REPELLENT ACTIVITIES AND FORMULATION OF *OCIMUM* SUAVE, *OCIMUM AMERICANUM* AND *EUCALYPTUS CITRIODORA* ESSENTIAL OILS AGAINST *ANOPHELES GAMBIAE*

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A Thesis Submitted to the Graduate School in Partial Fulfilment of the Requirements for the Award of the Degree of Master of Science Degree in Chemistry of Tharaka University

THARAKA UNIVERSITY

NOVEMBER 2024

DECLARATION AND RECOMMENDATION

Declaration

This thesis is my original work and has not been presented for any award of a diploma or conferment of a degree in any other institution.

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Recommendation

This thesis has been examined, passed and submitted with our approval as the University supervisors

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DEDICATION

I dedicate this work to my husband David Mwenga, my mother Martha Ciandige and my children Alex Mwenda and Patricia Kawira.

ACKNOWLEDGEMENT

I wish to acknowledge my supervisors; Dr. Alex Muthengi and Dr Fidelis Ngugi for their guidance and support towards the accomplishment of this work. I wish to acknowledge Mr. Eric Osoro for his guidance and assistance. Gratefulness also goes to the Faculty of Physical Science, Engineering and Technology and technologist of Tharaka University, University of Nairobi and ICIPE for their cooperation and support during the various stages of data collection, providing laboratory space, technological support and internet access for scientific journals. I wish to acknowledge the entire management of Tharaka University for giving me this opportunity to pursue education in this institution also awarding me internal research fund. Special regards to my husband David Mwenga and my children Alex Mwenda and Patricia Kawira for their financial and moral support. Finally, Glory and honour be to God for enabling me to complete my studies

ABSTRACT

Vector-borne diseases such as malaria and filariasis are public health problems that affect the socioeconomic status of all developing countries. Malaria is a serious disease globally, for instance, according to year 2019 and 2020 global malaria data; there were 227 million and 241 million malaria cases respectively, and approximately 627,000 deaths. In year 2020 about 95% of malaria cases and 96% of malaria deaths occurred in Africa. Mosquitoes are the main causes of malaria but there are several spices such as Aedes, Culex and Anopheles that transmit various diseases. Anopheles is the main vector for malaria which needs to be controlled in order to minimize the spread of malaria. Use of mosquito repellents is one of the ways used to prevent bites from the *A. gambiae*. In the market there are several repellents that are used in prevention of mosquito bites but they have negative effects such as allergy and dermatitis to the users. Therefore, the current study seeks to formulate an eco-friendly and non-toxic *A. gambiae* repellent for control of malaria. The plant samples were collected from *Mugui* village, Tharaka South Sub-County, Tharaka constituency in Tharaka Nithi County. The samples were hydro-distilled using a Clevenger apparatus to obtain the essential oils, and experimental tests done in a repellent testing chamber. The essential oils were then analyzed using Gas Chromatography-Mass Selective detector instrument (GC-MSD) to determine their chemical composition. The most abundance compounds in the essential oils were terpenoids: 1, 8-Cineole, *β*-Myrcene, *β*-Pinene. A blend of *O. suave* and *O. americanum* mixed in the ratio of 1:1 was more potent repellent (100.00±0.00) against *A. gambiae* suggesting that this may be due to additive or synergistic effects of individual constituents. The most potent single essential oil was *O. americanum* while *Eucalyptus citriodora* was the least potent. The values of repellence action were determined over control at a p-value of 0.05 and 0.01 by one-way Analysis of Variances (ANOVA) and separated using Student-Newman-Keels at P≤0.05 using SAS software.

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ABBREVIATIONS AND ACRONYM

AIDS	Acquired Immunodeficiency Syndrome
ANOVA	Analysis of Variances
DDT	Trichloro-2, 2-bis-(p-chlorophenyl) ethane
DEET	N, N-diethyl-m-toluamide
DHA-PQ	Dihydroartemisinin Piperaquine
EFAB	Extinguish Fire Ant-Bait
GC	Gas Chromatograph-Mass Spectrometry
GC-MS	Gas Chromatograph-Mass Spectrometry
GC-MSD	Gas Chromatography-Mass Selective Detector
GMMs	Genetically Modified Mosquitoes
HCH	Hexachlorocyclohexane
HIV	Human Immunodeficiency Virus
HPLC	High Performance Liquid Chromatography
ICIPE	International centre for Insect Physiology & Ecology
ICIPE	International Centre of Insect Physiology and Ecology
IGIs	Insect Growth Inhibitors
IGRs	Insect Growth Regulators
IPTp	Intermittent Preventive Treatment in Pregnancy
IRS	Indoor Residual Spraying
ITN	Insecticide Treated Mosquito
ITNs	Insecticide Treated mosquito-Nets
IVM	Integrated Vector Management
LLNs	Long Lasting Insecticide Treated bed Nets
MIM	Multilateral Initiative on Malaria
MVI	Malaria Vaccine Initiative
NACOSTI	National Commission for Science, Technology and Innovation
NIST	National Institute of Standards and Technology
PCM	Percentage Control Means
PCR	Polymerase Chain Reaction
PE	Protective Efficacy
PTM	Percentage Treatment Means
RD50	Dose Response at 50% confidence level

RD75	Dose Response at 75% confidence level
RD90	Dose Response at 90% confidence level
RT	Retention Time
SNK	Student-Newmann-Kuels
SE	Standard Error
SPSS	Statistical Package of Social Sciences
SSP-2	Sporozoite Sulfur Protein-2
SSP66	Sporozoite Surface Protein-Based Vaccine
UNDP	United Nation Development Program
UNICEF	United Nations Children's Emergency Fund
VOCs:	Volatile Organic Compounds

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Among the most significant public health issues and barriers to socioeconomic development of developing nations, particularly in the tropics, are vector-borne diseases like malaria and filariasis (Health Organization, 2019). There are many spices of mosquitoes such as Aedes, Culex, Anopheles, Culiseta, Mansonia, Psorophora, Wyeomyia, Toxorhynchites and many others that cause vector-borne diseases. According to World Health Organization, (2017), *Anopheles gambiae* spreads transmits malaria that harm 70 billion people annually throughout the world and also caused an estimated number of malaria deaths that stood at 627,000 in year 2020 in Kenya Africa. Children under the age of five are thought to mostly account for two thirds of deaths according to World Health Organization, (2020). Findings by Suwannayod *et al*. (2019) shows that there is no vaccination for malaria which is spread by *A.gambiae*; therefore, avoiding *A. gambiae* bites is one of the best ways to avoid spread of malaria

Berkeley *et al* (1996) study shows that the management of the adult *A. gambiae* population is aided by the use of plant extracts to reduce *A. gambiae* bites. Larval management has been an issue because of the numerous water bodies that result from agricultural irrigation systems, precipitation and natural calamities like floods or prolonged rain seasons. Traditionally, *A. gambiae* repellent herbs in western Kenya were the subject of ethno botanical investigations, which revealed that the plants are definitely efficient against malaria vectors when burned or thermally ejected with household charcoal stoves (Seyoum *et al*.2002). In addition, some plants were frequently applied by clipping the branches and putting them inside homes, primarily around beds However, this method was not effective because there is still spread of malaria, and branches are also vague. The current study intended to examine the effectiveness of essential oils extract from *Ocimium suave, Ocimium americium,* and *Eucalyptus citriodora* in repelling *A.gambiae.*

Previous study by Asadollahi *et al*. (2019) One of the advantages of using plant extracts, such as essential oils from *Ocimium suave*, *Ocimium americium*, and

Eucalyptus citriodora, in repelling *A. gambiae* is that they are environmentally friendly. Unlike chemical insecticides which can harm the environment and non-target species, plant-based repellents are natural and biodegradable. This is particularly important in regions where agriculture and wildlife are closely intertwined, as using plant-based repellents can help protect the ecosystem while also reducing the spread of vector-borne diseases. Through choosing plant-based repellents, communities can support sustainable practices that have minimal impact on the environment, promoting harmony between humans and nature. Another advantage of using plant extracts as repellents is that they are cost-effective. Plant-based repellents can be easily obtained from locally available plants, reducing the need for expensive chemical insecticides that may not be affordable for residents of developing nations. By utilizing plants that are already abundant in the local environment, communities can save money on vector control measures while still effectively protecting themselves from diseases like malaria. This cost-effective approach to vector control can help make public health interventions more accessible to communities in developing nations, ensuring that they have the resources they need to combat diseases transmitted by mosquitoes. Additionally, the availability of these plants makes them a practical solution for managing *A. gambiae* populations. Plants like *Ocimium suave, Ocimium americanum* and *Eucalyptus citriodora* are commonly found in tropical regions where *A.gambiae* infestations are prevalent. This means that communities can easily access these plants for use in repellent products without having to rely on imports or expensive manufacturing processes. Through utilizing local plants for vector control, communities can take advantage of the natural resources in their environment to address public health challenges. The localized approach to vector control also promotes self-sufficiency and empowers communities to address health issues using resources that are readily available to them.

A study conducted by Mboera *et al*. (2018) examined the efficacy of plant-based repellents in repelling mosquitoes in Tanzania. The study found that while certain plant extracts showed repellent effects against *A. gambiae,* the duration of protection provided by these repellents was limited compared to synthetic repellents. This suggests that further research is needed to optimize the effectiveness and longevity of plant-based repellent formulations for sustained *A. gambiae* control.

Further study by Vontas *et al.* (2018) investigated the mechanism of action of plant-based repellents on *A. gambiae*. The study revealed that plant extracts containing specific compounds, such as limonene and citronellal, disrupt the olfactory receptors in mosquitoes, interfering with their ability to locate hosts for blood-feeding. This disruption in the mosquito's sensory perception could contribute to repelling *A. gambiae* and reducing their biting behaviour. These findings underscore the importance of understanding the underlying mechanisms of action of plant-based repellents in order to develop more effective and long-lasting mosquito control strategies.

A number of studies on the effect of the essential oils on *A. gambiae* and other biting arthropods focused on the application of essential oil and identification of the active components rather than the free emitted volatile blends (Mathu *et al.*, 2015). Ndirangu *et al.* (2015) working on the essential oil of *Nigella sativa* L. seeds and (Ywaya *et al.* (2013) working on the essential oils of three plant species (*Ocimum gratissimum* L, *Hyptis suaveolens* L. and *Vitex keniensis* Turill) reported that *α*-pinene and *β*-pinene repels *An. gambiae*.

Previous studies have focused largely on anti-microbial activities of crude products and specific constituents of formulation of *Ocimum suave*, *Ocimum americanum* and *Eucalyptus citriodora* essential oils. Various crude extracts of this plant showed significant activity against all the bacteria tested. The leaf extract was reported to have the highest activity against Bacillus subtitles, Staphylococcus aureus and Proteus vulgaris than *Escherichia coli*, *Streptococcus pneumonia* and *Klebsiella pneumonia* (Para *et al*., 2013)

Formulation of *Ocimum suave, Ocimum americanum* and *Eucalyptus citriodora* essential oils oil were found to be fungicidal against *Candida albicans*, *Aspergillus albus* and Dermatophytic fungi (Para *et al*., 2013). Semi-field experiments showed that formulation of *Ocimum suave, Ocimum americanum* and *Eucalyptus citriodora* essential oils extracts has repellent and insecticidal activity against cabbage aphid (*Brevicoryne brassicae*) (Mersha *et al*., 2014), rice Weevil (*Sitophilus oryzae*), rice moth (*Corcyra cephalonica*) (Khani *et al*., 2012) and larvicidal activity against dengue vector, *Aedes aegypti* (Kumar *et al*., 2011). The essential oils from these plants has been reported to present a good activity against the following important postharvest deteriorating fungi: *Aspergillus flavus*, *Aspergillus glaucus*, *Aspergillus niger*, *Aspergillus ochraceous*, *Colletotrichum gloesporioides*, *Colletotrichum musae*, *Fusarium oxysporum* and *Fusarium semitectum* (Marcelo *et al*., 2012).

The essential oils of *Ocimum suave*, *Ocimum americanum* and *Eucalyptus citriodora* have been found to include tannin, phenols, steroids, alkaloids, and flavonoids. The phenolic chemicals prevent insects from feasting. A phenolic component from the wild groundnut prevented the development of *Spodoptera-litura.* A geometrid caterpillar's rate of tissue consumption may be altered by phenols in plant tissues (*Epirrita autumnata)* (Para *et al*., 2013). From this, it is clear that phenolic compounds from the formulation of *Ocimum suave*, *Ocimum americanum* and *Eucalyptus citriodora* essential oils will not allow insects to feed on treated environment (Para *et al*., 2013).

Other studies have explored the efficacy of plant-based repellents in combination with other control measures to enhance their effectiveness against *A. gambiae* populations. For example, a study by Ondiba *et al.* (2017) investigated the synergistic effects of combining plant extracts with larvicides for integrated *A. gambiae* management. The study demonstrated that the combination of plant-based repellents with larvicides resulted in improved *A. gambiae* control outcomes, suggesting that integrated approaches to vector control may offer enhanced protection against mosquito-borne diseases. Despite the potential benefits of plant-based repellents for *A. gambiae* control, challenges remain in scaling up their use for widespread public health interventions. Factors such as variability in plant extract composition, availability of plant species, and community acceptance of natural repellents may influence the implementation and sustainability of plant-based vector control strategies.

In addition to exploring the efficacy of plant-based repellents, research efforts have also been directed towards understanding the ecological implications of using natural products for *A. gambiae* control. Studies have investigated the impact of plant-based repellents on non-target species, such as beneficial insects and aquatic organisms, to assess the potential risks associated with their widespread use. Through evaluating the environmental consequences of plant-based repellents, researchers can develop eco-friendly *A. gambiae* control strategies that minimize harm to ecosystems while effectively targeting vector populations.

Recent advancements in technology have enabled the development of innovative formulations and delivery systems for plant-based repellents, such as microencapsulation and slow-release mechanisms. Such technological innovations aim to enhance the longevity and efficacy of plant-based repellents, providing sustained protection against *A. gambiae* bites and reducing the risk of disease transmission. Harnessing the natural repellent properties of these plant extracts can help communities to effectively protect themselves from *A. gambiae* bites and reduce the risk of malaria transmission. Such an evidence-based approach to vector control not only provides a sustainable solution to *A. gambiae* control but also offers a safe and reliable alternative to chemical insecticides. Plant-based repellents offer a promising avenue for vector control that can contribute to the prevention of mosquito-borne diseases in endemic areas. Through using these plant extracts in repellent products, communities can reduce the risk of *A. gambiae* bites and the transmission of diseases like malaria. This evidence-based approach to vector control provides communities with a proven method for protecting themselves from vector-borne diseases, offering a safe and reliable alternative to chemical insecticides. The goal of this thesis report is to develop an accessible, affordable and effective *A. gambiae* repellent, which is green, with no side effect to the user that can be used during the day and at night to avoid bites from *A. gambiae.* The chemical constituents in the oil were evaluated using Gas Chromatography coupled with Mass Spectrometry. A more effective product was formulated after the analysis of the ingredients responsible for repellence, since the essential oils from *O. suave, O.americanun* and *E.citriadora* plants have some components such as monoterpenoids which are said repel. *A. gambiae.*

The current study sort to extract, determine the amount, and chemical composition of the essential oils from *O. suave*, *O.americanun* and *E. citriadora* and explore its utilization as a novel repellent against *A. gambiae.* Furthermore, the study formulated a repellent against *Anopheles gambiae* from the potent blend of *Ocimum suave* and *Ocimum americanum* in the ratio of 1:1.

1.2 Statement of the Problem

The use of repellents to protect individuals from *A. gambiae* bites has been accepted as a component of a comprehensive integrated approach to prevent insect-borne diseases. However, the chemicals used to make majority of commercial repellents, like *N*, *N*-diethyl-metatoluamide (DEET) such as allethrin, *N*, *N*-diethyl mendelic acid amide, and dimethyl phthalate, have been reported to pose adverse health and environmental effects on synthetic fabric and plastic as well as toxic reactions such allergy, dermatitis effects, and adverse effects on the cardiovascular system and nervous system. The other methods of prevention and treatment of malaria such as use of Insecticide Treated Mosquito Nets (ITN), Anti malaria tablets like Artemether/Lumafantrine (AL) are expensive, and there is *plasmodium falciparum* resistance to most of these malaria drugs. Insecticide Treated Mosquito Nets prevent *A. gambiae* bites at night only. In this regard, the present study has developed an effective and environmentally friendly *A. gambiae* repellent formulated from the blend of essential oils of *O. suave and O. americanum.* The repellent can be utilized to deter *A. gambiae* bites, thus aid in control of malaria.

1.3 Objectives of the Study

1.3.1 General Objective

The general objective of the study was to examine the repellent activities of essential oils of *O. suave, O. americanum,* and *E. citriodora* against *A. gambiae* and formulation of a repellent product.

1.3.2 Specific Objectives

i. To extract the essential oils from *O. suave, O. americanun* and *E. citriodora* using hydro-distillation apparatus.
ii. To ascertain the chemical make-up of essential oils from *O. suave, O. americanun* and *E.citriodora* using GC-MS.
iii. To determine the repellence of *O.suave, O. americanun* and *E. citriodora* and their blends using Human-bit technique.

iv. To formulate *A. gambiae* repellent from essential oils of *O. suave, O. americanun* and *E. citriodora*.

1.4 Hypothesis

$H_{01:}$ There was no significant difference in the extraction of essential oils obtained from *O. suave, O. americanun* and *E. citriodora,* using hydro-distillation apparatus

$H_{02:}$ There was no significant difference in the chemical composition of essential oils from *O. suave, O.americanun* and *E. citriodora* using GC-MS

$H_{03:}$ There is no significant difference in the repellence of *O. suave, O. americanun* and *E. citriodora* and their blends using Human-bit technique.

$H_{04:}$ There is no significant difference in the formulation of *A.gambiae* repellent from essential oils of *O. suave, O. americanun* and *E. citriodora*

1.5 Significance of the Study

Previous study by Din *et a*l.(2017) shows that chemotherapy is still the primary method of preventing malaria, a deadly disease that affects over 40% of the world's population therefore there is a need to develop a better control of *A. gambiae* which is a vector for malaria. According to findings by WHO,(2022), chemotherapy is mostly done as an intermittent preventive treatment in pregnancy, in infants and sometimes it is done seasonally hence disadvantaging people who do not fall and the above category. Some drugs such as antimalarial drug efficacy is not in any way static, so that constant re-evaluation of drug response, drug acceptance significant side effects, and alternative way is needed. Therefore, the current study has utilized locally available resources which are taken to be waste to come up with a cost effective, readily available, economical, efficient, and simple to use *A gambiae* repellent using the essential oils from *O. sauve, O. americanum,* and *E. citriodora*. The study also made an attempt to formulate the repellent using leaves which are always a waste and the extract is effective and eco-friendly.

CHAPTER TWO

LITERATURE REVIEW

2.0 Overview

The application of essential oils as natural insect repellents has gained popularity in past few years due to concerns over the use of synthetic chemicals and their potential harmful outcome on human health and the environment. According to Tchouassi. *et.al*. (2019), essential oils derived from plants have been discovered to possess insecticidal and repellent qualities against a wide range of insect pests, including mosquitoes. Among the essential oils that have shown promise as mosquito repellents are those extracted from *Ocimum suave, Ocimum americanum,* and *Eucalyptus citriodora*. According to Ogoma.*et.al* (2018), *Ocimum suave,* also known as the tropical basil, is a plant species that belongs to the *Lamiaceae* family. It is native to tropical regions of Africa and Asia and has been traditionally used in herbal medicine for its various medicinal properties. Several research studies have investigated the insecticidal and repellent activities of the essential oil extracted from *O. suave* against mosquito species, including *A.gambiae*. In a study conducted by Tchouassi *et al*. (2019), the essential oil of *O. suave* was found to exhibit significant repellent activity against *A. gambiae*, with a repellence rate of over 90%.

Additionally, *Ocimum americanum,* commonly known as American basil, is another plant species belonging to the Ocimaceae family that has been investigated for its insecticidal and repellent properties. Essential oils extracted from *O. americanum* have shown promising results as mosquito repellents in various studies. For example, in a study by Ogoma *et al*. (2018), the essential oil of *O. americanum* was found to have strong repellent activity against *A. gambiae*, with a repellence rate of up to 95%. These findings suggest that *O. americanum* essential oil could be a potential natural alternative for mosquito repellent products.

Moreover, Eucalyptus citriodora is another plant species belonging to the Myrtaceae family. The essential oil of *E. citriodora* is well-known for its strong lemon-like scent and insect repellent properties. Several studies have demonstrated the efficacy of *E. citriodora* essential oil as a mosquito repellent, particularly against *A. gambiae*. In a study by Kweka *et al.* (2016) the essential oil of *E. citriodora* exhibited significant repellent activity against *A. gambiae* mosquitoes, with a repellence rate of over 90%.Based on Kweka *et al*. (2017), formulation of vital oil-based repellent products is a crucial step in utilizing the insecticidal and repellent characteristics of these plant extracts for practical use. Essential oils are highly concentrated liquids that can be irritating to the skin when applied directly, so it is essential to formulate them into safe and effective products for topical application Kweka *et al* (2017). Various formulations such as sprays, lotions, creams, and candles have been developed to enhance the efficacy and safety of essential oil-based mosquito repellents. In a study by Kweka *et al.* (2017), a repellent lotion containing a blend of essential oils, including *O .americanum* and *E. citriodora,* was formulated and tested for its repellent activity against *A. gambiae*. The lotion was found to be highly effective in repelling mosquitoes, with a repellence rate of over 95%.

2.1 *Ocimium Suave*

Native to Africa and India, the *O. suave* as shown in Figure 2.1 is a tropical shrub in the Labiate family. The plant has a number of indigenous uses such as adding taste to tobacco and using it as a body scent (Yuan *et al*. 2019 .). Tanzania is where it is primarily grown, and people regularly use it to send off mosquitoes. Its branches are placed on walls and roofs of shelters or burned. Mosquitoes, flies, and other insects are reported to repel away from the leaves. According to some claims, the plant has a variety of medical properties, and its extracts are used to cure coughs, gastrointestinal problems, eye and ear issues. (Din *et al*., 2017).

Hekla.*et al*. (2017), solely, stated that the fundamental components of this plant's composition have been identified in prior studies, which are only concerned with the essential oil. Following the evaluation by Wanzala (2017).

Eugenol and methyl eugenol as shown in Figure 2.2 is present in oils from a variety of sources in levels ranging from 62% and 56% of the oil from Western Kenya respectively.

Figure 2.1: *O. suave* plant

2.2 *Ocimium americanum*

The *O. americanum*, as shown in Figure 2.2, often known as labiate, is a member of the Lamiaceae family (Balakrishna *et al*. 1996). The plant is an herb with several sub-quadrangular striate branches that is pubescent and upright (Parrotta, 2001). Strongly scented herb with elliptic-lanceolate, whole, glabrous, and gland-dotted leaves; tuberculous, terete to four-angular branchlets. The leaves are also elliptic-lanceolate, whole or barely toothed, almost glabrous, and gland-dotted. Small, white, pink, or purple flowers are seen in more or less close-set whorls on spiciform racemes (Das Sarma *et al*.2011). According to legend, seeds in South America have Nublets that are punctuated, black, and narrowly ellipsoid. The three distinct chemo types of *O. americanum*, also known as the hoary basil or mosquito plant, are floral-lemony, camphorates, and spicy (Wendimu, A., & Tekalign, W, 2021) .From a pharmacological perspective, it's possible that the essential oil extracted from *O. americanum* had antibacterial action against Salmonella tryphosa, *Escherichia coli*, *Streptomyces pyogenes*, and *Staphylococcus aureus*. At 100 ppm concentration, there is anti-tubercular action against *Mycobacterium spy. Vibriocholerae, Staph. Albus, Sal. Paratyphoid* and *Xanthomonas campestris*. Were among the bacteria that the essential oil from the leaves showed antibacterial efficacy against. Additionally, *O. americanum* oil demonstrated antifungal effectiveness against a wide range of fungi, including certain human diseases (Ntezurubanza *et al*., 1988).

Figure 2.2: *O. americanum* plant

2.3 *Eucalyptus citriodora*

Numerous species of the Australian native genus *E. citriodora* (family Myrtaceae), as shown in Figure 2.3, also known as the gum tree, are found all over the world (Brooker and Kleinig 2004 *)*. Tall eucalyptus trees typically have aromatic, evergreen foliage that contains volatile essential oil. The oil is valuable commercially and ranks highly in both quantity and quality. It is utilized in the pharmaceutical and perfume industries. Numerous volatile monoterpenes, including cineole, citronellal, citronellal, limonene, linalool, and terpene, make up its chemical structure (Brooker . and Kleinig 2004).

However, the quantity and makeup of oil differs depending on the species, metabolic phases, in addition to the location and climatic conditions. Around 1792 saw the first introduction of eucalyptus to India, and today it is grown on roughly 32.6 M ha primarily for commercial purposes and as part of numerous reforestation initiatives (Khanuja, 2001.). Forest red gum (*E. tereticornis*), river red gum (*E.camaldulensis* Dehnh.), Tasmanian blue gum (*E.globules Labill*.), and lemon scent eucalyptus are among the species most frequently planted throughout the nation (*E.citriodora* Hook.). One of them, *E. citriodora,* is a tall, beautiful tree with a canopy of drooping leaf that is planted in North Indian plains in parks, gardens, along roadsides, and in farmer fields as part of various forestry and agroforestry schemes. The tree exhibits distinct morphological phases depending on its age (Chalchat *et al*., 2000).

Citronellal, which has significant commercial value, is present in large amounts in the tree's oil (extracted from the leaves, stem, and buds). Additionally, it has a broad range of biological actions, including phytotoxic, insecticidal, nematocidal, and insecticidal (Ramezani *et al*.2005), as well as fungicidal and insecticidal. However, the oil is taken from the young and old foliage for commercial and other uses. Since the tree is ever green, its leaves continue to fall throughout the year after they reach senescence, and new, young, and immature foliage takes their place. As a result, a matrix of foliage, including recently fallen senescent (yellow in colour) leaves and old undecomposed leaf litter covers the ground around *E. citriodora* (Brooker and Kleinig 2004). However, little is known about the composition and chemical makeup of the volatile oil found in these leaf kinds, which may also be a valuable bio resource for commercial exploitation and knowledge of their function in controlling vegetation around and under the tree by inhibiting plant growth (Singh, 2005).

Figure 2.3: *E. citriodora* plant

2.4 Essential Oil Components from Various Ocimium Species

Many Ocimium species vital oils have undergone chemical testing Known to repel *A. gambiae*, Eugenol, as shown in Figure 2.4, has been identified to be a significant constituent of essential oils of Ocimum plants (Chogo & Crank, 2001).

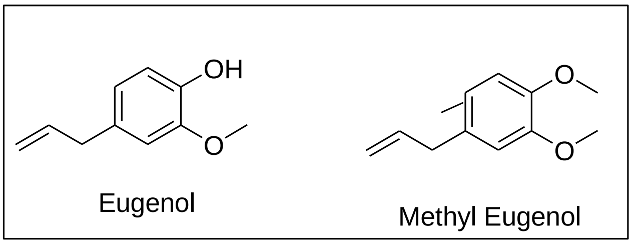


Figure 2.4: Structures of some compounds found in *O. suave* essential oil

With multiple morphologies, variations and chemotypes, *O. basilicum* is ubiquitous and its essential oils exhibit significant chemical diversity. Analysis of *O. basilicum and O. basil var. purpuracens* minimum from northeastern Brazil represented the true estragole chemotype, and his other two species analyzed were revealed to be of the linalool chemotype. (De Vasconcelos *et al*. 2003). Ten *O. bangladeshi* species were analyzed and other volatile oil constituents were described (Mondello *et al*., 2002).This study is going to concentrate on chemical makeup of *O. americanum*, which is widely distributed in most parts of the country Kenya (Li *et al*. 2003).

2.5 Essential Oils from Eucalyptus citriodora

The terpenoids, monoterpenes, sesquiterpenes, and diterpenes, as well as a variety of alcohol, ketones, and aldehydes terpenoids, as well as commonly occurring aromatic components (eugenol, safrole) derived from the phenylpropanoid pathway; make up significant oils, which are volatile plant oils that can be steam distilled. Alkanes, aliphatic alcohol, ketones, and aldehydes may be produced in some species (Adams, 2005). The oils may occasionally contain substances with nitrogen atoms (methylanthranilate in lemon oils) and sulphur atoms (dimethylsulphide in onions) (Williams, 2006).

Essential oils are secondary metabolic by-products of plants that are known to serve a number of vital purposes, such as attracting pollinators and dispersing diaspores, protecting against microorganisms, fungus, insects, and herbivores, and inhibiting seed germination and plant growth. Special cells or clusters of cells, such as the cytoplasm of cells, the vacuoles of epidermal cells, the mesophyll cells of petals, and glandular cells, are where the oils are generated. As seen in Table 2.1, they are typically observed to predominate in one specific plant component or organ.

Table 2.1: Essential Oils in Plant Parts

Plant organ	Essential oil contained
Leaves	Citronella oil
Flowers	Bergamot oil
Roots	Vetiver oil
Rhizome	Ginger oil
Wood	Sandalwood oil
Bark	Cinnamon tree oil
Fruit and seeds	Nutmeg oil

They continue to be utilized as raw materials in a variety of industries, such as nutrition, spices, aromatherapy, cosmetics, perfumes, and phytotherapy (Wendimu & Tekalign, 2022). A better and more targeted application is made possible by a detailed understanding of the composition of essential oils. A number of factors, including the plant's geographic origin, climate and seasonal fluctuations, and production method, influence its composition. The chemical makeup of essential oil can also be significantly impacted by the maturity of the plant at the stage of oil extraction and the presence of chemotypic variations, indicating that the physiological states and/or ecological conditions may interfere with the existence of substances that are biologically active in the plant (Lahlou & Berrada, 2003). These differences are particularly significant for the resulting natural products since, as Figure 2.5 illustrates, an essential oil's worth is closely correlated with its chemical makeup.

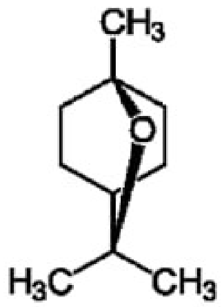
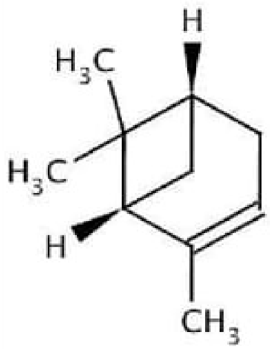
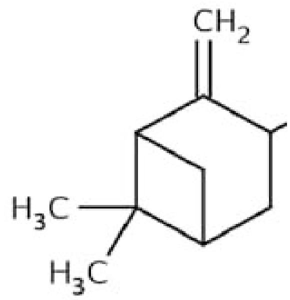
Name	1,8-cineol	α-pinene	L-pinocarveol
			
Synonyms	Eucalyptol 1,8-epoxy-p-menthane Cajeputol	α-pinene oxide (1*R*,5*R*)-2-Pinene	10-Pinen-3-ol
Formula	$C_{10}H_{18}O$	$C_{10}H_{16}$	$C_{10}H_{16}O$
Average Mass (g/mol)	154.24	136.23	152.23
Roles	Flavouring agent A food additive that is used to added improve the taste or odour of a food.	Plant metabolite	Plant metabolite

Figure 2.5: Essential Oils in *E.citriodora*

2.6 Methods of Oil Extraction

There are several ways that can be employed in extraction of vital oils from plant organs which include hydro-distillation, super critical, steam distillation, super critical, solvent extraction and expression effleurage. In this study hydro-distillation was used because it is economical.

2.6.1 Hydro-Distillation

Using water for obtaining essential oils from plant materials is known as hydro-distillation. Water is heated to boiling, and the steam is passed through plant material containing aromatic compounds. The aromatic compounds are vaporized and carried off with the steam, and as the steam cools, the essential oils condense on the surface of the water. The significant oils are then skimmed off the surface of the water or separated using a separatory funnel. This method has been utilised for centuries to obtain essential oils from plants and has been also utilised in the generation of fragrances and perfumes. Hydro-distillation is a form of steam distillation, which is the most common method of obtaining essential oils from plant. The process of hydro-distillation is relatively simple and does not require any specialized equipment (Nchu *et al*. 2012). The plant material is placed in a container, and water is added to the container. The container is then heated until the water reaches a boiling point. The steam is then passed through the plant material, which releases the aromatic compounds into the steam. As the steam cools, the essential oils condense onto the surface of the water. The essential oils are then skimmed off the surface of the water or separated using a separatory funnel. Hydro-distillation is a simple and cost-effective method of extracting essential oils from plant materials. The process is gentle and does not damage the delicate aromatic compounds in the plant material, resulting in a pure essential oil. Additionally, the procedure does not require any specialized equipment, making it a popular choice for obtaining the essential oil.

As illustrated in Figure 2.6, essential oils are produced by the process of hydro-distillation, which involves compressing aromatic plant substances in a still, adding enough water and bringing it to a boil. Alternatively, live steam is introduced into the plant charge. The oil that is essential is liberated from the plant tissue's oil-producing glands by the action of steam and hot water. Indirect cooling with water causes the water and oil vapour mixture to condense. The Distillate from the condenser enters a separator where the oil and distillate water mechanically separate. The following primary physicochemical processes are involved in the hydro-distillation of plant material: (Ntezurubanza *et al.*1988).

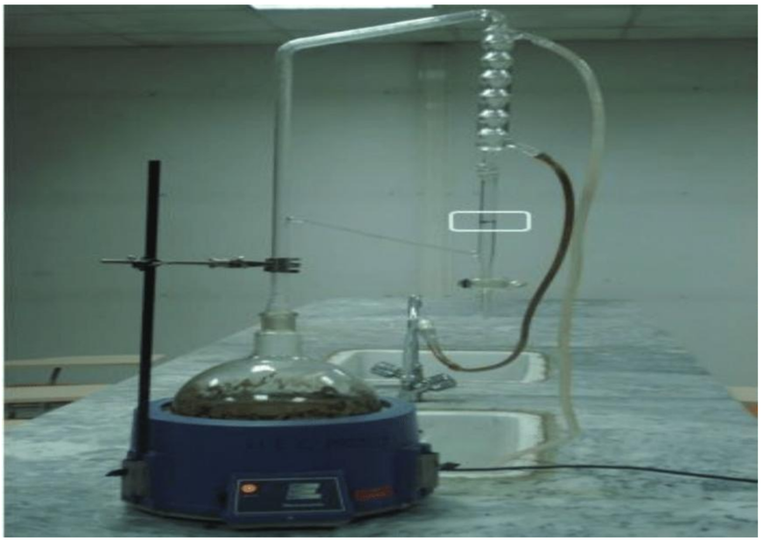


Figure 2.5: Hydro-distillation apparatus

2.7 Repellent Activity

Abenezer *et al*. (2021) designed a study to find about the repellence efficacy of smoke generated by combusting *Eucalyptus camaldulensis, Azadirachta indica* and *Ocimum forskolin* plants to moderate *Anopheles gambiae* biting action. They dissipated smoke by burning crushed powder mixer of the plant leaves and thermal ejection on the traditional stoves in experimental huts against *A. arabinoses* and *A. egyptus*. Additionally, they designed a four-by-four Latin-square which they used to allocate treatment and control experimental huts over different nights. The mosquito density reduction in the treatment huts was utilized for estimating the percentage of repellence of the haze produced by the combustion of plant mixes that were powdered. They determined that huts with burning powder had a smaller amount of A. arabinoses (93.75%, $P < 0.001$) and A. Aegyptus (92%, $P < 0.001$) respectively than huts without any treatment. Generally speaking, plant-mixed powders evaluated using both application techniques provided a high level of protection (>90%) against both tested mosquito species and showed promise as a substitute insect control technique.

Laura *et al*. (2020) discussed and presented a summary review of latest data on creation and effectiveness of plant-based repellents against *A. gambiae* as well as. Their capability to new advancements in the field. These publications were obtained by systematically searching several databases, namely Scopus, PubMed/Medline and Google scholar. Interest-grabbing results included protection duration, percentage repellence, and other characteristics discovered in repellent chemicals. The inclusion criteria were met by a total of 27 trials. The empirical studies found that citronella had a 100% protection rate against *A. gambiae* for 8 to 14 hours, followed by Rhizophora microdata oils, Ligusticum sinense extract, Dalbergia sissoo and pine. Additionally, >90% repellence against various species of Anopheles was demonstrated by essential oils from plants including lavender, camphor, catnip, geranium, jasmine, narrow-leaved eucalyptus, lemongrass, chamomile, cinnamon oil, juniper, cajuput, soya bean, rosemary, niaouli, olive, tagetes, violet, sandalwood, litsea, galbanum, and the review concluded that some plants' essential oils and extracts may be articulated. They found that plant oils could serve as viable alternatives to synthetic repellents in the future as they are relatively safe, affordable, and are abundantly available in many regions of the world.

Comparing plant-based products to synthetic insecticides, there is consensus that they are less hazardous (Regnault-Roger *et al*. 2012; Wendimu, A., & Tekalign, W. 2020). By extracting essential oils from plant leaves using the hydro-distillation process, this inquiry will be conducted to evaluate the repellence activities of *O. suave*, *O. americanum*, and *E. citriodora* oils spray against *A. gambiae*.

2.7.1 Repellents in the Market

Rehman *et al*. (2014) studied the plant-based repellents for the control of insects and rodents. The study found that plant-based repellents are effective in controlling insect and rodent pests, with the most effective being those containing essential oils. These repellents can be applied directly to plants or can be used as part of a larger integrated pest management system. The most effective repellents contained essential oils, with a success rate of up to 98%. Moreover, plant-based repellents were significantly better at repelling pests than synthetic chemical repellents, with a success rate of up to 75% (Rehman *et al*. 2014). The study also found that plant-based repellents tend to be more environmentally friendly than synthetic chemical repellents, and can be used safely around food crops and other sensitive areas.

The study on the use of plant-derived repellents as an alternative to synthetic chemicals to protect stored grains found that plant-derived repellents, such as essential oils, spices, and natural extracts, were effective in reducing insect infestations (Souto *et al.* 2021). They had a repellent effect on stored grain pests and were more cost- effective than synthetic chemicals. Plant-derived repellents were effective in reducing insect infestations by an average of 77.7% (Souto *et al*.2021). They were 20-30% more cost-effective than synthetic chemicals, and had no adverse effects on human health. Additionally, they had minimal environmental impact and did not pose any health risks to humans. When it comes to the use of plant-based repellents for the control of agricultural pests Souto *et al*. (2021) found that plant-based repellents, such as essential oils, spices, and natural extracts, were effective in controlling agricultural pest infestations. The plant-derived repellents were found to be more cost- effective than synthetic chemicals, and had minimal environmental impact. They wereeffective in reducing pest infestations by an average of 80%.

Benelli *et al*. (2019) conducted a study on repellent activities and formulation of *O. suave, O. americanum* and *E. citriodora* essential oils against *A. gambiae*. The study evaluated the efficacy of the essential oils as a repellent against the *A. gambiae*, as well as the efficacy of different formulations of the essential oils. It tested the essential oils in their pure forms, as well as in a formulation of 1:1:1 and 1:2:2 ratios of *O. suave, O. americanum*, and *E. citriodora*, respectively. Their results showed that the pure essential oils had the greatest repellent effect against the *A. gambiae*, with the 1:1:1 formulation showing the lowest repellent activity. The authors concluded that the essential oils could be used as a natural alternative to synthetic repellents, but that the 1:1:1 formulation was not as effective.

A study by Paulraj and Ignacimuthu.(2022) on evaluation of the repellence of *O. suave, O. americanum* and *E. citriodora* essential oils against *A. gambiae* sought to evaluate the repellence of the essential oils against *A.gambiae*. The authors tested the essential oils in their pure forms, as well as in a formulation of 1:1:1 and 1:2:2 ratios of *O. suave, O. americanum*, and *E. citriodora*, respectively. Their results showed that the pure essential oils had the highest repellence against the *A. gambiae*, with the 1:1:1 formulation showing the lowest repellence. The study concluded that the essential oils could be used as a natural alternative to synthetic repellents, but that the 1:1:1formulation was not effective.

Another weakness can be attributed to the formulations of essential oils. According to Okoli *et al*. (2022) pure form of the essential oils had the greatest repellent effect against *A. gambiae*. However, the 1:1:1 and 1:2:2 formulations showed lower repellence, indicating that the formulations are not as effective as the pure forms of the essential oils. This suggests that further research is needed to develop more effective formulations of essential oils, in order to replace synthetic repellents in the market.

Kimutai *et al*. (2017) conducted a study on the efficacy of repellents against Sand-flies and concluded that N, N-diethyl-m-toluidine (DEET) citronella oil, and lemon eucalyptus oil were the most effective repellents against sand-flies. However, DEET had a shorter protection time (around 4 hours) than the other two repellents (trials with citronella oil and lemon eucalyptus oil showed protection times of 8 hours).

DEET had a repellence of 91.8% against sand-flies after 4 hours of exposure, while citronella oil and lemon eucalyptus oil had repellences of 94.6% and 92.2% respectively after 8 hours of exposure. Additionally, repellents were more effective when combined with other repellents, as this increased the protection times.

2.7.2 The Need to Improve Repellents in the Market

The effectiveness of the repellents in the market needs to be improved. Currently, many of them are not effective for long periods of time, and may need to be re-applied after a short period of time. This can be inconvenient for users, and can be potentially hazardous if the user re-applies too much of the repellent, leading to potential skin irritation or other health risks (Tropis & Donated, 2019). Additionally, the repellents can be very expensive, making them inaccessible for many people. Furthermore, many repellents available in the market are synthetic chemicals, which may have negative environmental and health impacts. Therefore, natural repellents, such as those derived from essential oils, may be better and safer alternatives.

Additionally, the safety of repellents in the market needs to be improved. Most of the repellents in the market contain synthetic chemicals, which can be hazardous to humans if used incorrectly (Tuetun *et al*. 2005). Some repellents also contain ingredients that are toxic to aquatic life, which can have negative effects on the environment (Cho *et a*l., 2005). Therefore, natural repellents derived from essential oils may be a safer option for both humans and the environment. Many of repellents need to be improved since they have a strong, unpleasant scent, which can be unpleasant to humans and may not be effective at repelling insects. Natural repellents derived from essential oils may be able to provide a more pleasant scent, while still being effective at repelling insects (Dorman & Deans, 2022). Current repellents also provide limited protection and tend to wear off quickly, often within an hour or two, leaving people vulnerable to mosquito-borne illnesses. This is especially concerning for people who live in areas where mosquitoes are prevalent and the risk of contracting a mosquito-borne illness is high. Long-lasting protection would be beneficial for those who are at high risk of being exposed to mosquitoes.

There is also a need improve their ability to provide protection against a wide range of mosquito species. Currently, repellents are effective against some species of mosquitoes but not others. This means that people who are exposed to multiple mosquito species are not always protected (Tropis & Donated, 2019). Improving repellents to provide protection against a wide range of mosquito species would be beneficial for those who are at risk of contracting a mosquito-borne illness.

Chemicals like *N, N*-diethyl-metatoluamide (DEET), allethrin, *N, N*-diethyl mendelic acid amide, and dimethyl phthalate, as shown in Figure 2.7, are used to make the majority of commercial repellents (Patel *et al*. 2012). These chemical repellents pose a risk to the general public's health and should only be used under strict supervision. This is due to their negative effects on synthetic fabric and plastic as well as toxic reactions such as allergy, dermatitis, and adverse effects on the. cardiovascular system and nervous system (Diaz, 2016)

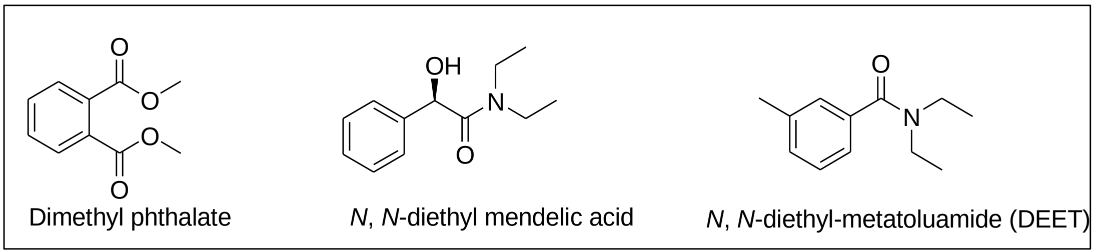
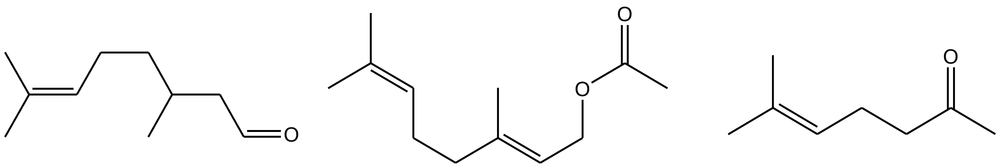


Figure 2.6: Compounds used to make majority of *A. gambiae* repellents

Prolonged and frequent use of synthetic repellents in *A. gambiae* control has resulted to development of resistance to insecticides, resurgence in *A. gambiae* populations and adverse impact on target organisms (Liu N *et al.*2016)). Therefore, employing natural *A. gambiae* repellent products as a replacement to create new eco-friendly repellents could be a peaceful way to rectify the negative impacts on the environment and human health. Interest in plant-based repellents has increased recently since they are a plentiful source of bioactive phytochemicals that are risk-free and biodegrade into nontoxic by-products and may be tested for insecticidal and mosquito-repelling properties (Pavela, 2015). Plant extracts or essential oils have repellent properties against malaria vectors around the world. Most plants produce various compounds to preventing attack from phytophagous (plant eating) insects. These chemicals fall into several categories, including repellents, feeding deterrents, toxins, and growth regulators (SP Carroll, 2006).

Basing on chemical properties these compounds can be grouped into five major categories, namely nitrogen compounds, terpenoids, phenolics, proteinase inhibitors, and growth regulators (Edriss *et al.* 2016). Although the primary functions of these compounds in plant defines mechanism against phytophagous insects remain unclear, many plant compounds are also effective against and other *A. gambiae* biting Diptera, especially volatile components released as a consequence of herbivory. The fact that several of these compounds are repellent to hematophagous insects could be an evolutionary relict from a plant-feeding ancestor, as many of these compounds evolved as repellents to phytophagous insects, and this repellent response to potentially toxic compounds is well conserved in the lineage of Diptera (Asadollahi *et al*. 2019). When leaves are injured by herbivores, plants frequently create volatile "green leaf volatiles," and numerous studies have shown that odour receptors strongly respond to this class of volatiles, which includes citronellal, geranyl acetate, and 6- methyl-5-hepten2-one as shown in Figure 2.8 (Logan *et al*. 2010). However, because to their great vapour toxicity to insects, it is likely that many plant volatiles are deterrent or repellent (Asadollahi *et al*. 2019.). Man has been taking advantage of this plant material's natural repulsiveness for thousands of years, most commonly by hanging damaged plants inside of homes practice that is still common in underdeveloped nations today (Asadollahi *et al*. 2019.). Additionally, plants have been used asrudimentary fumigants for ages.



Citronellal **Geranyl acetate** **6-Methyl-5-hepten2-one**

Figure 2.7: Class of volatiles

Most of the *A. gambiae* control approaches like Insecticide Treated Mosquito Nets (ITN) are not effective in controlling malaria (Wendimu, A., & Tekalign, W. 2021). This is because ITN method protects people from *A. gambiae* bites while sleeping. Therefore, there is need to formulate a green and effective *A. gambiae* repellent product for protecting people from *A. gambiae* bites while resting, working or doing other activities. This proposal study will formulate an *A. gambiae* repellent for control of malaria.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Collection of Material

Plants leaves samples from *O. suave, O. americanun* and *E. citriodora* were collected from *Mugui* in Tharaka South Sub-County, Tharaka constituency in Tharaka Nithi County GPS location 0^{o}3′4′42.7842″S,37^{o}51′58.75092″E, as shown in Figure 3.1. The leave samples were identified by a taxonomist from National Museums of Kenya and a voucher specimen deposited at Tharaka University herbarium. The leaves were washed with clean water, to remove any dust that could contaminate the oil, air-dried to prevent loss of volatile essential oils. The samples were then crushed to smaller pieces using a mechanical grinder to increase surface during extraction.

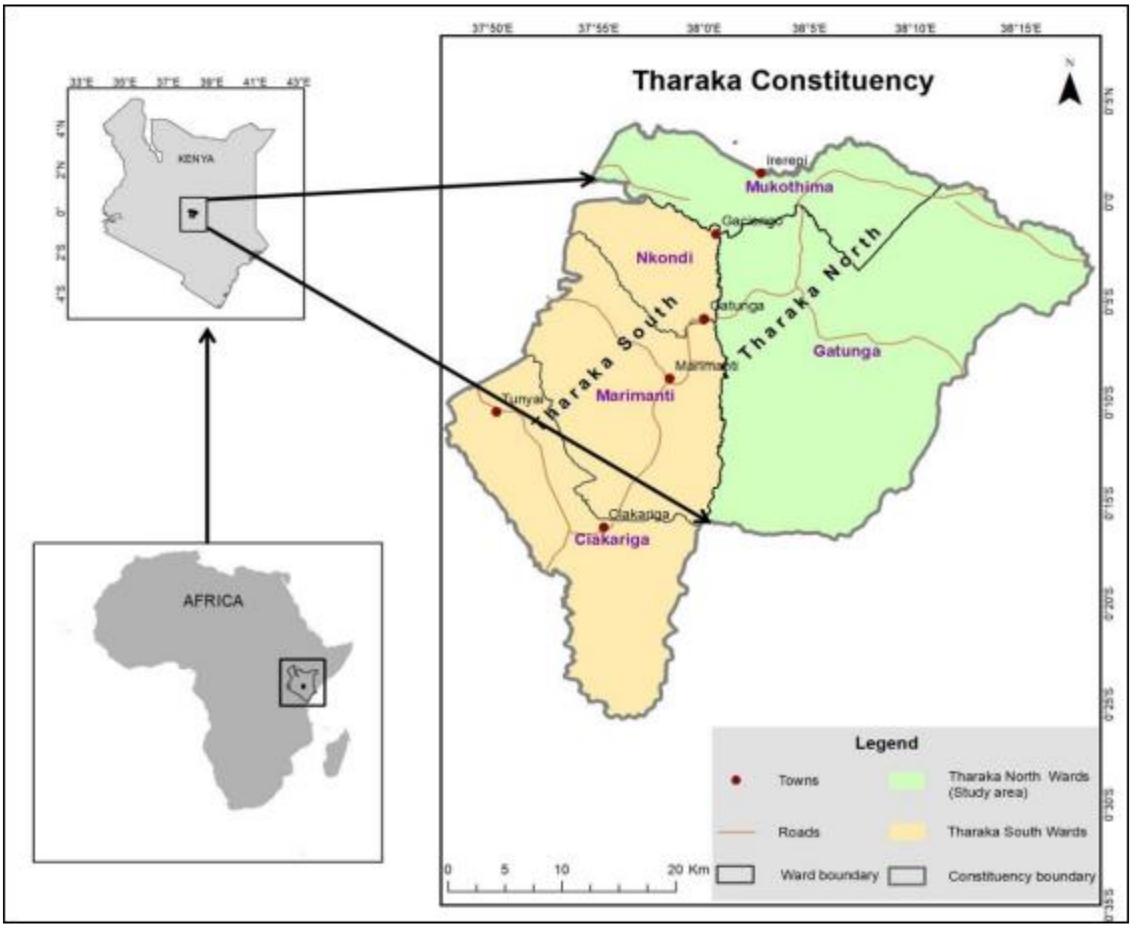


Figure 3.1: Tharaka Constituency

3.2 Extraction of Essential Oils

Extraction of essential oils was done using hydro-distillation apparatus, as shown in Figure 3.2. Five hundred grams of clean, dry and crushed plant leaves were weighed packed in a round bottomed flask and sufficient quantity of water added. The distillate obtained made up of the aqueous layer and organic layer were collected separately where the organic layer (essential oil) was allowed to dry over anhydrous Sodium Sulphate. The dry essential oil was weighed and stored in a lidded vallale in the refrigerator at 4 ^{o}C for use in both chemical and experimental analysis.

Figure 3.2: Extraction of Essential Oils

3.3 Chemical Analysis of Essential Oils

The essential oils of *O. suave, O. americanum and E. citriodora* were analyzed using GC-MS gas chromatograph operating at the following temperature set on the computer: 70 ^{0}C for 4 min, ramp at 4^{0}C/min to 22 ^{0}C for 5 min; carrier gas, N_2. The computer-based method of peak area normalization without any correction factors were used to estimate the relative concentrations of the various elements. Peaks found were compared to data from a GC-MS analysis.(Wendimu, A., & Tekalign, W. 2020.) Injector and detector temperatures were 100 ^{0}C and 120 ^{0}C, respectively; oven temperature was programmed from 40 ^{0}C (isothermal for 7 min) to 13 ^{0}C (isothermal for 12 min) at 50 C/min; with helium as the carrier gas.

Essential oils released from the leaves of eucalyptus plants were trapped using head space entrainment with a super Q trap as an adsorbent and transparent oven bag (355 mm×508 mm, Classic Consumer Products, Englewood, NJ, USA) pre-sterilized at 100 °C for 12 hours. The adsorbent traps were made of a 3 cm long Teflon® tube filter trap packed with 30 mg of super Q polymer (80–100 mesh size; ARS) held in place between two plugs of glass wool (Mburu *et al*. 2010).Before head space entrapping, each adsorbent trap was cleaned by flushing it ten times with 1mL of dichloromethane (HPLC grade, 99.9%, Sigma-Aldrich). Purified nitrogen gas (BOC Gases, Nairobi, Kenya) was passed through each trap for 3 minutes to dry them, and then sealed with Teflon thread tape on both ends to prevent contamination as described by (Mburu *et al.* 2010). The essential oils were collected on an air entrainment system; using purified medical air (BOC Gases) that passed through activated carbon filters (ARS) with continuous flow rate of 170 mLmin-1. The foliage parts of the plant were enclosed in pre-sterilised oven bag and tied with a string slightly above the pot's soil level. The adsorbent trap was firmly held in place at the open end of a tube connected to the vacuum pump (Vacuum Brand, MZ 2C, Wertheim, Germany).

The essential oils were diluted in GC-grade dichloromethane (DCM) (Sigma–Aldrich, St. Louis, MO, USA) (100 ng/µl), centrifuged at 14,000 rpm for 5 min, dried over anhydrous Na_2SO_4 and analysed (1.0 µL) by GC-MS (7890A gas chromatograph; Agilent Technologies, Inc., Santa Clara, CA, USA) coupled to a 5975 °C mass selective detector (Agilent Technologies, Inc., Santa Clara, CA, USA). The GC was fitted with a (5-phenyl)-methylpolysiloxane (HP5 MS) low bleed capillary column of (30 m × 0.25 mm i.d., 0.25 µm; J&W, Folsom, CA, USA). Helium was the carrier gas, and the column flow rate was 1.25 mL/min. The GC's inlet temperature was maintained at 270 °C, while that of the transfer line was set at 280 °C. The column oven's temperature was pre-set to rise from 35 °C to 285 °C, with the former temperature being held for 5 minutes followed by a 10 °C increase for every minute until the temperature reached 280 °C, where it was maintained for 20.4 min. The mass selective detector was maintained at quadruple (180 °C) and ion source (230 °C). Electron impact (EI) mass spectra was obtained at the acceleration energy of 70 eV with fragment ions being analyzed over 40–550 m/z mass range in the full scan mode, having a solvent delay time set at 3.3 min. The extracting solvent (DCM), blank machine runs was similarly analyzed and their peaks excluded from analysis. The essential oils components were identified by comparing their fragmentation patterns and retention times to those of known authentic samples where available and their reference spectra published by library–MS databases: National Institute of Standards and Technology (NIST) 05, 08, and 11. Authentic sample of methyl octadecenoate (0.2–125 ng/μL) was subsequently analyzed under same GC- MS conditions to obtain a linear calibration curve [y = 7E + 06x – 4E + 07; (R^2 = 0.9757)] that was used for external quantification of the essential oil components. The data are expressed as µg/µl of oil and also expressed as abundance %. Analysis was carried out in triplicates using different batches of the oils.

3.4 Repellent Test Procedure

A. gambiae used was obtained from the insectary in International Centre for Insect Physiology & Ecology (ICIPE), Nairobi, Kenya. Human-bait technique, as shown in Figure 3.3, was used to gauge the extracted oils' level of repellence. Evaluations were conducted in a 6x6x3 m room with a humidity level of 60-80 % and a temperature range of 25-29 ^{o}C. Three human participants having a 3 by 10 cm area marked with a permanent marker on each forearm were used. For efficacy, the testing time was lasting up to eight hours during the day and at night. *A. gambiae* repellence was examined between 0800 hrs. and 1600 hrs.

Figure 3.3: Repellent Testing

On the designated area of first volunteer's forearm, around 0.1 ml of the test repellent was administered, and on the other forearm, the same repellent was applied with 5% ethanol. On the second and the third volunteer’s forearm was applied using the same procedure as the test repellents as a blank control, while the other forearm was left untreated. During the test, the forearm was covered with groves. Each volunteer had to put the test forearm in *A. gambiae* cage (40x40x40 cm), containing 50 *A. gambiae*, for the first three minutes of every half-hour exposure. The repellence test went on until *A. gambiae* landed on or bites the exposed part of the hand. The test continued until at least two bites happen within three minutes, or until a bite happens followed by a confirming bite (second bite) in the subsequent exposure time. The protection time was measured between the time of applying the repellents first and the second subsequent bite. The three repellents from the various plant species went through the same process, and the effectiveness of each oil was noted. The repellence of the oil extracts against the *A. gambiae* was gauged using the median protection time. One-way ANOVA was used to compare the three oils' repellence against one another and assess how ethanol affects repellents before losing their effectiveness.

The following formula (Sharma & Ansari 1994; Yap *et al* 1998) was used to determine the percentage repellence in the trials done.

% Repellence = $\frac{C-T}{C} \times 100$ equation 1

Where: T is total number of *A. gambiae* bites in the treated areas

C is total number of *A. gambiae* bites in the untreated (control) areas.

3.5 Procedure for Formulation of *Anopheles gambiae* Repellent

To prepare the *A. gambiae* repellent, a blend of *O. suave* and *O. americanum* essential oils was created by measuring out equal amounts of each oil in the ratio 1:1 ratio. A clean and dry glass container was selected to combine the essential oils in 0.3 ml of the essential oil blend was carefully measured and added to the container, 3 drops of Tween 80 (a surfactant commonly used in formulations to help evenly disperse ingredients) were added to the essential oil blend in the container then ethanol (acting as the solvent in this formulation) was gradually poured into the container while gently stirring the mixture. The addition of ethanol was continued until the total volume reached 3 ml. The mixture was thoroughly stirred and mixed to ensure that all ingredients were homogeneously combined. This step is important to guarantee that the repellent formulation is effective in repelling *A. gambiae*. Once the mixture was well mixed then the final *A. gambiae* repellent formulated was stored in an airtight container. It was essential to store the repellent in a cool, dark place to maintain its stability and efficacy. The final *A. gambiae* repellent formulation was then labelled and stored in a safe place, ready for further testing and use. This detailed procedure was followed meticulously to ensure the accurate formulation of the repellent and to maintain its effectiveness in repelling *A. gambiae*.

3.6 Data Analysis and Presentation

The mean % repellence data was normalized by logarithmic transformation before being subjected to analysis of variance (ANOVA). The means between treatments were separated using Student-Newman-Keuls at $P \leq 0.05$ using SAS software so that to determine the potent essential oil.

3.7 Analysis of Essential Oils of *Eucalyptus citriodora*, *Ocimum americanum* and *Ocimum suave* by Gas Chromatography Mass Selective Detection (GC-MSD)

3.7.1 Identification of Emitted Volatiles Constituents in the Essential Oils.

The essential oils were eluted and analyzed using an HP 7890B series GC (Agilent Technologies, Wilmington, DE, USA) coupled to a gas chromatogram 5977A series mass selective detector (GC-MSD) (Agilent technologies) that was fitted with a 7693 series auto sampler detector. Each peak represented the signal produced when a compound in the injected emitted volatiles elute through the GC column to the detector. The identity of the constituents was based on comparison with the mass spectral fragmentation patterns provided in the libraries.

3.7.2 The Volatiles Emitted by the Essential Oils.

Constituents' quantification was done using external standards and calibration graphs (Figure 3.2 and 3.3). The quantification was based on comparison of the peak area of each component to that of the external standards ® Chemicals Co, St Louis, MO, USA) for identification of monoterpenoids and sesterquiterpenes, respectively. Different concentrations of 100 ngµl -1, 75 ngµl-1, 25 ngµl -1, 1 ngµl-1, 0.1 ngµl-1, 0.001 ngµl1 of 1,8-cineole and β-caryophyllene were prepared and ran under the same GC-MSD conditions as described in Section 3.10.1.

The graph of concentration against time was plotted and applied in finding the unknown concentrations. The synthetic blends of these compounds were constituted sequentially starting with the highest concentration according to their relative percentage abundance in the emitted volatiles 1,8- cineole (27.68±2.27), α-pinene (3.43±0.24), β-pinene (14.86±0.77) and β- myrcene (3.56±0.34), as follows: 1,8-cineole (143µl) + α-pinene (17µl)+ β-pinene (71µl) + β- myrcene (19µl) (blend 1), 1,8-cineole (155µl)+ α-pinene (18µl)+ β-pinene (77µl) (blend 2), 1,8-cineole (200µl)+ α-pinene (23µl)+ β-myrcene (27µl) (blend 3), 1,8-cineole (153µl)+ β-pinene (77µl) + β-myrcene (20µl) (blend 4), β-pinene (167µl) + α-pinene (39µl)+ β-myrcene (44µl) (blend 5) and DEET (250µl) as a positive control. This was followed by serial dilution to obtain the consecutive concentrations (Odalo *et al*., 2005).

3.8 Laboratory *Anopheles gambiae* Repellence Assays

The repellence was evaluated using the human-bait technique to simulate the condition of human skin to which repellents will be eventually applied (Health Organization, 1996) 5–7-day-old female *A. gambiae* that were reared at ICIPE under standard conditions were used. Three human volunteers who had under gone through ethical consenting process as authorized by Ethical Review were used in the bioassays.

The synthetic standards and their blends were prepared in percentage concentrations levels of 0.001, 0.01, 0.10, 1 and 100% in HPLC grade ethanol. 1 mL of the test solution was dispensed with a syringe on one of the forearms of each volunteer from the elbow to the wrist while the rest of the arm was covered with a glove. Ethanol (1 mL) was dispensed on the other arm to act as a negative control. Repellence of DEET (a positive control) was also undertaken at similar concentrations. The arms were washed before application of the next concentration with soap, rinsed with tap water and dried using tissue paper for 10 minutes. The different sample concentrations were tested sequentially starting with the lowest. Each concentration was screened with a fresh batch of *Anopheles gambiae* after which they were sacrificed. Although the number of *Anopheles gambiae* bites was low due to short exposure duration time, the volunteers were provided with insect bite cream in case of any minor bites and associated irritation (WHO, 2013).

3.9 Data Analysis of the Experimental Results

Percentage protective efficacy (PE) data from the four replicates during semi-field experiments (Section 3.3) was calculated for each number of test plant(s) using the formula % Repellency = (C-T)/C×100. Where C represents the number of *Anopheles gambiae* landing on the control, and T represents the number of *Anopheles gambiae* landings on the treated arm, respectively. The data was transformed and subjected to analysis of variance (ANOVA) and the means compared using the student–Newman–Kuels (SNK) test (IBM, SPSS software version 21). Significant variation for phytochemical relative composition of the emitted volatiles by the plant during the day and night were analyzed using student t- test at 95% confidence level.

The % protective efficacy (PE) data from laboratory bioassays (Section, 3.9), was calculated using the formula PE=PCM/PTM Where PCM and PTM is the % control and treated means, respectively (WHO, 1996). Dose-response relationships values (RD50, RD75 and RD90) were determined by probit analysis using the formula Probit [P (Dose1)] = ß0 + xß1+ î (Busvine, 1971; Finney, 1971).

Where: ß0 = Coefficient of the model representing y-intercept

ß1 = Coefficient of the model representing dose 1 Dose 1 = Log10 (dose)

î = Error term in the data set of the predictor (Regressor) variable (x) P = Repellence probability

3.10 Ethical Considerations

The researcher received an introductory letter from Tharaka University, an ethical clearance from Chuka University's ethics committee and a research permit from the National Commission for Science, Technology and Innovation (NACOSTI) as shown in Appendix 2, 3 & 4 respectively and administration approval from the appropriate field authorities. This was to ensure that none of the respondents' experiences affected the results negatively since the study was using plant leaves and branches, the researcher tried as much as possible not to harm the environment by using only the required quantity. In addition, standard laboratory procedures were followed to ensure no harm to the staff or the environment. Literature cited in this study was acknowledged to avoid the issue of plagiarism. Human beings were informed before exposing them to *A. gambiae* in order to volunteer and they signed certificate of consent as shown in Appendix 1.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 GC-MSD Results

The GC-MSD analysis of essential oil of *O. suave, O. americanum and E. citriodora* gave the mass spectra as shown in Figure 4.1-4.3 and their chemical composition, retention time and relative abundance is also shown in Table 4.1-4.3.

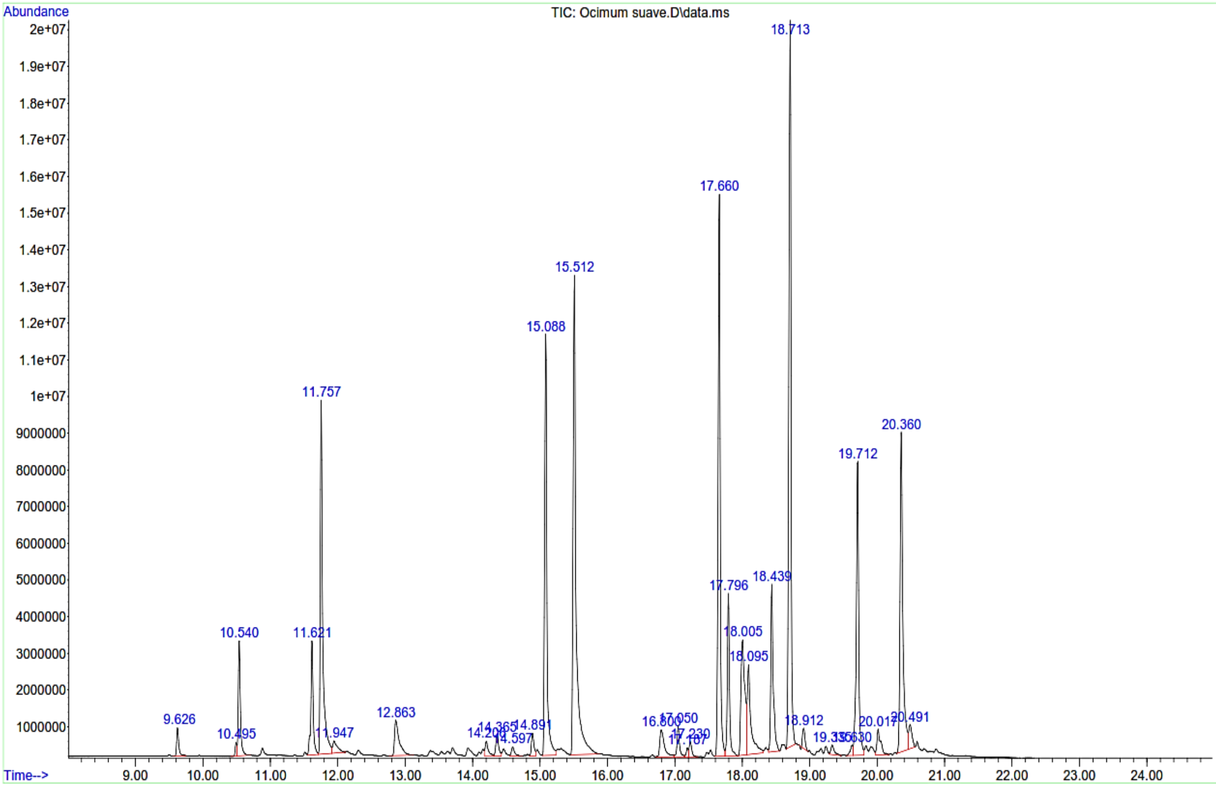


Figure 4.1: The GC-MSD Total Ion chromatogram of essential oil of essential oil of *O. suave*

The GC-MSD results revealed that *O. suave* plant leaves contained forty-nine compounds (Figure 4.1 & Table 4.1). The major components in *O. americanum* plant leaves were β-Bisabolene, α-Pinene, Geranial and Neral.

Table 4.1: GC-MSD results for *Ocimum suave*

NO	RT	Compound Name	CAS	Relative %
1	9.50	Thujene	000099-83-2	0.82
2	9.63	α-Pinene	000080-56-8	1.16
3	9.95	Camphene	000079-92-5	0.80
4	10.54	β-Pinene	000127-91-3	2.26
5	10.88	Myrcene	000123-35-3	1.02
6	11.36	δ-2-Carene	000554-61-0	0.79
7	11.52	p-Cymene	000099-87-6	0.81
8	11.62	1,8-Cineole	000470-82-6	2.03
9	11.76	α-Pinene	000080-56-8	4.53
10	11.95	9.42 (E)-β-Ocimene	003779-61-1	1.26
11	12.14	γ-Terpinene	000099-85-4	0.93
12	12.31	Sabinene hydrate	017699-16-0	0.96
13	12.69	Terpinolene	000586-62-9	0.81
14	12.87	Linalool	000078-70-6	1.91
15	13.38	allo-Ocimene	007216-56-0	1.01
16	13.54	epiphotocitral A	1000365-93-8	0.86
17	13.63	Citral	005392-40-5	0.85
18	13.70	3,3-Dimethyl-hepta-4,5-dien-2-one	1000190-54-1	1.00
19	13.94	(Z)- Isocitral	072203-97-5	1.00
20	14.21	(E)-Isocitral	055722-59-3	1.23
21	14.37	α-Terpineol	000098-55-5	1.05
22	14.47	(1R)-(-)-Myrtenal	018486-69-6	0.88
23	14.82	(Z)-Neral	000106-26-3	0.80
24	14.89	Nerol	000106-25-2	1.21
25	15.09	Neral	000106-26-3	5.77
26	15.31	Geraniol	000106-24-1	1.12
27	15.51	Geranial	000141-27-5	9.40
28	16.38	2,4-Dioxaspiro[5.5]undec-8-ene, 7,11,11-trimethyl-	069745-74-0	0.78
29	16.67	α-Cubebene	017699-14-8	0.79
30	16.81	Eugenol	000097-53-0	1.45
31	17.05	α-Copaene	003856-25-5	1.17
32	17.23	β-Cubebene	013744-15-5	1.10
33	17.53	(Z)-α-Bergamotene	018252-46-5	0.91
34	17.66	(E)-Caryophyllene	000087-44-5	5.86
35	17.79	(E)-β-Bergamotene	018252-46-5	2.53
36	18.00	(Z)-Isoeugenol	005912-86-7	3.05
37	18.09	α-Humulene	006753-98-6	2.76
38	18.44	Germacrene D	023986-74-5	3.14
39	18.60	γ-Muurolene	030021-74-0	1.07
40	18.71	β-Bisabolene	000495-61-4	11.20
41	18.91	α-Copaene	003856-25-5	1.42
42	19.24	Elemicin	000487-11-6	0.93
43	19.34	Nerolidol 2	1000285-43-6	1.08
44	19.71	Caryophyllene oxide	001139-30-6	4.75
45	19.84	2-(1-Hydroxycycloheptyl)-furan	115754-89-7	0.91

Table 4.1 (Continued)

46	20.02	Humulene epoxide II	019888-34-7	1.31
47	20.20	Naphthalene, 1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)-	016728-99-7	0.80
48	20.36	Isoelemicin	000487-12-7	5.65
49	20.60	Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethylidene)-, (4aR-trans)-	000515-17-3	1.07

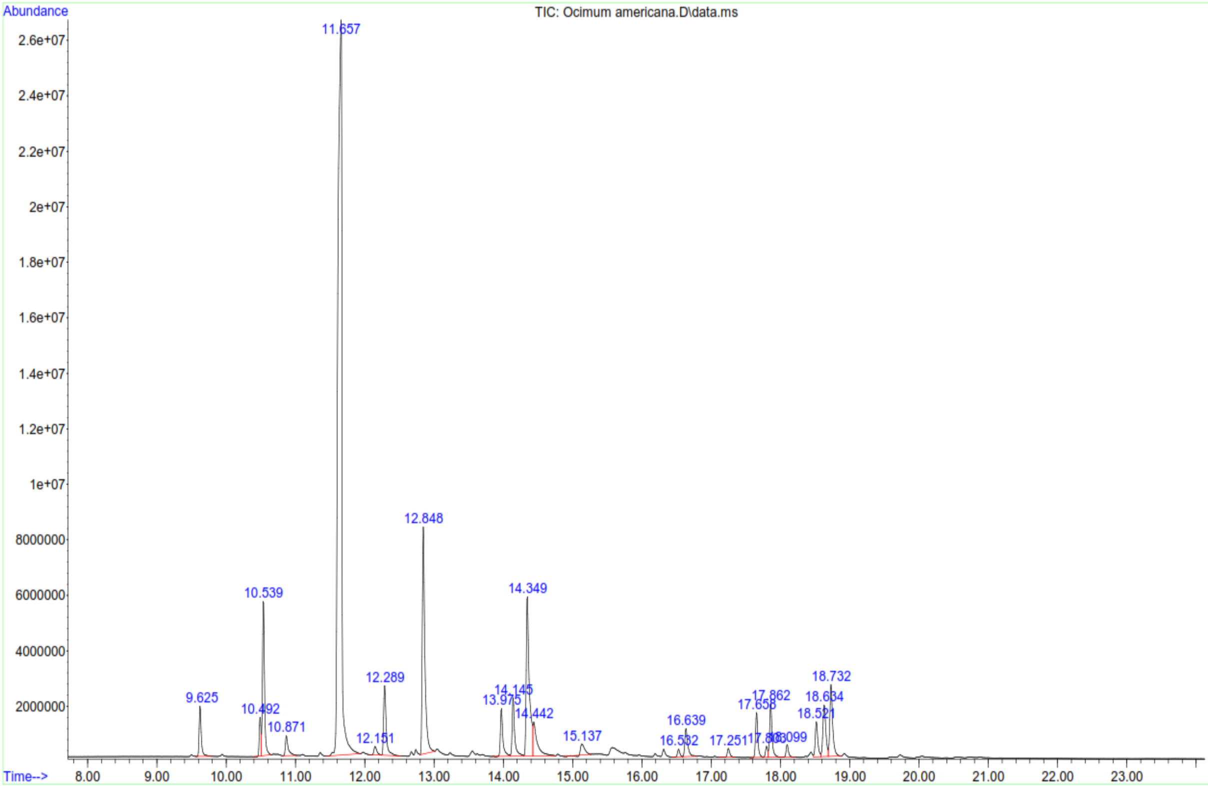


Figure 4.2: The GC-MSD Total Ion Chromatogram (TIC) of essential oil of essential oil of *O. americanum*

The GC-MSD results revealed that *O. americanum* plant leaves contained forty-four compounds (Figure 4.2 & Table 4.2). The major components in *O. americanum* plant leaves were 1,8-Cineole, α-Terpineol and Linalool.

Table 4.2: GC-MSD results for *Ocimum americanum*

NO	RT	Compound Name	CAS	Relative %
1	9.50	Thujene	000099-83-2	1.03
2	9.63	α-Pinene	000080-56-8	2.06
3	9.94	Camphene	000079-92-5	1.06
4	10.54	β-Pinene	000127-91-3	4.28
5	10.87	Myrcene	000123-35-3	1.55
6	11.10	p-Mentha-1(7),8-diene	000499-97-8	1.09
7	11.24	Octanal	000124-13-0	0.99
8	11.36	δ-2-Carene	000554-61-0	1.09
9	11.65	1,8-Cineole	000470-82-6	25.96
10	11.98	(E)-β-Ocimene	003779-61-1	1.26
11	12.15	γ-Terpinene	000099-85-4	1.30
12	12.29	Sabinene hydrate	017699-16-0	2.77
13	12.67	Terpinolene	000586-62-9	1.08
14	12.85	Linalool	000078-70-6	6.54
15	13.05	1-Octen-1-ol, acetate	077149-68-9	1.44
16	13.56	(E)- Epoxy-ocimene	028977-57-3	1.26
17	14.14	Terpinen-4-ol	000562-74-3	2.14
18	14.35	α-Terpineol	000098-55-5	5.91
19	14.79	Fenchyl acetate	013851-11-1	1.09
20	14.97	Nerolidol 1	1000285-43-5	1.09
21	15.14	Neral	000106-26-3	1.67
22	15.38	Geraniol	000106-24-1	1.41
23	15.58	Geranial	000141-27-5	2.13
24	16.20	δ-Terpinyl acetate	093836-50-1	1.07
25	16.32	Myrtenyl acetate	001079-01-2	1.19
26	16.53	2-Oxabicyclo[2.2.2]octan-6-ol, 1,3,3-trimethyl-, acetate	057709-95-2	1.15
27	16.64	α-Terpinyl acetate	000080-26-2	1.76
28	16.83	2,6-Octadien-1-ol, 3,7-dimethyl-, acetate, (Z)-	000141-12-8	1.12
29	17.06	Dauca-5,8-diene	142928-08-3	0.98
30	17.25	β-Elemene	000515-13-9	1.26
31	17.66	(E)-Caryophyllene	000087-44-5	1.95
32	17.86	α-Guaiene	003691-12-1	2.32
33	18.10	α-Humulene	006753-98-6	1.26
34	18.44	Germacrene D	023986-74-5	1.14
35	18.52	β-Selinene	017066-67-0	1.70
36	18.64	Aciphyllene	087745-31-1	2.42
37	18.74	α-Bulnesene	003691-11-0	2.98
38	18.92	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)-	000483-76-1	1.18
39	19.61	γ-Muurolene	030021-74-0	1.01
40	19.73	Caryophyllene oxide	001139-30-6	1.11
41	20.05	γ- Gurjunene	022567-17-5	1.18
42	20.55	α-Guaiene	003691-12-1	1.03
43	20.88	Guaia-1(10),11-diene	1000374-19-7	1.00
44	21.19	Isolongifolene, 9,10-dehydro-	1000151-67-1	1.01

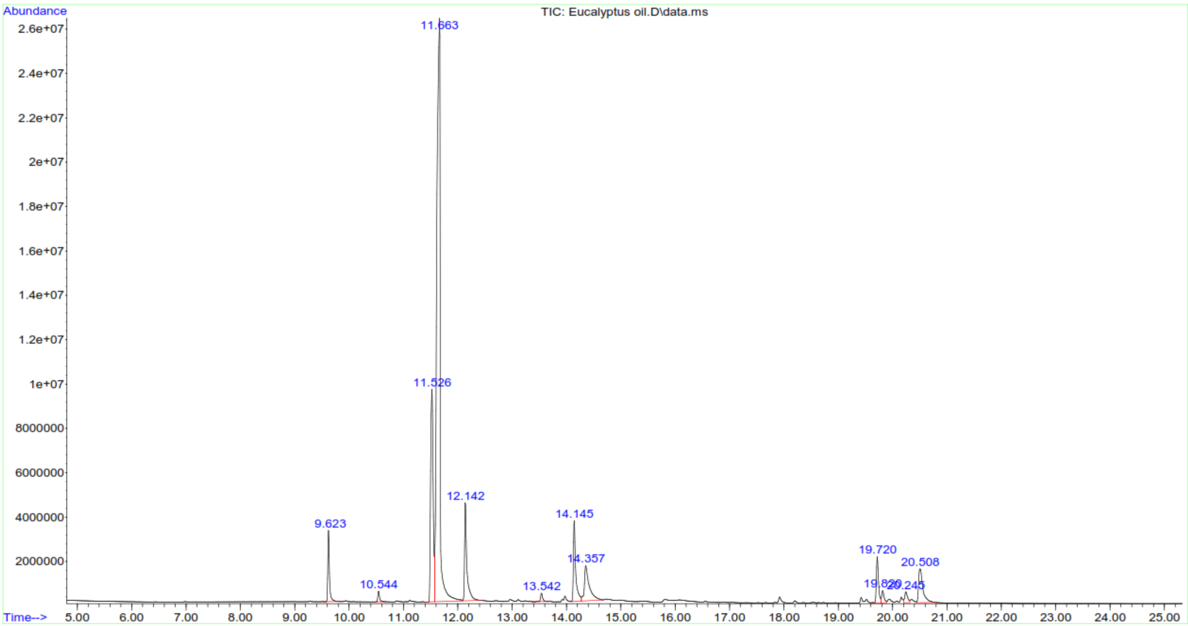


Figure 4.3: GC-MSD Total Ion chromatogram of essential oil of *E. citriodora*

The GC-MSD results revealed that *E. citriodora* plant leaves contained fifty-two compounds (Figure 4.3 & Table 4.3). The major components in *E. citriodora* plant leaves were 1,8-Cineole, o-Cymene and γ-Terpinene.

Table 4.3: GC-MSD results of chemical components from the essential oils of *E. citriodora.*

No	RT	Compound Name	CAS	Relative %
1	9.28	Tricyclene	000488-97-1	1.04
2	9.50	Thujene	002867-05-2	1.01
3	9.62	α-Pinene	000080-56-8	2.49
4	9.94	Camphene	000079-92-5	1.08
5	10.08	3-Thujen-2-ol, stereoisomer	003310-03-0	1.10
6	10.55	β-Pinene	000127-91-3	1.41
7	10.89	Myrcene	000123-35-3	1.11
8	11.12	α-Phellandrene	000099-83-2	1.10
9	11.36	p-Mentha-2,4(8)-diene	000586-63-0	1.02
10	11.52	o-Cymene	000527-84-4	6.69
11	11.66	1,8-Cineole	000470-82-6	24.55
12	12.14	γ-Terpinene	000099-85-4	4.43
13	12.71	δ-2-Carene	000554-61-0	1.20
14	12.97	3-methyl-3-methylbutylbutanoate	000659-70-1	1.29
15	13.11	endo-Fenchol	014575-74-7	1.12
16	13.25	cis-p-Mentha-2,8-dien-1-ol	003886-78-0	1.07
17	13.34	α-Campholenal	004501-58-0	1.03
18	13.54	(Z)-Pinocarveol	019889-99-7	1.31
19	13.69	Camphenilanol	000465-31-6	1.06
20	13.97	Isoborneol	000124-76-5	1.40
21	14.14	Terpinen-4-ol	000562-74-3	3.65
22	14.36	α-Terpineol	000098-55-5	3.67
23	14.74	Sabinol	000471-16-9	1.34
24	14.99	(E)-p-mentha-1(7),8-dien-2-ol	1000374-16-7	1.36
25	15.44	2-isopropyl-5-methyl-3-cyclohexen-1-one	1000155-47-0	1.27
27	15.82	Thymol	000089-83-8	1.35
28	16.09	3-Methyl-4-isopropylphenol	003228-02-2	1.77
29	16.56	2-Oxabicyclo[2.2.2]octan-6-ol, 1,3,3-trimethyl-, acetate	057709-95-2	1.19
30	16.88	Fumaric acid, cyclohex-3-enylmethyl isohexyl ester	1000345-14-2	1.04
31	17.02	Epizonarene	041702-63-0	1.00
32	17.53	10s,11s-Himachala-3(12),4-diene	060909-28-6	1.00
33	17.67	(E)-Caryophyllene	000087-44-5	1.00
34	17.84	Zonarene	041929-05-9	1.01
35	17.92	Aromadendrene	000489-39-4	1.19
36	18.20	allo-Aromadendrene	025246-27-9	1.05
37	18.36	1,2,3,4-tetrahydro-6,7-dimethylnaphthalene	001076-61-5	0.99
38	18.54	β-Selinene	017066-67-0	1.01
39	18.72	Dihydro-β-agarofuran	005956-09-2	1.00

Table 4.3 (Continued)

40	18.86	2-isopropyl-5-methyl-9-methylene-bicyclo[4.4.0]dec-1-ene	150320-52-8	0.99
41	18.95	1,2,3,4-tetrahydro-1,6-dimethyl-4-(1-methylethyl)-, (1S-cis)-Naphthalene	000483-77-2	1.00
42	19.35	Globulol	051371-47-2	0.99
43	19.43	Viridiflorol	000552-02-3	1.16
44	19.53	(E)-Cadina-1(6),4-diene	020085-11-4	1.13
45	19.72	γ-Gurjunene	022567-17-5	2.21
46	19.82	Azulene, 1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1R-(1.alpha.,3a.beta.,4.alpha.,7.beta.)]-	022567-17-5	1.31
47	19.94	1H-Indene, 1-ethylideneoctahydro-7a-methyl-, (1E,3a.alpha.,7a.beta.)-	056324-68-6	1.17
48	20.08	Caryophyllene oxide	001139-30-6	1.04
49	20.16	α-Bulnesene	1000374-19-9	1.12
50	20.24	β-Gurjunene	017334-55-3	1.35
51	20.35	1,2,4-Metheno-1H-indene, octahydro-1,7a-dimethyl-5-(1-methylethyl)-, [1S-(1.alpha.,2.alpha.,3a.beta.,4.alpha.,5.alpha.,7a.beta.,8S*)]-	022469-52-9	1.14
52	20.51	1H-Indene, 1-ethylideneoctahydro-7a-methyl-, (1Z,3a.alpha.,7a.beta.)-	056324-69-7	3.01

4.2 Repellence Activity Test for the Plants Leaf Extracts.

Repellence of essential oils of *E. citriodora, O. suave, O. americanun,* and blend against *A. gambiae* are shown in Table 5 and Figure 4.3 & 4.4.

Table 4.4: Repellence of different essential oils against *A. gambiae.*

Eucalyptus citriodora	89.33±4.40^{a}	67.33±3.36^{b}	54.00±2.45^{c}	28.33±2.04^{d}
Ocimum americanum	96.67±3.33^{a}	92.67±4.52^{a}	89.81±4.26^{a}	80.33±1.53^{b}
Ocimum suave	88.67±4.67^{a}	85.33±3.74^{a}	75.33±2.44^{b}	58.67±3.74^{c}
Blend 1	100.00±0.00^{a}	96.00±4.00^{a}	96.00±4.00^{a}	96.00±4.00^{a}
Blend 2	90.23±3.40^{a}	86.67±2.95^{a}	85.33±3.54^{a}	83.62±4.22^{a}
Blend 3	89.32±4.2^{a}	85.43±3.5^{a}	86.22±3.2^{a}	81.78±3.90^{a}
Ballet	100.00±0.00^{a}	96.67±3.33^{a}	96.67±3.33^{a}	96.67±3.33^{a}

Values expressed as mean±standard error of the mean (n=5). Values with similar lower-case letters along the column are not significantly different using one-way ANOVA and Tukey's post hoc (p>0.05). Blend 1: 1:1 ratio of *O. suave* & *O americanum*. Blend 2- *E. citriodora* & *O. Americanum* Blend 3- *E.citriodora & O. suave,* Ballet: Mosquito Repellent. Table 4.4 shows there is no significant difference in the repellence activities of blend of *O. suave* & *O. americanum* in the ratio of 1:1 and the existing mosquito repellent in Kenyan shops, Ballet (SNK, *p*≥0.05, 95% CL).

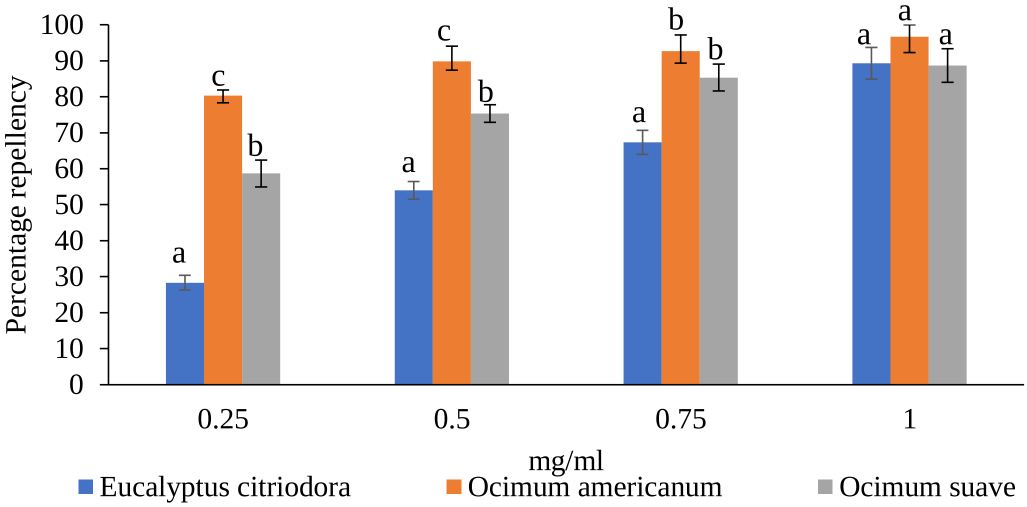


Figure 4.4: Mean (±SE) percentage repellence of *E.citriodora*, *O.suave*, & *O.americanum.*

Rankings of different essential oils against *A. gambiae* show that there was no significance difference in the repellence activities of different essential oils with time (SNK, *p*≥0.05, 95% CL). Figure 4, shows the mean repellence of essential oils of *E. citriodora, O. suave, O. americanun* against *A. gambiae*.

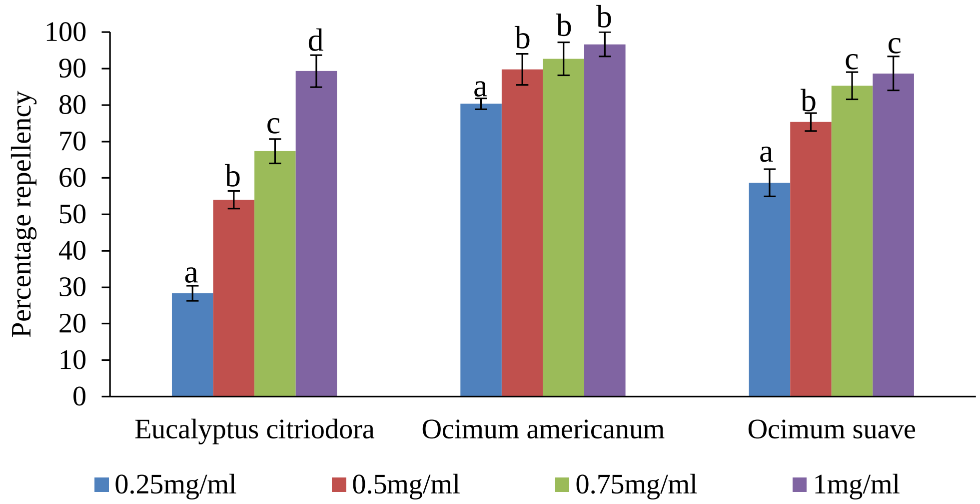


Figure 4.5: Mean (±SE) percentage repellence of *E. citriodora, O. suave & O. americanum.*

It's evident that there was more repellence in the blends than in single essential oils (Table 4.5 & Figure 4.6). Among the plants essential oils, there was significant ($p<0.05$) increase in repellence between the exposure of the cohort replicate against *A. gambiae* when *O. suave* and *O. americanum* essential oils were blended and used in the ratio of 1:1 (Table 4.5 & Figure 4.6). However, there was a drop of repellence with the exposure of the cohort of *A. gambiae* to blends with *E. citriodora*. Thus, the level of repellence from essential oil of each plant appears to be negatively affected by the presence of other con-specific plants (Table4. 5 & Figure 4.6).

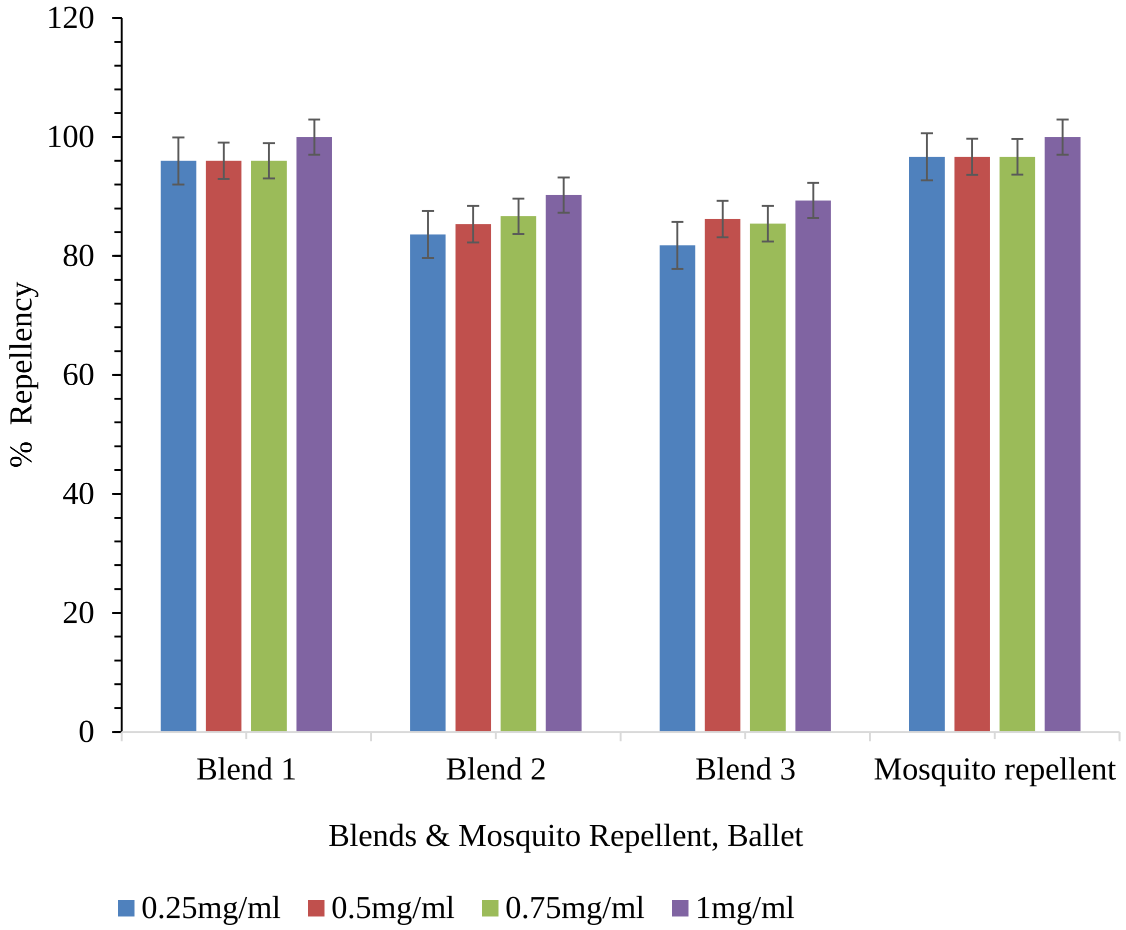


Figure 4.6: Mean (±SE) percentage repellence of the blends & Ballet (Mosquito repellent) at various concentrations. Blend 1: 1:1 ratio of *O. suave* & *O. americanum*.

Blend 2- *E. citriodora* & *O. americanum* Blend 3- *E. citriodora & O. suave,* Ballet: Mosquito Repellent

For a given dose, there were varying degrees of dose-dependent responses. All of the individual essential oils tested had significant repellent effects against *A. gambiae*. However, a blend of these compounds had more repellent activities against *A. gambiae* as shown in Table 4.5 & Figure 4.6. Among the tested essential oils assayed the most repellent were *O. suave* & *O. americanum* with a repellence activity of 85.33% and 92.67% at a concentration of 0.75 g/mL. The blend of *O. suave, & O. Americanum* in the ratio of 1:1 was the most potent repellent with a mean percentage repellence of 96% at a concentration of 0.75 g/mL (Table 5 & Figure 6). Similarly, this study utilized a positive control, a mosquito repellent in Kenyan supermarkets/shops and chemists, Ballet. There was no significant difference in the repellence of the mosquito repellent Ballet and that of the blend of *O. suave, & O. Americanum* in the ratio of 1:1 (Table 4.5 & Figure 4.6). Its worthy noting that *O. americanum* was the most potent single essential oil, hence the biggest contributor in the high potency of the blend (Table 4.5 & Figure 4.6). The blends had more repellence than single essential oils, and the most potent blend was *Ocimum suave* and *Ocimum americanum* in the ratio of 1:1. However, there was a drop of repellence with the blends with *E. citriodora* against *A. gambiae*. Thus, the level of repellence from essential oil of each plant appears to be negatively affected by the presence of other con-specific plants. The high repellence of the blends of *Ocimum suave* and *Ocimum americanum* essential oils against *A. gambiae* than those of the individual essential oil could be due to additive or synergistic effects of individual constituents in the two essential oils. The GC-MSD results revealed that the major compounds on *Eucalyptus citriodora* and *Ocimum americanum* plants leaves was 1,8-Cineole, while *β*-Bisabolene was the major component/compounds in the *Ocimum suave* plant leaves.

4.3 Discussion

The repellents from plant extracts, are green, environmentally friendly, biodegradable and non-toxic (Chatterjee *et al*.2023; Mworia *et al*.2021). Many researchers have conducted studies on repellence to determine the efficacy of ethno-botanical plants for space fumigation against human biting arthropods (Priya *et al*., 2023). This research study was done to determine the potency of *Ocimum suave Ocimum americanum* and *Eucalyptus citriodora* essential oils and their blends against *A. gambiae*. The qualitative and quantitative differences in diurnal and nocturnal emitted volatiles from *M. piperita* plant could be regulated by storage organs, their rate of synthesis and volatility (Escobar-Bravo *et al*., 2023). Terpene accumulation and glandular trichome development in this plant is controlled by the rate of biosynthesis, which is regulated by individual pathway enzymes and structural genes of day, and stage of development (Kortbeek *et al*., 2023). Light decreases the diffusion resistance of stomata during the day and the availability of glyceraldehyde-

3-phosphate, a precursor of terpene formed during photosynthesis (Tariq *et al*., 2023). Non-flowering, 6-week-old plants which were measured under light and temperature had lower volatilization rate during the light period than the dark period (Toht, 2017).

Light intensity has been reported to have an effect on the quality and quantity of the induced odour blend. The relative amount of *β*-myrcene, (*Z*) -3-hexenyl acetate, and *β*-bisabolene significantly decreased with increases in light intensity(Yactayo-Chang *et al*., 2024). Nocturnal emission of floral volatiles is more often found to be controlled by endogenous factors than diurnal emission which may be more influenced by prevailing light and temperature conditions(Abbas *et al*., 2023). Previous studies have shown increased night-time concentrations of limonene and *β*-phellandrene within baronial flower tissues (Paradiso & Proietti, 2022). The rapid increase in emission of both volatiles in the dark phase of the alternating light: dark treatment is a clear indication that production and emission of these volatiles is controlled by light (Paradiso & Proietti, 2022). Diurnal changes in tissue concentrations of particular volatiles also showed relatively higher concentrations of ionone at night-time, the emission of volatiles is influenced to a great extent by the emission of major volatiles, ionone's and dodecyl acetate.

The repellent effect of the emitted volatiles is attributed to the higher percentage of terpenoids in *O. suave* and *O. americanum* respectively. This shows that active compounds gain synergism between themselves resulting to an increase in repellency (Yoon & Tak, 2023). Thus, subtractive assays provide additional insight into the relative contributions of these compounds to the repellence of the two-component blend. The current study shows that multiple deployment of formulation of *Ocimum suave,* and *Ocimum americanum* essential oils plant can provide space protection against *A. gambiae* up to a certain level, after which no further enhancement in repellence occurs. Thus, the level of emission of volatiles from each plant appears to be negatively affected beyond three con-specific plants (Fellowes *et al*., 2023). This could be due to intra-plant communication that leads to suppression of emission of volatiles. Although, the repellence is below substantial levels that can significantly repel the host, their simplicity may provide an alternative of reducing malaria incidences in resource limited people (Martín-Cacheda *et al*., 2023).

CHAPTER FIVE

SUMMARY, CONCLUSION AND RECOMMENDATIONS

5.1 Summary

The synergistic effect of the emitted volatiles, particularly 1,8-cineole, has been shown to increase the repellence of a two-component blend against Anopheles gambiae. Subtractive assays have provided insight into the contributions of individual compounds to the overall repellence. Crude extracts and formulations of *Ocimum suave, Ocimum americanum*, and Eucalyptus citriodora essential oils have demonstrated antimicrobial, fungicidal, and insecticidal activities. These formulations have shown effectiveness against bacteria, fungi, aphids, weevils, moths, and dengue vectors. The presence of alkaloids, flavonoids, steroids, tannins, and phenols in these essential oils contribute to their insect-repellent properties, inhibiting feeding and development of insects.

The deployment of multiple plants containing these essential oils can provide space protection against Anopheles gambiae, although there may be a limit to the level of repellence achieved. Beyond a certain number of plants, there is no further enhancement in repellence, potentially due to intra-plant communication leading to the suppression of emission of volatiles. While the repellence levels may not be substantial enough to significantly repel, the essential oils of the three plants can serve as a simple and cost-effective method to reduce malaria incidences in resource-limited areas. Thus, the combination of essential oils and their active components show promise in repelling anopheles gambiae and other pests, highlighting the potential for natural repellents in pest control strategies.

5.2 Conclusion

Different research has been conducted in various countries to determine the efficacy of ethno- botanical plants for space fumigation against human biting arthropods. This research was conducted to determine the repellence of the volatiles emitted by formulation of *Ocimum suave* and *Ocimum americanum* essential oils against *A. gambiae* and the phytochemical composition of the plant. The study concludes the following:

First, a blend of *Ocimum suave* and *Ocimum americanum* essential oils is a repellent to *A. gambiae* during its active times. Hence, blends of *Ocimum suave* and *Ocimum americanum* should be used to offer protection against *A. gambiae* bites thus reducing spread of malaria while *E. citriodora* essential oils had the lowest repellence against *A. gambiae.*

Secondly, the day and night phytochemical compositions of the emitted volatiles showed significantly large qualitative and quantitative differences. The monoterpenes and sesquiterpenes were 60.5% and 25%, 54.5% and 23.3% during night and day respectively.

Finally, repellence of blends (blend 1; 100.00±0.00) of *Ocimum suave*, and *Ocimum americanum* essential oils against *A. gambiae* was higher than those of the individual essential oil 56.40±6.40), suggesting that the repellence of essential oil emission of the plant may be due to additive or synergistic effects of individual constituents.

5.3 Recommendations

The following are recommended from this research and for further research

i. The repellence of the emitted volatiles was evaluated in a choice set ups in two screen houses. Full field trials need to be undertaken to rule out any possible differences in repellence due to the overlap of repellence range of the treatment with that of the control and behaviour of A. gambiae when they are constrained.
ii. Gas Chromatography linked Electroantennography analysis of the essential oil should be conducted so as to identify all compounds perceived by the antennae A. gambiae, which can then be assayed as a full blend to determine its repellence, and in subtractive modes to determine the relative contribution of each component

REFERENCES

Abbas, F., Zhou, Y., O'Neill Rothenberg, D., Alam, I., Ke, Y., & Wang, H.-C. (2023). Aroma components in horticultural crops: chemical diversity and usage of metabolic engineering for industrial applications. *Plants*, *12*(9), 1748

Aklilu, S., Ephantus, W., Wilber, L., Gerry F., Hassanali, A. & Bart G. (2002). Repellency of live potted plants against *Anopheles gambiae* from human baits in semi-field experimental huts. *American Journal of Tropical Medicine and Hygiene* 67(2), 191–195.

Aklilu, S., Kabiru, E., Knols, B. & Hassanali, A. (2003). Field efficacy of thermally expelled or live potted repellent plants against African malaria vectors in Western Kenya. *Tropical Medicine and International Health*, *8*, 1005–1011.

Alonso, P. (2004). Efficacy of RTS, *S/AS02A* vaccine against *P. falciparum* infection disease in young children, a randomized control trial. *Lancet*, *16*, 1411-1420.

Amenya, D., Bonizzoni, M. & Isaacs, A. (2010). Comparative fitness assessment of *Anopheles stephensi* transgenic lines receptive to site specific integration. *Insect Molecular Biology*, *19*, 263–269.

Arrow, K., Panosian, C. & Gelband, H. (2004). *Saving lives, buying time: Economics of malaria drugs in an age of resistance. Institute of Medicine (U.S.). Committee on the Economics of Antimalarial Drugs. National Academies Press*, pp 141.

Asadollahi, A., Khoobdel, M., Zahraei-Ramazani, A., Azarmi, S. & Mosawi, S. (2019). *Effectiveness of plant-based repellents against different Anopheles species: A systematic review. Malaria Journal*, 18 (1): 436-447.

Bahamondez, C., Álvarez, O., & Itzelcoaut, M. (2010). *Global forest resources assessment 2010 main report. Food and Agriculture Organization of the United Nations.*

Baird, J., David, J., Hasin, B., Micheal, J., Budi, S., Sofya, M. & Thomas, L. (1995). Premaquine for prophylaxis of malaria among non-immune immigrants in Irian Jaya, Indonesia. *American Journal of Tropical Medicine and Hygiene*, *52*, 479-484.

Barasa, S., Ndiege, I., Lwande, W. & Hassanali, A. (2002). Repellent activities of stereo- isomers of *p*-Menthane-3,8-diols against *Anopheles spp. Journal of Medical Entomology*, *39*, 736-741.

Barnard, D. (1999). *Repellency of essential oils to mosquitoes* (Diptera: Culicidae).

Becker, N., Dusan, P., Zgomba, M., Boase, C., Madon, M., Dahl, C. & Kaiser, A. (2010). Medical importance of mosquitoes. *Mosquitoes and their control*. Springer-Verlag Berlin Heidelberg, *3*, pp 25-42.

Beier, J., Keating, J., Githure, J., Macdonald, M., Impoinvil, D. & Novak, R. (2008). Integrated vector management for malaria control. *Malaria Journal*, *7* (suppl.1), S4, 1-10.

Benelli, G. (2015). Research in mosquito control: current challenges for a brighter future. *Parasitology research*, 114(8), 2801-2805.

Benelli, G., Canale, A. & Conti, B. (2014). Eco-friendly control strategies against the Asian tiger mosquito, Aedes albopictus (Diptera: Culicidae): repellency and toxic activity of plant essential oils and extracts. *Pharmacologyonline*, 23(1), 44-51.

Benelli, G., Pavela, R., Maggi, F., Wandjou, J., Koné-Bamba, D., Sagratini, G., & Caprioli, G. (2019). Insecticidal activity of the essential oil and polar extracts from Ocimum gratissimum grown in Ivory Coast: Efficacy on insect pests and vectors and impact on non-target species. *Industrial Crops and Products*, 132(12), 377-385.

Bernard, R. (1999). *Repellence of the essential oils to mosquitoes* (Diptera: Culicidae). *Journal of Medical Entomology*, *36*, 320-328, 625-629.

Breman, J. (2001). The ears of the hippopotamus: manifestations, determinants, and estimates of the malaria burden. *American Journal of Tropical Medicine and Hygiene*, *64* (1– 2), 1–11.

Brooker, I. & Kleinig, D. (2004). *Field guide to eucalypts*: Northern Australia: Volume three. Bloomings Books.

Brown, M. & Hebert, A. (1997). Insect repellents: An overview. *Journal of American Academic Dermatology*, *36*, 243–249.

Busvine, J. (1971). *A critical review of the techniques for testing insecticides*, 2^{nd} editioncommon health agricultural bureaux England, pp 263-277.

Chatterjee, S., Bag, S., Biswal, D., Paria, D. S., Bandyopadhyay, R., Sarkar, B., Mandal, A., & Dangar, T. K. (2023). Neem-based products as potential eco-friendly mosquito control agents over conventional eco-toxic chemical pesticides-A review. *Acta Tropica*, *240*, 106858.

Canyon, D. & Hii, J. (1997). The gecko: An environmentally friendly biological agent for mosquito control. *Medical and Veterinary Entomology*, *11* (4), 319–323.

Carroll, S. & Loye, J. (2006). A registered botanical mosquito repellent with DEET- Like efficacy. *Journal of medical Mosquito Control Association*, *22*, 507-514.

CDC (2010). Anopheles mosquitoes. *Malaria* (04 September 2014).

Chalchat, J., Garry, R., Sidibé, L. & Harama, M. (2000). Aromatic plants of Mali (V): chemical composition of essential oils of four Eucalyptus species implanted in

Mali: Eucalyptus camaldulensis, *E. citriodora*, E. torelliana and E. tereticornis. *Journal of Essential Oil Research, 12*(6), 695-701.

Chandra, G., Bhattacharjee, I., Chatterjee, S. & Ghosh, A. (2008). Mosquito control by larvivorous fish. *Indian Journal of Medicine Resource*, *127,* 13–27.

Chavasse, D. & Yap, H. (2019). *Chemical methods for the control of vectors and pests of public health importance* (No. WHO/CTD/WHOPES/97.2). World Health Organization.

Clements, A. (1992). *The biology of mosquitoes: Development, Nutrition and Reproduction*. London: Chapman and Hall, *1*, pp 1-509.

Coetzee, M., Hunt, R., Wilkerson, R., Della, T., Coulibaly, M. & Besansky, N. (2013). *Anopheles coluzzii* and *Anopheles amharicus*, new members of the *Anopheles gambiae* complex. *Zootaxa, 3619,* 246-274.

Collins, D. & Brady, J. (1993). Assessment of the efficacy of quwenling as a mosquitorepellent. *Phytotherapy Research*, *7,* 17–20.

Curtis, C. (1986). Physiology and etiology of malaria infection cycle. *Journal of AmericanMosquito Control Association, 66*, 411-418.

Dalziel, J. (1937). *The useful plants of west Tropical Africa, The crown agents for the colonies*, London, pp 52-560.

Dawes, B. (1973). *Advances in parasitology,* Academic Press Inc. London Limited, II, pp 131.

Debbab, A., Mosaddak, B., Aly, A., Hakiki, A. & Mosaddak, M. (2007). Chemical Characterization and toxicological evaluation of the essential oil of *Mentha piperita* L. Growing in Morocco. *Scientific Study Reserve*, *8,* 281–288.

Dethier, V. (1956). Repellents. *Annual Revolutionary Entomology*, *1,* 181-202.

Diaz, J. (2016). Chemical and plant-based insect repellents: efficacy, safety, and toxicity. *Wilderness & Environmental Medicine, 27*(1), 153-163.

Dorman, H. & Deans, S. (2022). Antioxidant and antimicrobial properties of extracts from cinnamon and ginger. *Journal of agricultural and food chemistry*, 48(11), 549-552.

Dudareva, N., Pichersky, E. & Gershenzon, J. (2004). Biochemistry of plant volatiles. *Plant Physiology*. *135*:1893–1902.

Dwivedi, S., Khan, M., Srivastava, S., Syamasunnder, K. & Srivastava, A. (2004). Essential oil composition of different accessions of *Mentha piperita* L. grown on the northern plains of India. *Journal and Flavor and Fragrance*, *19,* 437–440.

Edoh, D., Mshinda, H., Jennifer, J. & Max, B. (1997). Pyremethamine resistant *P. falciparum* parasites among Tanzanian children. A facility-based study using polymerase chain reaction. *American Journal of Tropical Medicine and Hygiene*, *57*, 342-347.

Edriss, A., Alabjar, Z. & Satti, A. (2012). Phytochemical screening of important secondary metabolites in some extracts of two Sudanese plants. *Global Advanced Research Journal of Environmental Science and Toxicology*, *1*(8), 199-202.

Enayati, A. & Hemingway, J. (2010). Malaria management: past, present, and future. *Annual Review of Entomology*, *55,* 569–591

Enayati, A., Lines, J., Maharaj, R. & Hemingway, J. (2009). Suppressing the vector in Feachem, R. G. A., Phillips, A. A. and Targett, G. A. (eds.). Shrinking the Malaria Map: A prospectus on malaria elimination. San Francisco. *The Global Health Group*, pp 140–154.

Escobar-Bravo, R., Lin, P.-A., Waterman, J. M., & Erb, M. (2023). Dynamic environmental interactions shaped by vegetative plant volatiles. *Natural product reports, 40*(4), 840-865.

Farnsworth, N. & Bingel, A. (1977). New natural products and plant drugs with pharmacological, biological or therapeutic activity. Problems and prospects of discovering new drugs from higher plants by pharmacological screening. Springer-Verlag, Berlin, pp 1- 22.

Farooq, U. & Mahajan, R. (2004). Drug resistance in malaria. *Journal of Vector Borne Diseases, 41*, 45-53.

Federici, B. (1995). The future of microbial insecticides as a vector control agents. *Journalof American Mosquito Control Association, 11*, 260–268.

Fellowes, M. D., van Alphen, J. J., Shameer, K., Hardy, I. C., Wajnberg, E., & Jervis, M. A. (2023). Foraging behaviour. In *Jervis's Insects as Natural Enemies: Practical Perspectives* (pp. 1-104). Springer.

Finney, D. (1971). *Probit analysis*, 3rd ed. Cambridge University Press, Cambridge, pp 9- 158.

Focks, D., Sackett, S. & Bailey, D. (1982). Field experiments on the control of *Aedes aegypti* and *Culex quinquefasciatus* by *Toxorhynchites rutilus* (Diptera: Culicidae). *Journal of Medical Entomology*, *19* (3), 336–339.

Fontaine, R., Pull, J., Payne, D., Pradhan, G. & Pearson, H. (1979). Evaluation of Fenitrothion (OMS 43) for malaria control in a large scale: *Epidemiology in Kisumu*, Kenya, 76, pp 645.

Fradin, J. & Day, G. (2002). Efficacy of DEET and its analogues in mosquito control. *Annual Review of Entomology*, *16*, 207-209.

Fradin, M. (1998). Mosquitoes and mosquito repellents: A clinician's guide. *Journal of International Medicine, 128,* 931–940.

Franke, K., Saca, J., Wang, R., Chareonut, Y., Apella, E. & Alexander, J. (1999). Pan DR binding sequence provides T- cell help for induction of protective anti-bodies. *American Journal of Tropical Medicine and Hygiene*, *57,* 109-116.

Fredena, G., Dovine, B., Catherine, J., Manivah, S., Lilian, C., Martin, D. (1997). *In-vitro* resistance of *P. falciparum* to atovaquone and correlation with other anti-malarials. A comparison between Africa and Asia. *American Journal of Tropical Medicine and Hygiene*, *56*, 517-526.

Garnham, P. (1980). *Malaria in its various vertebrate hosts*. In Kreier, J. P. (eds.) Malaria. Academic Press, New York, *1*, pp 95–144

Gillet, J. (1971). *Mosquitoes*, Weiden field and Nicol son, Wesley street London, *5*,pp1

Gillet, J. (1972). *Common African mosquitoes and their medical importance*, William Heinemann medical books Ltd London, pp 26.

Greenwood, B., Bojang, K., Whitty, C. & Tangett, G. (2007). Malaria. *Lancet*, *365*, 1487-1498.

Gupta, K. & Louis, C. (1994). Role of repellents in vector control and disease prevention. *American Journal of Tropical Medicine and Hygiene*, *50*, 82-86.

Hallmon, C., Schreiber, E., Vo, T. & Bloomquist, M. (2000). Field trials of three concentrations of Laginex T M as a biological larvicide compared to Vectobac T M-12AS as a biocontrol agent for *Culex quinquefasciatus. Journal of American Mosquito Control Association*, *16*, 5–8.

Hansted, L., Jakobsen, H., Olsen, C. (1994). Influence of temperature on the rhythmic emission of volatiles from Ribesnigrum flowers in situ. *Plant Cell and Environment 17*: 1069-1072.

Hemingway, J., Field, L., & Vontas, J. (2002). An overview of insecticide resistance. *Science*, 298(5591), 96-97.

Hikal, W., Baeshen, R. & Said-Al Ahl, H. (2017). Botanical insecticide as simple extractives for pest control. *Cogent Biology*, 3(1), 1404274.

Hoffman, S. & Doolan, D. (2000). Malaria vaccine targeting infected hepatocytes. *Nature Medicine*, *6*, 1228-1229.

Isman, M. (2000). Plant essential oils for pest and disease management. *Crop protection*, 19(8-10), 603-608.

Jennifer, C. (2011). *Spermless mosquitoes hold promise to stop malaria*. *BBC News*. Scientists have created spermless mosquitoes in an effort to curb the spread of malaria. http://www.bbc.com/news/science-environment-14422440, accessed on 21st August, 2023.

Jerten, T., Marters, W. & Takken, W. (1996). Model simmilations to estimate malaria risk under climate change. *Journal of Medical Entomology*, *33*, 361-371.

Joy, D., Feng, X., Mu, J., Furuya, T., Chotivanich, K., Krettli, A., Ho, M., Wang, A., White, N., Suh, E., Beerli, P., & Su, X. Z. (2003). Early origin and recent expansion of *Plasmodium falciparum*. *Science*, *300*, 318–321.

Karunamoorthi, K. (2011). Vector control: a cornerstone in the malaria elimination campaign. Clinical Microbiology and Infection. *European Society of Clinical Microbiology and Infectious Diseases* (CMI), *17*, 1608–1616.

Karunamoorthi, K. & Sabesan, S. (2009). Field trials on the efficacy of DEET impregnated anklets, wristbands, shoulder, and pocket strips against mosquito vectors of disease. *Parasitology Reserve*, *105*, 641–645.

Karunamoorthi, K., Ilango, K. & Endale, A. (2009). Ethno-botanical survey of knowledge and usage custom of traditional insect/mosquito repellent plants among the Ethiopian Oromo ethnic group. *Journal of Ethno-pharmacology*, *125*, 224–229.

Karunamoorthi, K., Ilango, K. & Murugan, K. (2010). Laboratory evaluation of traditionally used plant-based insect repellents against the malaria vector *Anopheles arabiensis* Patton. *Parasitology Resource*, *106*, 1217-1223.

Karunamoorthi, K., Mulelam, A. & Wassie, F. (2008). Laboratory evaluation of traditional insect/mosquito repellent plants against *Anopheles arabiensis*, the predominant malaria vectorin Ethiopia. *Parasitology Reserve*, *103*, 529–534.

Karunamoorthi, K., Mulelam, A. & Wassie, F. (2009). Assessment of knowledge and usage custom of traditional insect/mosquito repellent plants in Addis Zemen Town, South Gonder, North Western Ethiopia. *Journal of Ethno-pharmacology*, *121*, 49–53.

Kettle, D. (1995). *Medical and veterinary entomology*. CAB International, 2 nd edn. Oxon, UK, pp 725.

Khani, M., Muhamad, R. & Omer, D. (2012). Insecticidal effects of peppermint and black pepper essential oils against rice weevil (*Sitophilus oryzae* L.) and rice moth (*Corcyra cephalonica*) (St.). *Journal of Medicinal Plants*, *11* (43), 97-110.

Kimutai, A., Ngeiywa, M., Mulaa, M., Njagi, P., Ingonga, J., Nyamwamu, L. & Ngumbi, P. (2017). Repellent effects of the essential oils of Cymbopogon citratus and Tagetes minuta on the sandfly, Phlebotomus duboscqi. BMC *research notes*, 10(1), 1-9.

Kirk, R. & Othmer, D. (1981). *Encyclopedia of Chemical Technology*, 3rd Edn. John Wiley and Sons, Toronto, *13*, pp 413-483.

Knipling, E. (1955). Possibilities of insect control or eradication through use of sexually sterile males. *Journal of Economics and Entomology*, *48*, 459–462.

Knox, E., Gilman, F. & Fradin, J. (2003). Biological control of malaria vector for developing countries. *Journal of American Mosquito Control Association*, *43*, 742-746.

Kortbeek, R. W., Galland, M. D., Muras, A., Therezan, R., Maia, S., Haring, M. A., Schuurink, R. C., & Bleeker, P. M. (2023). Genetic and physiological requirements for high-level sesquiterpene-production in tomato glandular trichomes. *Frontiers in Plant Science*, *14*, 1139274.

Kreier, F. (1983). Plant metabolites that repel mosquitoes. *Journal of American Mosquito Control Association*, *142*, 102-110.

Krumholz, & Louis, A. (1948). Reproduction in the Western mosquito fish, *Gambusia affinis* (Baird and Girard), and its uses in mosquito control. *Ecological Monographs*, *18* (1),pp 1–43.

Kumar, A. & Dutta, G. (1987). Indigenous plant oils as larvicidal agent against *Anophelesstephensi* mosquitoes. *Curricullum of Science*, *56*, 959–960.

Kumar, P., Chaudhary, A. & Choudhury, A. (2016). Hydro-diffusion: An efficient process for extraction of oil from marine biomass. *Journal of the American Oil Chemists' Society*, 93(3), 387-393.

Kumar, S., Wahab, N. & Warikoo, R. (2011). Developmental inhibition dengue fever mosquito *Aedes aegypti* L. Asian of *Spodoptera litura* (Fab.) larvae by a novel Pacific. *Journal of Tropical Biomedicine, 1* (2), 85-88.

Lacey, L. (1998). Development of Entomopathogens as microbial control agents of mosquitoes and black flies: 30 Years of Progress. Proc. VI *Simpósio de Controle Biológico (SICONBIOL), Rio de Janeiro*, pp 23-28.

Lacey, L. A. and Lacey, C. M. (1990). The medical importance of Rice-land mosquitoes and their control using alternatives to chemical insecticides. *Journal of American Mosquito Control Association, 2*, 1–93.

Leisnham, P. (2012). Taking bite out of mosquito research, university of Maryland. https://www.enst.umd.edu/news, accessed on 21st August, 2016.

Lengeler, C. (2002). Insecticide-treated bed nets and curtains for preventing malaria (Cochrane Review 1). Oxford: Update Software, pp 1-3.

Lewis, M., Silva, M., Sanborn, J., Pfeifer, K. & Shreider, J. P. (2000). N, N-diethyl-meta- toluamide (DEET). *Risk characterization document. Medical Toxicology and Worker Health and Safety Branches*: California environamental protection agency, Pesticide Registration Branch, Sacramento, CA, USA, pp 1-62.

Lin, T. & Juliano, J. (2010). Drug-Resistant Malaria: The Era of ACT. *Current Infectious Disease Reports*, *12* (3), 165-173.

Lindsay, J. & Birley, M. (1996). Climate change malaria transmission. *Journal of*

Tropical Medicine and Parasitology, *90*, 573-588.

Lindsay, R. L., Heal, J. D. and Surgeone, G. A. (1990). Evaluation of efficacy of 3% citronella candles and 5% citronella incense for protection against field populations of *Aedes* mosquitoes. *Journal of American Mosquito Control Association*, *12,* 293-294.

Lindsay, S. & Gibson, M. (1988). Bed nets revisited; an old idea, a new angle, *Parasitoogy Today*, *4,* 270-272.

Liu, N. (2015). Insecticide resistance in mosquitoes: impact, mechanisms, and research directions. *Annual Review of Entomology*, 60(7), 537-559.

Liu, N., Xu, Q., Zhu, F., & Zhang, L. (2006). Pyrethroid resistance in mosquitoes. *Insect Science*, 13(3), 159-166.

Lofgren, C., Dame, D. & Breeland, S. (1974). Release of chemo sterilized males for the control of *Anopheles albimanus* in El Salvador. III. Field methods and population control. *American Journal of Tropical Medical and Hygiene, 23*, 288–297.

Loreto, F., Ciccioli, P., Cecinato, A., Brancaleoni, E., Frattoni, M., Fabozzi, C. & Tricoli, D. (1996). Evidence of the photosynthetic origin of monoterpenes emitted by *Quercus ilex* L. leaves by ^{13}C labeling. *Plant Physiology 110*:1317–1322.

Lukwa, N., Masedza, C., Nyazema, N. & Mwaiko, G. (1994). *Efficacy and duration of activity* of *Lippia javanica* Spreng, *Ocimum cnum* Sims and commercial repellent against *Aedes Aegypti*, *In Proc. First IOCD symp*, Victoria Falls Zimbambwe, pp 321-325.

Mac Tavish, H. (1995). Factors affecting yield and composition of floral extract from Boronia megastigma Nees. PhD thesis, University of Tasmania, Australia.

Makundi, E., Mboera, L., Malebo, H. & Kitua, A. (2007). Priority setting on malaria interventions in Tanzania: strategies and challenges to mitigate against the intolerable burden. *American Journal of Tropical Medicine and Hygiene, 77*, 106-111

Manish, S., Duraipandian, T., Ashish, K. & Mahabir, P. (2009). Synthesis and anti-malaria evaluation of cyclic *β*-amino acid containing dipeptides. *Molecules*, *13*, 432–443.

Marbiah, L., Young, K. & Kung, K. (1988). Use of *Artemisia* and *Calamus* to control mosquitoes among rural population in China. *Journal of American Mosquito Control Association*, *184,* 12-19.

Marcelo, M., Gulab, N., Onkar, D., Carolina, M., Rosimeire, C. & Vânia, M. (2012). Composition, antifungal activity and main fungitoxic components of theessential oil of *Mentha piperita* L. *Journal of Food Safety 32*: 29–36.

Margarita, U., Aluizio, P. & Claudio, J. (1998). Evaluation of SPf66 malaria vaccine efficacy in Brazil. *American Journal of Tropical Medicine and Hygiene, 58*, 378-385.

Mark D. & Fradin, K. (2003). Use of electric vapourizers to dispense pyrethrins in mosquito control. *Journal of American Mosquito Control Association*, *142*, 34-42.

Marquardt, C. & Demance, R. (1985). Macmillan publishing company Newyork USA. *Journal of Parasitology*, *57*, 550-555.

Martins, M., Costa, S., Neves, C., Cavaleiro, C., Salgueiro, L. & Costa, M. (2004). Olive oil flavored by the essential oils of *Mentha piperita* and *Thymus mastichina. Local Food Quality and Preference*, *15*, 447–452.

Martín-Cacheda, L., Vázquez-González, C., Rasmann, S., Röder, G., Abdala-Roberts, L., & Moreira, X. (2023). Plant genetic relatedness and volatile-mediated signalling between Solanum tuberosum plants in response to herbivory by Spodoptera exigua. *Phytochemistry, 206*, 113561.

Mathu, R. (2015). Chemical composition of *Ocimum kilimandscharicum* and *Ocimum americanum* and their bioactivities against *Sitophilus zeamais*. Kenyatta University, Kenya, Msc. Thesis. pp 1-68.

Matsumara, F. & Brown, A. (1961). Biochemistry of malathion resistance in *Culex tarsalis. Journal of Economics*, *54*, 1176-85.

Mawada, A., Waleed, K. & Galalb, M. (2005). Evaluation of repellence and larvicidal activity of *Ocimum basilicum* L. and *Cymbopogon citratus* D. C against *Culex quinquefasciatus. International Journal of Aromatheraphy*, *2*, 243-246.

Mayeku, P. (2006). Variation in chemical composition and mosquito repellent efficacy of essential oil of *Conyza newii* from different regions in Kenya. Kenyatta university, Kenya. Msc. Thesis. Pp 1-61.

Mburu, M., Ndung'u, W., Nguya, K. & Hassanali, A. (2010). Comparison of volatile blends and gene sequences of two isolates of *Metarhizium anisopliae* of different virulence and repellency toward the termite *Macrotermes michaelseni*. *The Journal of Experimental Biology*, *214*, 956-962.

Mc Conkey, M., Gershenzon, J., & Croteau, R. (2000). Developmental regulation of monoterpene biosynthesis in the glandular trichomes of peppermint (*Mentha* × *piperita* L.). *Plant Physiology 122*: 215–223.

Mckay, D. & Blumberg, J. (2006). Review of the bioactivity and potential health benefit of peppermint tea (*Mentha piperita* L.). *Phytotherapy Research*, *20*, pp 619–633.

Mendis, L., Claudia, J. & Brown, A. (2001). Malaria strains: profile of Africa. *American Journal Tropical Medicine Hygiene*, *64*, 271-279.

Mersha, W., Ayele, N, Fentahun, G., Getinet, M., Kassu, K. & Nagappan, R. (2014). Repellent and insecticidal activity of *Mentha piperita* Linn. Plant Extracts against Cabbage Aphid [*Brevicoryne brassicae* Linn. (Homoptera: Aphididae)]. *American-Eurasian Journal ofScientific Research, 9,* 150-156.

Metcalf, C. & Flint, W. (1962). *Destructive and useful insects*, 4th Edn. McGraw Hill, New York, pp 389-391, 1004.

Mittal, P. & Subbarao, S. (2003). Prospects of using herbal products in control of mosquito vectors. *Indian Council of Medical Research Bulletin*, *33*, 1–11.

Monath, T. (1988). *The arboviruses: Epidemiology and ecology*, CRC Press, Boca Raton, FL, pp 1–5.

Motoyama, N., Dauterman, W., Flapp, F. (1997). Genetic studies gluthione dependent resistant strains of house fly, *Musca domestica. Pesticides Biochemistry Physiology*, *7*, 443- 450.

Mouchet, J., Manguin, S., Sircoulou, J., Leventure, S., Faye, O., Ouapa, A., Carnevale, P., Julvez, J. & Foutemille, D. (1988). Evolution of malaria in Africa for the past 40 years. Impact of climatic and human factors. *Journal of the American Mosquito Control Association,14*, 212-230.

Mworia, J. K., Kibiti, C. M., Ngeranwa, J. J., & Ngugi, M. P. (2021). Anti-inflammatory potential of dichloromethane leaf extracts of Eucalyptus globulus (Labill) and Senna didymobotrya (Fresenius) in mice. *African Health Sciences*, *21*(1), 397-409.

Ndirangu, G. (2015). Repellence of essential oil of *Nigella sativa* L. seeds against *Anopheles gambiae* and identification of the active blends. Kenyatta University, Msc. Thesis. Pp 1-51.

Nerio, L. S., Olivero-Verbel, J., & Stashenko, E. (2010). Repellent activity of essential oils: a review. *Bioresource technology*, 101(1), 372-378.

Niinemets, U., Loreto, F. & Reichstein, M. (2004). Physiological and physicochemical controls on foliar volatile organic compound emissions. *Trends in Plant Science 9*: 180–186.

Norris, D. (2004). Mosquito-borne diseases as a consequence of land use change. *EcoHealth*, 1(1), 19-24.

Nosten, F. & White, N. (2007). Artemisinin-based combination treatment of *falciparum* malaria. *American Journal of Tropical Medicine and Hygiene, 77,* 181-192.

Odalo, J., Omolo, O., Malebo, H., Angira, J., Njeru, P., Ndiege, I. & Hassanali, A. (2005). Repellency of essential oils of some plants from the Kenyan coast against *Anopheles gambiae. Acta Tropica*, *95*, 210–218.

Okoli, B. J., Eltayb, W. A., Gyebi, G. A., Ghanam, A. R., Ladan, Z., Oguegbulu, J.

C., & Abdalla, M. (2022). In Silico Study and Excito-Repellent Activity of Vitex negundo L. Essential Oil against Anopheles gambiae. *Applied Sciences*, 12(15), 7500.

Omolo, D., Okinyo, D., Ndiege, O., Lwade, W. & Hassanali, A. (2004). Repellency of essential oils of some Kenyan plants against *Anopheles gambiae. Phytochemistry*, *65*, 2797- 2802.

Palanisami, S., Natarajan, E., & Rajamma, R. (2014). Development of eco-friendly herbal mosquito repellent. *Journal of Innovative Biology*, 1(3), 132-136.

Para, S., Thulasi, S., Penchalaneni, J. & Challagundla, V. (2013). Antibacterial Activity and Phytochemical Analysis of *Mentha piperita* L. (Peppermint). An important multi-purpose medicinal plant. *American Journal of Plant Sciences*, *4*, 77-83.

Patel, E., Gupta, A. & Oswal, R. (2012). A review on: mosquito repellent methods. *IJPCBS*, 2(3), 310-317.

Pates, H., Lines, D. & Miller, E. (2002). Personal protection against mosquitoes in Dar- es salaam, Tanzania, by using a kerosene oil lamp to vaporize transfluthrin *Medical and Veterinary Entomology*, *16*, 277-284.

Patz, J., Graczyk, T., Geller, N. & Victor, A. (2000). Effects of environmental change on emerging parasitic diseases. *International Journal for Parasitology*, *30*, 1395- 1405.

Paulraj, M. & Ignacimuthu, S. (2022). Plant Volatile Oils and Compounds as Ecofriendly Mosquito Control Products: Review on Recent Developments. Quality of Life (Banja Luka)-Apeiron, 22(1-2), 65-79.

Pavela, R. (2015). Essential oils for the development of eco-friendly mosquito larvicides: a review. Industrial Crops and Products, 76(3), 174-187.

Phillips-Howard, P., Nahlen, B. & Wannemuehler, K. (2003). Impact of permethrin-treated bed nets on the incidence of sick child visits to peripheral health facilities.*American Journal of Tropical Medicine and Hygiene*, *68*, 38–43.

Powells, R. (1989). Malaria and babesiosis. In: *Tropical Medicine and Parasitology.* Goldsmith, R. and Heyman, D. (eds). Prentice-Hall Int. Inc., pp 303-326.

Paradiso, R., & Proietti, S. (2022). Light-quality manipulation to control plant growth and photomorphogenesis in greenhouse horticulture: The state of the art and the opportunities of modern LED systems. *Journal of Plant Growth Regulation*, *41*(2), 742-780.

Priya, S. S., Vasantha-Srinivasan, P., Altemimi, A. B., Keerthana, R., Radhakrishnan, N., Senthil-Nathan, S., Kalaivani, K., Chandrasekar, N., Karthi, S., & Ganesan, R. (2023). Bioactive molecules derived from plants in managing dengue vector Aedes aegypti (Linn.). *Molecules*, *28*(5), 2386.

Ramezani, H., Singh, H., Batish, D., & Kohli, R. (2002). Antifungal activity of the volatile oil of Eucalyptus citriodora. *Fitoterapia*, 73(3), 261-262.

Rasooli, I., Gachkar, L., Yadegarinia, D., Rezaei, M. & Alipoorastaneh, S. (2008). Antibacterial and ant-oxidative characterization of essential oils from *Mentha piperita* and *Mentha spicata* grown in Iran. *Acta Alimentally, 37*, 41–52.

Ratemo, J. (2010). Fighting Malaria via SMS. *The Standard*, pp 3. RBM (2005). World Malaria report 2005. WHO Geneva, pp 293.

Rehman, J., Ali, A., & Khan, I. (2014). Plant based products: Use and development as repellents against mosquitoes: A review. *Fitoterapia*, 9(5), 65-74.

Reiter, P. (2000). From Shakespeare to Defoe: Malaria in England in the little ice age. *Emerging Infectious Diseases*, *6* (1), 1-11.

Ridley, R. (1997). *Plasmodium* drug discovery and development, an industrial perspective. *Experimental Parasitology, 87*, 293-304.

Robert, I., Krieger, M., Dinoff, T. & Xiaofei, Z. (2003). Octachlorodipropyl ether mosquito coils are inadequately studied for residential use. *Environment Health Perspective*, *111*, 280- 286.

Roll Back Malaria, (2002). Africa Malaria Day: Mobilizing Communities to Roll Back Malaria, http://www.rollbackmalaria.org/microsites/wmd2011/abuja 2002 first.html, accessedon 21st August, 2016.

Ronald, E., Jan, J. & Rigg, J. (1985). Toxic encephalopathy in children after brief exposure to insect repellent. *Can Medical Association Journal, 132*, 155–156.

Ross, R. (1897). Some peculiar pigmented cells found in two mosquitoes fed on malaria blood.*British Medical Journal*, *2*, 1786-1788.

Sarma, D. & Babu, A. (2011). Pharmacognostic and phytochemical studies of Ocimum americanum. *Journal of Chemical and Pharmaceutical Research, 3*(3), 337-347.

Schreck, C., Fish, D. & McGovern, T. (1995). Activity of repellents applied to skin for protection against *Amblyomma americanum* and *Ixodes scapularis* ticks (Acari: Ixodidae). *Journal of Amblyomma and Mosquito Control Association, 11*, 136-140.

Seo, J., Lee, Y., Kim, S., Cha, C., Ahn, J. & Hur, H. (2005). Biodegradation of the instcticide N,N-Diethyl-*m*-Toluamide by fungi: Identification and Toxicity of metabur. *Archives of Environmental Contamination and Toxicology, 48*, 323-328.

Seyoum, A., Kabiru, W., Lwande, W., Killeen, F., Hassanali, A., & Knols, G. (2002). Repellency of live potted plants against Anopheles gambiae from human baits in semi-field experimental huts. *The American Journal of Tropical Medicine and Hygiene*, 67(2), 191-195.

Seyoum, A., Palsson, K., Kung'a, S., Kabiru, E., Lwande, W., Killeen, G., Hassanali, & Knols, B. (2002). Traditional use of mosquito-repellent plants in western Kenya and their evaluation in semi-field experimental huts against *Anopheles gambiae*: Ethno- botanical studies and application by thermal expulsion and direct burning. *Transactions of the Royal Society of Tropical Medicine and Hygiene, 96*, 225–231.

Seyoum, A., Pålsson, K., Kung'a, S., Kabiru, E., Lwande, W., Killeen, G. & Knots, B. (2020). Traditional use of mosquito-repellent plants in western Kenya and their evaluation in semi-field experimental huts against Anopheles gambiae: ethnobotanical studies and application by thermal expulsion and direct burning. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 96(3), 225-231.

Seyoum, A., Pålsson, K., Kung'a, S., Kabiru, E. W., Lwande, W., Killeen, G., & Knots, B. (2002). Traditional use of mosquito-repellent plants in western Kenya and their evaluation in semi-field experimental huts against Anopheles gambiae: ethnobotanical studies and application by thermal expulsion and direct burning. Transactions of the Royal Society of Tropical Medicine and Hygiene, 96(3), 225-231.

Shambaugh, D. & Brown, R. (1958). *Advances in Pest Control Research*. John Wiley and Sons Inco., New York, *1*, pp 277.

Sharma, V. & Ansari, M. (1994). Personal protection from mosquitoes (Diptera: Culcidae) by burning neem oil in kerosene. *Journal of Medical Entomology, 31*, 505-507.

Shin-Ho, K., Min-Ki, K., Dong-Kyu, S., Doo-Jin, N., Jeong-Oh, Y., Changman, Y. & Gil- Hah, K. (2009). Comparative repellency of essential oils against *culex pipiens pallens* (Diptera: culicidae). *Journal of Korean Society and Applied Biological Chemistry*, *52* (4), 353-359.

Singh, H., Batish, D., Setia, N., & Kohli, R. (2005). Herbicidal activity of volatile oils from Eucalyptus citriodora against Parthenium hysterophorus. *Annals of Applied Biology*, 146(1), 89-94.

Souto, A., Sylvestre, M., Tölke, E., Tavares, J., Barbosa-Filho, J., & Cebrián-Torrejón, G. (2021). Plant-derived pesticides as an alternative to pest management and sustainable agricultural production: Prospects, applications and challenges. *Molecules, 26*(16), 4835.

Staute, J., Kester, K., Kizych, D., & Hall, T. (1998). Long-term efficacy and immune responses following Immunization with RTS malaria vaccine. *Journal of Infectious Diseases, 4,* 1139-1144.

Steffan, W. & Evenhuis, N. (1981). Biology of Toxorhynchites. *Annual Review of Entomology, 26*, 159.

Strickman, D. (2007). Older synthetic active ingredients and current additives.Incorporated: Debboun, M., Frances, S. and Strickman, D. (Edits.).

Insect repellents: Principles, methods and uses. Boca Raton. CRC Press, pp 361-383.

Sukumar, K., Perich, M. & Boobar, L. (1991). Botanical derivatives in mosquito control: *A review Journal of the American Mosquito Control Association, 7*, 210–237.

Takken, W. & Knols, B. (2009). Malaria vector control: Current and future strategies. *Trends in Parasitology, 25*, 101-104.

Tariq, A., Zeng, F., Graciano, C., Ullah, A., Sadia, S., Ahmed, Z., Murtaza, G., Ismoilov, K., & Zhang, Z. (2023). Regulation of metabolites by nutrients in plants. *Plant Ionomics: Sensing, Signaling, and Regulation*, 1-18.

Tawatsin, A., Asavadachanukorn, P., Thavara, U., Wongsinkongman, P., Bansidhi, J., Boonruad, T., & Mulla, M. (2006). Repellency of essential oils extracted from plants in Thailand against four mosquito vectors (Diptera: Culicidae) and oviposition deterrent effects against Aedes aegypti (Diptera: Culicidae). *Southeast Asian Journal of Tropical Medicine and Public Health, 37*(5), 915.

Tawatsin, A., Wratten, S., Scott, R., Thavara, U. & Techadamrongsin, Y. (2001). Repellency of volatile oils from plants against three mosquito vectors. *Journal of Vector Ecology, 26*, 76-82.

Ter, K., Dianne, J., Terlouw, S., Kariuki, P., Phillips-Howard, L., Mirel, W., Hawley, Jennifer, F., Ya Ping Shi, M., Kolczak, A., Lal, J., & Bernard, L. (2003). Impact of permethrin-treated bed nets on malaria, anemia, and growth in infants in an area of intense perennial malaria transmission in western Kenya. *American Journal of Tropical Medicine and Hygiene*, *68* (Suppl 4), 68–77.

Toht, D. (2017). *Backyard Homesteading: A Back-to-basics Guide to Self-sufficiency*. Fox Chapel Publishing.

Tropis, M. & Donated, E. (2019). Hydro-distillation for Essential Oils Extraction. *Journal of Essential Oil Bearing Plants*, 18(2), 255-262.

Undeen, A. & Dame, D. (1987). Measurement of the effect of microsporidian pathogens on mosquito larval mortality under artificial field conditions. *Journal of American Mosquito Control Association, 3*, 91–93.

Verma, R., Rahman, L., Verma, R., Chauhan, A., Yadav, A. & Singh, A. (2010). Essential oil composition of Menthol Mint (*Mentha arvensis)* and Peppermint (*Menthapiperita*) Cultivars at different stages of plant growth from kumaon region of Western Himalaya. *Journal of Medicinal and Aromatic Plants, 1*, 13-18.

Wakelin, D. (1996). Immunity to Parasites: How Parasitic Infections are controlled. Cambridge University Press, pp 1-2004.

Wangai, L., Kamau, K., Munyekenye, G., Nderu, D., Maina, E., Gitau, W., & Otieno,

F. (2020). Efficacy of plant-based repellents against anopheles mosquitoes: a systematic review. *Biomed. Science, 6*(4), 44-65.

Wanzala, W. (2017). Potential of traditional knowledge of plants in the management of arthropods in livestock industry with focus on (Acari) ticks. Evidence-Based Complementary and Alternative Medicine, 35(5), 345-421.

Webb, Jonathan (2014). GM laboratory mosquitoes may aid malaria fight. BBC News, Science and Environment, Retrieved 11th June, 2014.

Weill, M., Lutfalla, G., Mogensen, K., Chandre, F., Berthomieu, A., Berticat, C. & Raymond, M. (2003). *Insecticide resistance in mosquito vectors. Nature, 423*(6936), 136-137.

Wendimu, A. & Tekalign, W. (2020). Infusion extraction of toxin from chili pepper (Capsicum baccatum) for bedbug protection. *Asian J Biotechnology, 12*(2):65–74

Wendimu, A., & Tekalign, W. (2020). Field Efficacy Evaluation of some Ethno medicinal Plants Smoke Repellency against Anopheles arabinoses and Aedes Aegyptus, *Research Square Journal, 2*(1), 4721-7528

Wendimu, A., & Tekalign, W. (2021). Field efficacy of ethnomedicinal plant smoke repellency against Anopheles arabiensis and Aedes aegypti. *Heliyon, 7*(7), e07373.

White, G. (1975). Notes on a catalogue of Culicidae of the Ethiopian region. *Mosquito Systematics, 7,* 303-344.

White, N., Nosten, F., Looaraasuwam, S., Watkins, W., Marsh, K., Snow, R., Kokwaro, G., Ouma, J., Hien, T., Molyneux, M., Newbold, C., Reubush, T., Danish, M., Green, B., Anderson, R. & Olliaro, P. (1999). Averting a malaria disaster. *Lancet, 353,* pp 1965-1967.

WHO (1980). Environmental management for vector control. Third report of the WHO expert committee on vector biology and control. Technical report series. No. 649. Geneva.

WHO (1989). Geographical distribution of arthropod-borne diseases and their principal vectors. Geneva: World Health Organization, WHO/VBC/89, *967*, pp 7-22.

WHO (1996). *Evaluation and testing of insecticides*. Report of the WHO informalconsultation, Geneva, pp 11-19.

WHO (1996). *Fighting disease, Fostering development.* Report of the director-general WHO Geneva, pp 5-15.

WHO (1997). World malaria situation in 1994. *Weekly epidemiological record*, *36* (72), pp. 269-276.

WHO (1998). *Roll Back Malaria, a global partnership*. WHO, Geneva, pp 49-62. WHO (1999). Fact sheet on MMU "*WHO Background Document* Nov. 1999.

WHO (2000). *Global surveillance in control of Leishmaniasis.* WHO, Geneva. WHO/CDS/CSR/ISR/2000.1, pp 121-127.

WHO (2001). *Antimalarial drug combination therapy.* Report of a WHO technical consultation. Geneva. pp 4-5.

WHO (2002). *The world health report. Reducing risks, promoting health life.* WHO, Geneva,pp 1-248.

WHO (2003). *Malaria vector control; Decision making criteria and procedures for judicious use of insecticides, Geneva*. WHO/CDS/WHOPES/2002.5 *Review, 1,* 1-101.

WHO (2009). *Progress and prospects for the use of genetically modified mosquitoes to inhibitdisease transmission.* Geneva, pp 1-54.

WHO (2013). *Guidelines for efficacy testing of spatial repellents*. Geneva, pp 1-48

WHO (2014). *Fact sheet on the world malaria report.* http://www.who.int/malaria/ media/world malaria report 2014/en/, accessed on 21st August, 2016.

WHO (2015). *World Malaria Report.* World Health Organization, pp 1-280.

WHO (2016). Artemisinin and artemisinin-based combination therapy resistance. *GlobalMalaria Programme*, pp 1-12.

Wiggleworth, V. (1976). *Insects and Life of Man.* John Wiley and Sons Inc, New York, pp 95-102.

World Health Organization. (2009). Guidelines for efficacy testing of mosquito repellents for human skin (No. WHO/HTM/NTD/WHOPES/2009.4). World Health Organization.

World Health Organization. (2021). World malaria report 2020: 20 years of global progress and challenges. Geneva: WHO; 2020. License: CC BY-NC-SA, 3.

Wright, R. H. (1975). Why mosquito repellents repel. *Journal of Scientific American, 233* (1),104-111.

Xue, R., Ali, A. & Day, J. (2007). Commercially available insect repellents and criteria for their use. Incorporated: Debboun, M., Frances, S., and Strickman, D. (Edits.). Insect Repellents: Principles, methods and uses. Boca Raton. CRC Press, pp 405-415.

Yactayo-Chang, J. P., Broadhead, G. T., Housler, R. J., Resende Jr, M. F., Verma, K., Louis, J., Basset, G. J., Beck, J. J., & Block, A. K. (2024). Maize terpene synthase 1 impacts insect behavior via the production of monoterpene volatiles β-myrcene and linalool. *Phytochemistry, 218*, 113957.

Yap, H., Jahangir, K., Chong, A., Adanan, C., Chong, N., Malik, Y., & Rohaizat, B. (2019). Field efficacy of a new repellent, KBR 3023, against Aedes albopictus (SKUSE) and Culex quinquefasciatus (SAY) in a tropical environment. *Journal of the Society for Vector Ecology*, 23(1), 62-68.

Yeye Toure (2001). Malaria vector control in Africa: strategies and challenges. Malaria in Africa, Report from a symposium held at the AAAS annual meeting, San Francisco. Retrieved from www.aaas.org/international/africa/malaria/toure. html on 21 August 2016.

Yoon, J., & Tak, J.-H. (2023). Potential utilization of the brewery's hop wastes as an insecticidal synergist and repellent against Spodoptera frugiperda. *Journal of Pest Science*, *96*(4), 1441-1454.

Yuan, L., Yang, X., Yu, X., Wu, Y., & Jiang, D. (2019). Resistance to insecticides and synergistic and antagonistic effects of essential oils on dimefluthrin toxicity in a field population of Culex quinquefasciatus Say. Ecotoxicology and Environmental Safety, 169(3), 928-936.

Ywaya, D. O. (2013). *Repellency Efficacy of Essential Oils of Selected Plants Growing in western Kenya and their Eag-active Constituent Blends against Anopheles Gambiae* Kenyatta University].

Ywaya, D. (2013). Repellency efficacy of essential oils of selected plants growing in western kenya and their EAG-active constituent blends against *Anopheles gambiae.* Kenyatta University, Kenya, Msc. Thesis. pp 1-62.

Zadikoff, C. (1979). Toxic encephalopathy associated with use of insect repellent. *Journal of Paediatric, 95*, 140–142.

Zaim, M. & Jambulingam, P. (2007). Global insecticide use for vector-borne disease control. WHO/CDS/NTD/WHOPES/GCDPP/2007.2.

Zaim, M., Ladonni, H. and Ershadi, M. R. Y. (1988). Field application of Romanomermis culicivorax (Mermithidae: Nematoda) to control anopheline larvae in southern Iran. *Journal of American Mosquito Control Association, 4,* 351–355.

Zeb, A., Ullah, F., Ayaz, M., Ahmad, S., & Sadiq, A. (2017). Demonstration of biological activities of extracts from Isodon rugosus Wall. Ex Benth: Separation and identification of bioactive phytoconstituents by GC-MS analysis in the ethyl acetate extract. BMC *Complementary and Alternative Medicine*, 17(1), 1-16

Zucker, J. & Campell, C. (1992). Smear negative cerebral malaria due to mefloquine resistant *Flalciparum* malaria acquire in Amazon. *Journal of Infectious Disease, 166,* 1456- 145.

APPENDICES

Appendix 1: Certificate of Consent

This research involves repellent activities and formulation of *Ocimum suave, Ocimum americanum and Eucalyptus citriodora* essential oils against *Anopheles gambiae*. Plant(s) were placed in one of the exposed area, a human volunteer was allowed to sit in for the day and for night. The hands of the participant from the fingers to the elbow were exposed for 3 hours to *Anopheles gambiae*; the number of *Anopheles gambiae* that land on the exposed area was counted and then shaken off before imbibing any blood. The *Anopheles gambiae* that was used did not have any disease-causing agent. An insect bite cream was provided to the participants in case of any minor bites and associated irritations. The results are expected to form the basis of downstream development for development of appropriate potted plants with potent repellent volatile opportunity to ask questions about it, and any questions that the researcher have asked has been answered to his/her satisfaction. The people concerned signed the informed consent below.

I have read the foregoing information, or it has been read to me. I have had the consent voluntarily to participate as a participant in this research and understand that I have the right to withdraw from the research at any time without in any way affecting my medical care.

Print name of participant:______________________________________

Signature of participant: _______________________________________

Date:____

Appendix 2: Institutional Introductory Letter.

THARAKA UNIVERSITY

P.O BOX 193-60215,
MARIMANTI, KENYA

Telephone: +(254)-0202008549
Website: https://tharaka.ac.ke
Social Media: tharakauni
Email: info@tharaka.ac.ke

OFFICE OF THE DIRECTOR
BOARD OF POSTGRADUATE STUDIES

REF: TUN/BPGS/PL/06/23 **20th September, 2023**

To Whom It May Concern,

Dear Sir/Madam,

RE: INTRODUCTORY LETTER FOR ALICE CIANDIGE KARITHI, ADMISSION NUMBER: SMT11/00564/20

The above named is our postgraduate student undertaking a Master of Science degree programme in **Chemistry**. The student has finished coursework and is expected to collect data. The title of the research is "**repellent activities and formulation of *Ocimum suave*, *Ocimum americanum* and *Eucalyptus citridora* against *Anopheles gambiae.*"** The study will be conducted in **Tharaka- Nitbi County.**

The candidate has defended the proposal successfully at the Faculty and has submitted the required number of corrected copies to the Office of the Director, Board of Postgraduate Studies. The candidate is expected to begin collecting data, analyse and write a report on the findings. The study is expected to be completed by September, 2024.

Any assistance accorded to him will be highly appreciated.

Thank you in advance.

Yours faithfully,

THARAKA UNIVERSITY
BOARD OF POSTGRADUATE STUDIES AND RESEARCH
29 SEP 2023
DIRECTOR
P.O. Box 193-60215, MARIMANTI

Dr. Denis Obote, Ph.D.
Director,
Board of Postgraduate Studies.

Appendix 3: Ethical Clearance

CHUKA UNIVERSITY

Knowledge is Wealth *(Sapientia divitia est)* Akili ni Mali

CHUKA UNIVERSITY INSTITUTIONAL ETHICS REVIEW COMMITTEE

Telephones: 020-2310512/18 P. O. Box 109-60400, Chuka
Direct Line: 0772894438 Email: info@chuka.ac.ke, Website: www.chuka.ac.ke

20th September, 2023

REF: CUIERC/ NACOSTI/423
TO: **Ciandige Alice Karithi**

RE: Repellent Activities and Formulation of *Ocimum suave, Ocimum americanum* and *Eucalyptus citriodora* Essential Oils Against *Anopheles gambiae*..

This is to inform you that *Chuka University IERC* has reviewed and approved your above research proposal. Your application approval number is *NACOSTI/NBC/AC-0812.* The approval period is 20th September, 2023 – 20th September, 2024.

This approval is subject to compliance with the following requirements;

i. Only approved documents including (informed consents, study instruments, MTA) will be used
ii. All changes including (amendments, deviations, and violations) are submitted for review and approval by *Chuka University IERC.*
iii. Death and life threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to *Chuka University IERC* within 72 hours of notification
iv. Any changes, anticipated or otherwise that may increase the risks or affected safety or welfare of study participants and others or affect the integrity of the research must be reported to *Chuka University IERC* within 72 hours
v. Clearance for export of biological specimens must be obtained from relevant institutions.
vi. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal.
vii. Submission of an executive summary report within 90 days upon completion of the study to *Chuka University IERC.*

Prior to commencing your study, you will be expected to obtain a research license from National Commission for Science, Technology and Innovation (NACOSTI) https://oris.nacosti.go.ke and also obtain other clearances needed.

Yours sincerely

Dr. Benjamin Kanga
SECRETARY

Chuka University is……. …Inspiring Environmental Sustainability for Better Life

Appendix 4: Research Permit

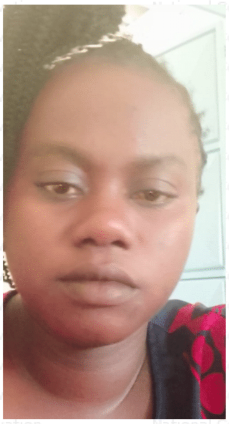
REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: **104351**

Date of Issue: **26/October/2023**

RESEARCH LICENSE



This is to Certify that Ms.. ALICE karithi ciandige of Tharaka University, has been licensed to conduct research as per the provision of the Science, Technology and Innovation Act, 2013 (Rev.2014) in Tharaka-Nithi on the topic: REPELLENT ACTIVITIES AND FORMULATION OF OCIMUM SUAVE, OCIMUM AMERICANUM AND EUCALYPTUS CITRODORA ESSENTIAL OILS AND ANOPHELES GAMBIAE. for the period ending : 26/October/2024.

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