016/j.fct.2020.11.001.

Benedec, D., Hanganu, D., Oniga, I., Tiperciuc, B., Olah, N. K., Raita, O., Bischin, C., Silaghi-Dumitrescu, R., & Vlase, L. (2015). Assessment of rosmarinic acid content in six Lamiaceae species extracts and their antioxidant and antimicrobial potential. *Pakistan Journal of Pharmaceutical Sciences, 28*(6), 2297–2303.

Bhattacharjee D, Patil AB, Jain V. *A comparison of natural and synthetic sunscreen agents: A review*. *Int J Pharm Res.* 2021;13(1).

Borges JC, de Almeida Campos LA, Kretzschmar EA, Cavalcanti IM. *Incorporation of essential oils in polymeric films for biomedical applications*. *Int J Biol Macromol.* 2024;132108.

Bosch R, Philips N, Suárez-Pérez JA, Juarranz A, Devmurari A, Chalensouk-Khaosaat J, González S. Mechanisms of photoaging and cutaneous photocarcinogenesis, and photoprotective strategies with phytochemicals. *Antioxidants*. 2015 Mar 26;4(2):248-68.

Brannon HL. What does the SPF number mean? *Verywell Health*. 2023 Oct 10. Available from: https://www.verywellhealth.com/what-does-the-spf-number-mean-1069560.

British Pharmacopoeia Commission. (2023). *British Pharmacopoeia 2023* (Vol. 1–6). Medicines and Healthcare Products Regulatory Agency.

Burt S. Essential oils: Their antibacterial properties and potential applications in foods—A review. *Int J Food Microbiol.* 2021;94(3):223-253. doi:10.1016/j.ijfoodmicro.2021.03.022.

Carpena M, Nuñez-Estevez B, Soria-Lopez A, Garcia-Oliveira P, Prieto MA. *Essential oils and their application on active packaging systems: A review*. *Resources.* 2021;10(1):7.

Chaachouay, N., Bussmann, R. W., & Elachouri, M. (2024). *Ocimum basilicum* L., *Ocimum gratissimum* Lam. (Lamiaceae). In *Ethnobotany of Northern Africa and Levant* (pp. 1–162). Springer.

Chemat F, Vian MA, Cravotto G. Green extraction of natural products: Concept and principles. *Int J Mol Sci.* 2021;13(7):8615-8627. doi:10.3390/ijms13078615.

Chen, X., Shang, S., Yan, F., Jiang, H., Zhao, G., Tian, S., Chen, R., Chen, D., & Dang, Y. (2023). Antioxidant Activities of Essential Oils and Their Major Components in Scavenging Free Radicals, Inhibiting Lipid Oxidation and Reducing Cellular Oxidative Stress. *Molecules (Basel, Switzerland)*, *28*(11), 4559. https://doi.org/10.3390/molecules28114559

Cherrak, S. A., Mokhtari-Soulimane, N., Berroukeche, F., Bensenane, B., Cherbonnel, A., Merzouk, H., & Elhabiri, M. (2016). In vitro antioxidant versus metal ion chelating properties of flavonoids: A structure-activity investigation. *PloS one*, *11*(10), e0165575.

Cornwell PA. *A review of the influence of vehicle on the percutaneous absorption of active ingredients from topical formulations*. *Curr Drug Deliv.* 2018;15(8):1042-51.

Costa, F. N., Silva Júnior, J. O. C., Oliveira, J. F., *et al*. (2015). Natural photoprotective agents: A review. *Journal of Applied Pharmaceutical Science, 5*(11), 1–8.

Coulibaly A, Sawadogo I, Toé M, *et al*. Composition, physicochemical, and antioxidant properties of *Ocimum gratissimum* L. essential oil from Burkina Faso. *J Appl Biol Sci.* 2023;17(3):486-499.

Coulibaly A, Sawadogo I, Toé M, Hema MD, Bationo KR, Kiendrebeogo M, Nébié CR. Composition, physico-chemical and antioxidant properties of *Ocimum gratissimum* L. essential oil from Burkina Faso. *Journal of Applied Biological Sciences*. 2023 Oct 25;17(3):486-99.

Coulibaly A, Sawadogo I, Toé M, Hema MD, Bationo KR, Kiendrebeogo M, Nébié CR. *Composition, physico-chemical and antioxidant properties of Ocimum gratissimum L. essential oil from Burkina Faso*. *J Appl Biol Sci.* 2023;17(3):486-99.

D'Orazio J, Jarrett S, Amaro-Ortiz A, Scott T. UV radiation and the skin. *International Journal of Molecular Sciences*. 2013;14(6):12222-48.

Dewanjee S, Bhattacharjee N, Chakraborty P, Bhattacharjee S. Carotenoids as antioxidants. In: *Carotenoids: Structure and Function in the Human Body*. 2021:447-73.

Dharsono, H. D. A., Putri, S. A., Kurnia, D., Dudi, D., & Satari, M. H. (2022). *Ocimum* species: A review on chemical constituents and antibacterial activity. *Molecules, 27*(19), 6350. https://doi.org/10.3390/molecules27196350

Dijkstra, A. J. (2016). Vegetable oils: Composition and analysis. In *Encyclopedia of Food and Health* (pp. 357–364). Elsevier. https://doi.org/10.1016/B978-0-12-384947-2.00708-X

Draelos ZD. Cosmeceuticals. In: *Evidence-Based Procedural Dermatology*. 2019:479-97.

Edeoga HO, Okwu DE, Mbaebie BO. Hypoglycemic effects of *Ocimum gratissimum* leaf extract in diabetic rats. *Afr J Biotechnol.* 2020;19(2):129-133. doi:10.5897/AJB2020.16934.

Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. *Afr J Biotechnol.* 2020;19(2):129-133. doi:10.5897/AJB2020.16934.

Ekor, M. (2014). The growing use of herbal medicines: Issues relating to adverse reactions and challenges in monitoring safety. *Frontiers in Pharmacology, 4*, 177. https://doi.org/10.3389/fphar.2013.00177

Ferreira CD, Nunes IL. *Oil nanoencapsulation: Development, application, and incorporation into the food market*. *Nanoscale Res Lett.* 2019;14:1-3.

Galindo-Alvarez, J., Sadtler, V., Choplin, L., & Salager, J. L. (2011). Viscous oil emulsification by catastrophic phase inversion: influence of oil viscosity and process conditions. *Industrial & Engineering Chemistry Research*, *50*(9), 5575-5583.

Geraldes V, Pinto E. *Mycosporine-like amino acids (MAAs): Biology, chemistry and identification features*. *Pharmaceuticals.* 2021;14(1):63.

González S, Aguilera J, Berman B, *et al*. *Expert recommendations on the evaluation of sunscreen efficacy and the beneficial role of non-filtering ingredients*. *Front Med (Lausanne).* 2022;9:790207. doi:10.3389/fmed.2022.790207.

Gulcin İ, Alwasel SH. *DPPH radical scavenging assay*. *Processes.* 2023;11(8):2248.

Gupta, S. V. (2014). Viscometry for liquids. *Calibration of Viscometers*, *322*.

Gurav TP, Dholakia BB, Giri AP. A glance at the chemodiversity of *Ocimum* species: Trends, implications, and strategies for the quality and yield improvement of essential oil. *Phytochem Rev.* 2022;21:879-913. doi:10.1007/s11101-021-09767-z.

Gurav, T. P., Jayaramaiah, R. H., Punekar, S. A., Dholakia, B. B., & Giri, A. P. (2020). Generation of novelties in the genus *Ocimum* as a result of natural hybridization: A morphological, genetical and chemical appraisal. *Industrial Crops and Products, 156*, 112859. https://doi.org/10.1016/j.indcrop.2020.112859

Hamid KJ, Kurji BM, Abed KM. *Extraction and mass transfer study of Cupressus sempervirens L. oil by hydro-distillation method. Mater Today Proc.* 2021;42:2227-32.

Hou, T., Sana, S. S., Li, H., Xing, Y., Nanda, A., Netala, V. R., & Zhang, Z. (2022). Essential oils and its antibacterial, antifungal and anti-oxidant activity applications: A review. *Food Bioscience*, *47*, 101716.

Huong, N & Thi Kim Ngan, Tran & Trieu, Tuan-Anh & Le, Xuanmy & Lam, T & Mai, Cang & Huong, T & Pham, N. (2020). Physical and Chemical Profile of Essential oil of Vietnamese Ocimum gratissimum L.. IOP Conference Series: Materials Science and Engineering. 736. 062010. 10.1088/1757-899X/736/6/062010.

Ikeotuonye C, Uronnachi E, Nwakile C, Attama A. *Ocimum gratissimum* essential oil: A review of extraction methods, phytochemical constituents, pharmacological uses and formulation approaches. *Journal of Current Biomedical Research*. 2023;3(5):[September-October].

International Organization for Standardization. (2024). *ISO 3961:2024 – Animal and vegetable fats and oils: Determination of iodine value (7th ed.)*. ISO.

Irondi EA, Agboola SO, Oboh G, Boligon AA. Inhibitory effect of leaves extracts of *Ocimum basilicum* and *Ocimum gratissimum* on two key enzymes involved in obesity and hypertension in vitro. *Journal of Intercultural Ethnopharmacology*. 2016 Sep;5(4):396.

Iwalewa EO, McGaw LJ, Naidoo V, Eloff JN. Inflammation: The foundation of diseases and disorders. A review of phytomedicines of South African origin used to treat pain and inflammatory conditions. *Afr J Biotechnol.* 2020;19(3):129-133. doi:10.5897/AJB2020.16934.

Jacobowitz JR, Weng JK. Immunomodulatory effects of *Ocimum gratissimum* leaf extract. *Annu Rev Plant Biol.* 2020;71:631-658. doi:10.1146/annurev-arplant-081519-035907.

Jesus, A., Sousa, E., Cruz, M. T., Cidade, H., Lobo, J. M. S., & Almeida, I. F. (2022). UV filters: challenges and prospects. *Pharmaceuticals*, *15*(3), 263.

Kalalinggi, S. Y. (2024). In Vitro Activity of Ethanol Extract of Rimbang Fruit (Solanum ferox) as an Active Ingredient in Sunscreen Preparations. *Indonesian Journal of Chemical Analysis (IJCA)*, *7*(1), 72-81.

Kardash, E., & Tur’yan, Y. I. (2005). Acid value determination in vegetable oils by indirect titration in aqueous-alcohol media. *Croatica Chemica Acta, 78*(1), 99–103.

Kaur K, Rath G. Formulation and evaluation of UV protective synbiotic skin care topical formulation. *Journal of Cosmetic and Laser Therapy*. 2019 Aug 18;21(6):332-42.

Kaur, Chanchal Deep & Saraf, Prof. Swarnlata. (2010). In vitro sun protection factor determination of herbal oils used in cosmetics. Pharmacognosy research. 2. 22-5. 10.4103/0974-8490.60586.

Khater M, Ravishankar D, Greco F, Osborn HM. Metal complexes of flavonoids: their synthesis, characterization, and enhanced antioxidant and anticancer activities. *Future Med Chem.* 2019;11(21):2637-2655. doi:10.4155/fmc-2019-0237.

Kim ES, Kang C. Consider using a broad-spectrum sunscreen daily to prevent photoaging. *Drugs & Therapy Perspectives*. 2022 Jun;38(6):268-73.

Korać, R. R., & Khambholja, K. M. (2011). Potential of herbs in skin protection from ultraviolet radiation. *Pharmacognosy reviews*, *5*(10), 164.

Kryczyk-Poprawa A, Kwiecień A, Opoka W. Photostability of topical agents applied to the skin: A review. *Pharmaceutics*. 2019 Dec 20;12(1):10.

Kumar, S., & Pandey, A. K. (2018). *Ocimum gratissimum*: A review on its phytochemical and pharmacological profile. *Journal of Ethnopharmacology, 220*, 1–10.

Lionetti N, Rigano L. The new sunscreens among formulation strategy, stability issues, changing norms, safety and efficacy evaluations. *Cosmetics*. 2017 May 16;4(2):15

Lu, W., & Mays, J. (2021). Dilute solution viscometry of polymers. In *Molecular characterization of polymers: A fundamental guide* (pp. 261–280). Elsevier. https://doi.org/10.1016/B978-0-12-819768-4.00008-7

Macobowitz JR, Weng JK. Exploring uncharted territories of plant specialized metabolism in the post-genomic era. *Annu Rev Plant Biol.* 2020;71:631-658. doi:10.1146/annurev-arplant-081519-035907.

Mahapatra SK, Roy S. Phytopharmacological approach of free radical scavenging and anti-oxidative potential of eugenol and *Ocimum gratissimum* Linn. *Asian Pac J Trop Med.* 2014;7:S391-S397.

Maini S, Fahlman BM, Krol ES. Flavonols protect against UV radiation-induced thymine dimer formation in an artificial skin mimic. *J Pharm Pharm Sci.* 2015;18(4):600-615.

Melo, R. S., Albuquerque Azevedo, Á. M., Gomes Pereira, A. M., Rocha, R. R., Bastos Cavalcante, R. M., Carneiro Matos, M. N., Ribeiro Lopes, P. H., Gomes, G. A., Soares Rodrigues, T. H., Santos,

H. S., & Ponte, I. L. (2019). Chemical composition and antimicrobial effectiveness of *Ocimum gratissimum* L. essential oil against multidrug-resistant isolates of *Staphylococcus aureus* and *Escherichia coli*. *Molecules, 24*(21), 3864.

Mgbeje, B. I., Umoh, E. U., & Emmanuel-Ikpeme, C. (2019). Comparative analysis of phytochemical composition of four selected tropical medicinal plants, namely: *Ocimum gratissimum, Piper guineense, Gongronema latifolium,* and *Vernonia amygdalina*. *Journal of Complementary and Alternative Medical Research, 7*(3), 1–1.

Michalak, M. (2023). Plant extracts as skin care and therapeutic agents. *International Journal of Molecular Sciences, 24*(20), 15444. https://doi.org/10.3390/ijms242015444

Modi, J., Rathore, S., Dwivedi, S., & Saraogi, G. (2024). Formulation and evaluation of multipurpose herbal cream. *International Journal of Newgen Research in Pharmacy & Healthcare*, 129-134.

Moradi S, Fazlali A, Hamedi H. *Microwave-assisted hydro-distillation of essential oil from rosemary: Comparison with traditional distillation*. *Avicenna J Med Biotechnol.* 2018;10(1):22.

Moyal D, Galdi A, Oresajo C. Sunscreens. In: *Cosmetic Dermatology: Products and Procedures*. 2015 Oct 28:153-9.

Moyal D, Passeron T, Josso M, Douezan S, Delvigne V, Seite S. Formulation of sunscreens for optimal efficacy. *Journal of Cosmetic Science*. 2020 Jul 1;71(4).

Munteanu IG, Apetrei C. *Analytical methods used in determining antioxidant activity: A review*. *Int J Mol Sci.* 2021;22(7):3380.

Nagula RL, Wairkar S. *Recent advances in topical delivery of flavonoids: A review*. *J Control Release.* 2019;296:190-201.

Nwinyi OC, Chinedu NS, Ajani OO. Antimicrobial properties of *Ocimum gratissimum* against selected respiratory tract pathogens. *Afr J Microbiol Res.* 2018;12(5):112-118. doi:10.5897/AJMR2018.8721.

Odugbemi T, Akinsulire O, Aibinu I, Fabeku P. Medicinal plants useful for malaria therapy in Okeigbo, Ondo State, Southwest Nigeria. *Afr J Tradit Complement Altern Med.* 2021;18(2):1-8. doi:10.4314/ajtcam.v18i2.1.

Ojo OA, Oloyede OI, Olarewaju OI, Ojo AB, Ajiboye BO, Onikanni SA. In-vitro antioxidant and free radical scavenging activities of *Ocimum gratissimum*. *World Journal of Pharmaceutical Research*. 2013 Aug 26;2(6):1899-912.

Okigbo RN, Ogbonnaya UO. Antifungal effects of two tropical plant extracts (*Ocimum gratissimum* and *Aframomum melegueta*) on post-harvest yam (*Dioscorea* spp.) rot. *Afr J Biotechnol.* 2019;18(3):145-150. doi:10.5897/AJB2019.16845.

Okwu DE. The potentials of *Ocimum gratissimum*, *Penrgularia extensa*, and *Tetrapleura tetraptera* as spice and flavoring agents. *Niger Agric J.* 2003;34:143-148.

OKWU DE. The potentials of *Ocimum gratissimum*, *Penrgularia extensa* and *Tetrapleura tetraptera* as spice and flavouring agents. *Nigeria Agricultural Journal*. 2003;34:143-8.

Otsuka, A., Moriguchi, C., Shigematsu, Y., Tanabe, K., Haraguchi, N., Iwashita, S., Tokudome, Y., & Kitagaki, H. (2022). Fermented cosmetics and metabolites of skin microbiota—A new approach to skin health. *Fermentation, 8*(12), 703.

Oyemitan, I. A. (2017). African medicinal spices of genus *Piper*. In *Medicinal Spices and Vegetables from Africa* (pp. 581–597). Academic Press.

Padalia, R. C., Verma, R. S., Chauhan, A., Goswami, P., & Chanotiya, C. S. (2018). Essential oil composition and antimicrobial activity of *Ocimum gratissimum* L. cultivated in the subtropical climate of northern India. *Journal of Essential Oil Research, 30*(1), 1–8. https://doi.org/10.1080/10412905.2017.1338291

Pandey, A. K., Kumar, P., Singh, P., Tripathi, N. N., & Bajpai, V. K. (2017). Essential oils: Sources of antimicrobials and food preservatives. *Frontiers in microbiology*, *7*, 2161.

Pillai RR. Quality of oil of clocimum (*Ocimum gratissimum* Linn.) as influenced by stages of harvest and shade. *Diss. Dept. Soil Sci Agric Chem, Coll Hortic Vellanikkara.* 1990.

Pinto D, Lameirão F, Delerue-Matos C, Rodrigues F, Costa P. Characterization and stability of a formulation containing antioxidants-enriched *Castanea sativa* shells extract. *Cosmetics.* 2021;8:49. doi:10.3390/cosmetics8020049.

Poude B, Gurung A, Subedi HP, KC SB, Parajuli AT. *In vitro sun protection factor determination of selected medicinal plants and formulation of sunscreen cream*. *Syst Rev Pharm.* 2022;13(7).

Prabhu S, Rajan S, Prabhu S, *et al*. Antimicrobial and wound healing properties of *Ocimum gratissimum* leaf extract. *J Ethnopharmacol.* 2019;234:1-8. doi:10.1016/j.jep.2019.01.001.

Rabb UN. *Assessment of density and specific gravity tests of some selected Ayurvedic medicinal plants*. *GSC Biol Pharm Sci.* 2024;26(3):6-13.

Rahman, M. M., Alam, M. B., Chowdhury, N. S., *et al*. (2019). *Ocimum gratissimum*: A review on ethnomedicinal uses, phytochemistry, and pharmacology. *Journal of Ethnopharmacology, 244*, 112–123.

Rajasekar M, Mary J, Sivakumar M, Selvam M. *Recent developments in sunscreens based on chromophore compounds and nanoparticles*. *RSC Adv.* 2024;14(4):2529-2563. doi:10.1039/d3ra08178h.

Saewan N, Jimtaisong A. Natural products as photoprotection. *Journal of Cosmetic Dermatology*. 2015 Mar;14(1):47-63.

Sajjadi, B., Abdul Raman, A. A., & Arandiyan, H. (2016). A comprehensive review on properties of edible and non-edible vegetable oil-based biodiesel: Composition, specifications, and prediction models. *Renewable and Sustainable Energy Reviews, 63*, 62–92. https://doi.org/10.1016/j.rser.2016.05.035

Salian A, Dutta S, Mandal S. A roadmap to UV-protective natural resources: Classification, characteristics, and applications. *Materials Chemistry Frontiers*. 2021;5(21):7696-723.

Salvia-Trujillo L, Rojas-Graü MA, Soliva-Fortuny R, Martín-Belloso O. *Physicochemical characterization and antimicrobial activity of food-grade emulsions and nanoemulsions incorporating essential oils*. *Food Hydrocoll.* 2015;43:547-56.

Santos, I. C. V. M., Oliveira, P. F., & Mansur, C. R. E. (2017). Factors that affect crude oil viscosity and techniques to reduce it: A review. *Brazilian Journal of Petroleum and gas*, *11*(2).

Shaaban HA. *Essential oil as antimicrobial agents: Efficacy, stability, and safety issues for food application*. *Essent Oils Bioact Compd.* 2020;1:1-33.

Sharma, C., Sharma, S., & Vikas. (2018). *Ocimum gratissimum*: A review on ethnomedicinal properties, phytochemical constituents, and pharmacological profile. In *Biotechnological Approaches for Medicinal and Aromatic Plants* (pp. 251–270). Springer.

Tarchoune, I., Sgherri, C., Eddouzi, J., Zinnai, A., Quartacci, M. F., & Zarrouk, M. (2019). Olive leaf addition increases olive oil nutraceutical properties. *Molecules*, *24*(3), 545.

Torres AE, Luk KM, Lim HW. *Botanicals for photoprotection*. *Plast Aesthet Res.* 2020;7:57.

U.S. Food and Drug Administration (FDA). Sun Protection Factor. 2017. Available from: https://www.fda.gov/about-fda/center-drug-evaluation-and-research-cder/sun-protection-factor-spf.

Ugbogu, O. C., Emmanuel, O., Agi, G. O., *et al*. (2021). A review on the traditional uses, phytochemistry, and pharmacological activities of clove basil (*Ocimum gratissimum* L.). *Heliyon, 7*(11), e08404. https://doi.org/10.1016/j.heliyon.2021.e08404

Wang SQ, Xu H, Stanfield JW, Osterwalder U, Herzog B. Comparison of ultraviolet A light protection standards in the United States and European Union through in vitro measurements of commercially available sunscreens. *Journal of the American Academy of Dermatology*. 2017.

Wang Y, Ni X, Wen M, Lou S, Xiao W, Gao Z. Preparation of antioxidant konjac glucomannan-based films enriched with *Ocimum gratissimum* L. essential oil Pickering emulsion and its effect on walnuts preservation. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*. 2023 May 20;665:131220.

Williams JD, Maitra P, Atillasoy E, Wu MM, Farberg AS, Rigel DS. SPF 100+ sunscreen is more protective against sunburn than SPF 50+ in actual use: Results of a randomized, double-blind, split-face, natural sunlight exposure clinical trial. *Journal of the American Academy of Dermatology*. 2018 May 1;78(5):902-10.

Winkler, R. (2015). Iodine—a potential antioxidant and the role of Iodine/Iodide in health and disease. *Natural Science*, *7*(12), 548.

World Flora Online. (2024). *Ocimum gratissimum* L. *World Flora Online*. Retrieved October 19, 2024, from https://worldfloraonline.org/taxon/wfo-0000253342

Wulf HC, Philipsen PA. Improving photoprotection and implications for 25(OH)D formation. *Anticancer Research*. 2020 Jan 1;40(1):511-8.

Xu, D. P., Li, Y., Meng, X., Zhou, T., Zhou, Y., Zheng, J., Zhang, J. J., & Li, H. B. (2017). Natural Antioxidants in Foods and Medicinal Plants: Extraction, Assessment and Resources. *International journal of molecular sciences*, *18*(1), 96. https://doi.org/10.3390/ijms18010096

Yeddes W, Chalghoum A, Aidi-Wannes W, Ksouri R, Saidani Tounsi M. Effect of bioclimatic area and season on phenolics and antioxidant activities of rosemary (*Rosmarinus officinalis* L.) leaves. *J Essent Oil Res.* 2019;31(5):432-443.

Zahran, E. M., Abdelmohsen, U. R., Khalil, H. E., Desoukey, S. Y., Fouad, M. A., & Kamel, M. S. (2020). Diversity, phytochemical and medicinal potential of the genus *Ocimum* L. (Lamiaceae). *Phytochemistry Reviews, 19*, 907–9531.

Zulkarnain, A. K., & Jumina, J. (2023). Optimization of Carbopol, CMN-Na, gelatin, and *in vitro* activity test of 4-hydroxy chalcone gel as sunscreen. *Jurnal Food and Pharmaceutical Sciences, 11*(2). https://doi.org/10.22146/jfps.8237

APPENDICES

Appendix A: Essential Oil Calculations

Yield Calculation

Mass of dried leaves = 300 g

Volume of essential oil = 2.500 mL

Density of essential oil = 0.9036 g/mL

Mass of essential oil = Volume × Density

= 2.500 mL × 0.9036 g/mL

= 2.259 g

Percentage yield = (Mass of essential oil / Mass of dried leaves) × 100

= (2.259 g / 300 g) × 100

= 0.753%

Appendix B: Physicochemical Property Calculations

1. Specific Gravity Calculation

Weight of empty bottle (W1) = 10.234 g

Weight of bottle + distilled water (W2) = 20.234 g

Weight of bottle + essential oil (W3) = 19.337 g

Specific Gravity = (W3 - W1) / (W2 - W1)

= (19.337 - 10.234) / (20.234 - 10.234)

= 9.103 / 10.000

= 0.9036

2. Acid Value Calculation

Volume of KOH used (V) = 0.79 mL

Weight of sample (W) = 0.2 g

Molarity of KOH = 0.1 M

Acid Value = $(V \times 0.1 \times 56.1) / W$

= $(0.79 \times 0.1 \times 56.1) / 0.2$

= 2.22 mg KOH/g

3. Saponification Value Calculation

Volume of HCl for blank (B) = 25.6 mL

Volume of HCl for sample (S) = 18.4 mL

Weight of sample (W) = 0.2 g

Molarity of HCl (M) = 0.5 M

Saponification Value = $[(B - S) \times M \times 56.1] / W$

$= [(25.6 - 18.4) \times 0.5 \times 56.1] / 0.2$

= 203.45 mg KOH/g

Appendix C: Antioxidant Activity Calculations

1. DPPH Radical Scavenging Activity

$\% \text{ RSA} = [(Ac - As)/Ac] \times 100$

Where:

Ac = Absorbance of control

As = Absorbance of sample

Example calculation for 100 µg/mL concentration:

Ac = 0.987

As = 0.472

$\% \text{ RSA} = [(0.987 - 0.472)/0.987] \times 100$

$= (0.515/0.987) \times 100$

$= 52.12\%$

Appendix D: SPF Calculations

1. SPF Value Using Mansur Equation

$SPF = CF \times \Sigma[EE(\lambda) \times I(\lambda) \times Abs(\lambda)]$

Where:

CF = 10 (Correction factor)

$EE(\lambda)$ = Erythemal effect spectrum

$I(\lambda)$ = Solar intensity spectrum

$Abs(\lambda)$ = Absorbance values

Example calculation for 3% EO cream:

Wavelength (nm)	EE × I	Abs	EE × I × Abs
290	0.0150	0.197	0.002955
295	0.0817	0.208	0.016994
300	0.2874	0.198	0.056905
305	0.3278	0.189	0.061954
310	0.1864	0.167	0.031129
315	0.0839	0.158	0.013256
320	0.0180	0.145	0.002610

Sum of (EE × I × Abs) = 0.185803

SPF = 10 × 0.185803 = 2.24

2. Sample Spreadability Calculation

Weight = 100 g

For Base Cream:

Spreadibility = $\frac{D1+D2}{2} = \frac{2.10+2.60}{2} = 2.35cm$

For 1% EO

Spreadibility = $\frac{D1+D2}{2} = \frac{2.20+1.80}{2} = 2.00cm$

Appendix E: Sample Calculations and Raw Data Analysis

1. Viscosity Calculations

To determine the diluted viscosity, we use the equation:

$\eta = Kt\rho$

where:

- η = viscosity (cP)
- K = viscometer constant
- t = flow time (seconds)
- ρ = density of the sample (g/cm³)

Solving for k:

Known Values for Water at 25°C:

Viscosity of water = 0.89 cP,

Density of water = 0.997 g/cm³,

Flow time for water = 91 seconds

Rearrange the viscosity equation to solve for *k*:

$$= \frac{cP}{t\rho} = \frac{0.89}{91 \times 0.997} = \frac{0.89}{90.7} = 0.0098 \approx 0.01$$

Calculation of Diluted Viscosity

For the base cream:

K = 0.01 (viscometer constant, for the viscometer)

t = 21.5 sec

ρ = 1.05 g/cm³

η diluted = (0.1) (21.5) (1.05) = 2.15 cP

Similarly, for 1% EO:

η diluted = (0.1) (20.8) (1.00) = 2.08 cP

Calculation of estimated Viscosity

To determine the estimated true viscosity (η true), the Krieger-Dougherty equation is applied:

$$\eta_{\text{estimated}} = \eta_{\text{diluted}} \times \left(\frac{1 - \frac{\varphi_{diluted}}{\varphi_{\text{max}}}}{1 - \frac{\varphi_{\text{diluted}}}{\varphi_{\text{max}}}} \right)^{[\eta]\varphi_{\text{max}}}$$

Where:

ϕ max = 0.64 (maximum packing fraction)

ϕ original = 0.63 (original volume fraction)

Intrinsic viscosity parameter [η] = 4.5 (for droplet sphere/emulsion)

Computing for the base cream:

$$2.15 \times \left(\frac{\left(1 - \left(\frac{0.1007}{0.64}\right)\right)}{\left(1 - \left(\frac{0.63}{0.64}\right)\right)} \right)^{4.5\times 0.64} = 2.15 \times \left(\frac{1 - 0.1573}{1 - 0.9844}\right)^{2.88}$$

$$= 2.15 \times \left(\frac{0.8427}{0.0156}\right)^{2.88} = 2.15 \times 54.0^{2.88} = 2.15 \times 97565$$

$$= 209764.27$$

2. pH Value Standard Deviation

For 3% EO formulation:

Readings: 6.52, 6.54, 6.53

Mean = (6.52 + 6.54 + 6.53) / 3 = 6.53

Standard Deviation calculation:

$SD = \sqrt{[\Sigma(x - \mu)^2/n]}$

$= \sqrt{[(0.01^2 + 0.01^2 + 0^2)/3]}$

$= \pm 0.01$

Appendix F: Formulation Development and Stability Data

Table F1: DPPH Radical Scavenging Activity Raw Data

Concentration (µg/mL)	Essential Oil A1	Essential Oil A2	Essential Oil A3	%RSA (EO)	Ascorbic Acid A1	Ascorbic Acid A2	Ascorbic Acid A3	%RSA (AA)
1	0.472	0.475	0.470	44.83	0.478	0.475	0.476	44.31
2	0.436	0.438	0.435	49.07	0.394	0.396	0.395	53.99
5	0.466	0.465	0.467	45.50	0.308	0.310	0.309	64.01
10	0.436	0.438	0.435	49.07	0.304	0.303	0.305	64.52
20	0.432	0.434	0.433	49.41	0.304	0.303	0.305	64.52
50	0.431	0.430	0.432	49.58	0.289	0.290	0.288	66.21
100	0.409	0.410	0.408	52.12	0.312	0.313	0.311	63.50
200	0.400	0.401	0.399	53.14	0.298	0.297	0.299	65.20

Control absorbance (Ac) = 0.854

Table F2: UV Absorbance Data for SPF Calculation of Essential Oil

Wavelength (nm)	EE × I	Absorbance	EE × I × Abs
290	0.0150	0.185	0.002775
295	0.0817	0.184	0.015033
300	0.2874	0.182	0.052307
305	0.3278	0.180	0.059004
310	0.1864	0.178	0.033179
315	0.0837	0.175	0.014648
320	0.0180	0.172	0.003096
Total			**0.176600**

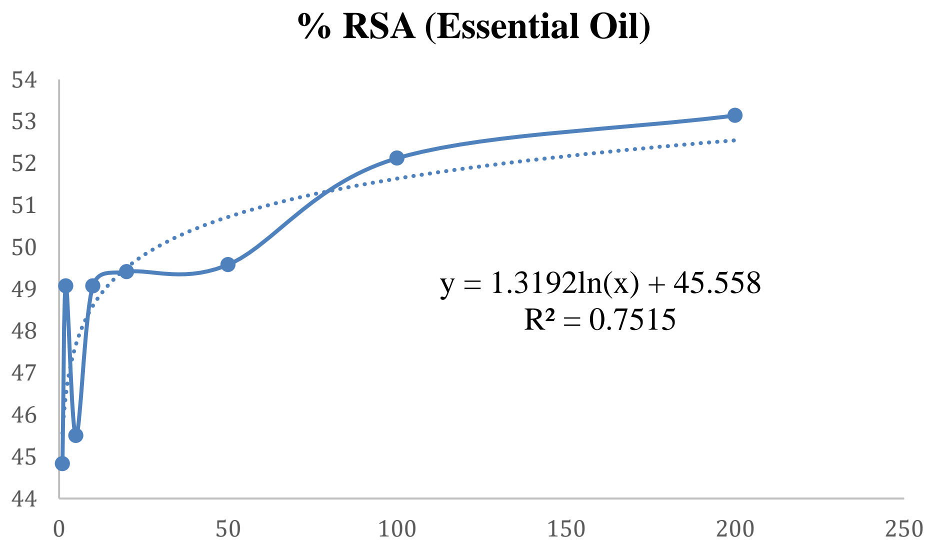
SPF = 10 × 0.1766 = 1.766

Table F3: Stability Study Data - pH Values Over Time

Time (hours)	Base Cream	1% EO	2% EO	3% EO	3% EO + CO
0	6.8	6.7	6.6	6.5	6.5
6	6.7	6.6	6.5	6.3	6.2
12	6.6	6.5	6.4	6.2	6.1
24	6.4	6.3	6.1	5.9	5.7

Appendix G: IC_{50} Extrapolation and Calculation

A. IC_{50} of the Essential Oil



Calculation:

Y set at 50

From the trendline equation, $y=1.3192 \cdot \ln(x)+45.558$

$50 = 1.3192\ln(x)+45.558$

$4.442 = 1.3192 \cdot \ln(x)$

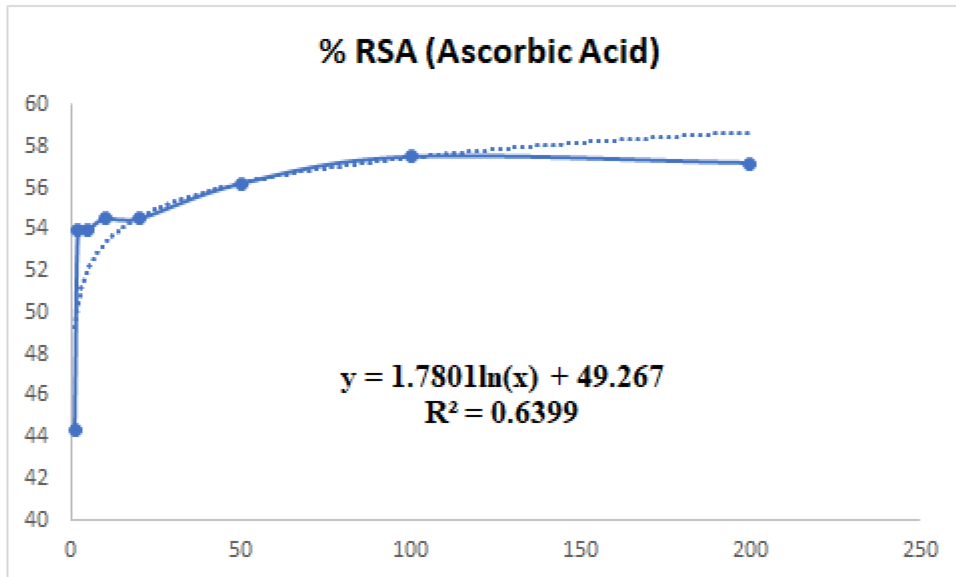
$\ln(x) = 4.442/1.3192$

$\ln(x) = 3.367$

$x = e^{3.367}$

$x \approx 28.96$μg/mL

B. IC_{50} value of Ascorbic Acid



Calculation:

$y = 50$

$50 = 1.7801 \ln(x) + 49.267$

$50 - 49.267 = 1.7801 \ln(x)$

$0.733 = 1.7801 \ln(x)$

$\ln(x) = 0.733/1.7801$

$\ln(x) \approx 0.411$

$x = e^{0.411}$

$x \approx e^{0.411} \approx 1.509\mu g/mL$

Appendix H: Extracted Essential Oil

Appendix I: Formulated Creams

Appendix J: Extraction and Formulation Process

79-1 MAGNETIC STIRRER WITH HEATER
PILOT LAMP
HEATE LAMP
AC 220V 50Hz
HEAT POWER: 250W
ON
OFF
POWER
ON
OFF
HEATER
JINOTECH INSTRUMENTS

Rite