# CHARACTERIZATION, INSECTICIDAL AND MOSQUITO REPELLENCY PROPERTIES OF ESSENTIAL OILS FROM *HYPTIS SUAVEOLENS* LEAVES

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(M.Sc/Scien/6449/2011-2012)

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# DECLARATION

I declare that the work in this Dissertation entitled 'CHARACTERIZATION, INSECTICIDAL AND MOSQUITO REPELLENCY PROPERTIES OF ESSENTIAL OILS FROM *HYPTIS SUAVEOLENS* LEAVES' has been performed by me in the Department of Biochemistry. The information derived from the literature has been duly acknowledged in the text and the list of references provided. No part of this dissertation was previously presented for another degree or diploma at any university.


Bala, Christiana Joseph

_____          _____          _____

Name of student                                      Signature                          Date

# CERTIFICATION

This dissertation report entitled CHARACTERIZATION, INSECTICIDAL AND MOSQUITO REPELLENCY PROPERTIES OF ESSENTIAL OILS FROM *HYPTIS SUAVEOLENS* LEAVES by CHRISTIANA JOSEPHBALA meets the regulations governing the award of Master of Science of Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

Dr A. B. Sallau

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(Chairman, Supervisory Committee)          Signature                      Date

Prof. E.Onyike

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(Member, Supervisory Committee)          Signature                      Date

Prof. I. A. Umar

_____          _____          _____

(Head of Department)                      Signature                      Date

Prof. Kabiru Bala

_____          _____          _____

(Dean, Postgraduate school)              Signature                      Date

## DEDICATION

To God Almighty, the Most high, the source of my strength and life and in memory of my late father Thlama G. Wakawa who laboured over us (his family) but could not wait to eat the labour of his hands.

# ACKNOWLEDGEMENTS

## ABSTRACT

Essential oils were extracted from the leaves of *Hyptis suaveolens*by hydro-distillation,Mosquitocidal effect was done through an experiment devised for the purpose of this research. The effect of the essential oils against mosquito larvae (larvicidal), mosquito repellency effectand physicochemical properties were determined using standard methods. Gas Chromatography-Mass Spectrometry (GCMS) and Fourier Transform Infra-Red (FTIR) were both done on the essential oils. Percentage yield of the essential oils was gotten as 0.05, for the larvicidal activity dose dependent mortality of the larvae was observed; there was low mortality rate at lower dosage and not significantly different from each other e.g. 6.25 and 12.50ppm had same $LC_{50}$ and $LC_{90}$ of 25.21 and 302.67 respectively, 25ppm had $LC_{50}$ and $LC_{90}$ of 9.58 and 352.46 respectively, while 50-1000ppm had $LC_{50}$ and $LC_{90}$ of 0.55 and 0.85 respectively. It was observed that the amount of air that enters and leaves the cage affect the rate at which mosquitoes were repelled. In a group of mosquitoes kept in a cage with all sides open (ASO) 65% of the mosquitoes were repelled within 30 minutes while for one side open (OSO) and all sides closed (ASC), 73% and 85% of the mosquitoes were repelled within the same time frame. In a second experiment, 100% of mosquitoes were repelled from the surface of rats with shaven skin where essential oil was applied. The essential oil also demonstrated dose dependent mosquitocidal activity with $LC_{50}$ and $LC_{90}$ values of 6 and 21ppm respectively. The essential oil has the following physicochemical properties; Iodine value 23.59$\pm$0.12g/100g, saponification 100.18$\pm$0.8mgKOH/g, Peroxide value 40.00$\pm$0.02meq/kg, Acid value 3.37$\pm$0.01mgKOH/g, Ester value 34.30$\pm$1.00mg/g and free fatty acids 0.15$\pm$0.57%. GC-MS analysis revealed Terpenes to be the major organic compound present in the essential oil which was confirmed by FTIR with the O=C-O-C stretch functional group indicating the presence of terpenes. Thus, the presences of terpenes

in Essential oil of *Hyptis suaveolens* may have contributed to its insecticidal and mosquitocidal repellency properties.

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List of Abreviations

| Acronym | Full meaning |
|---------|--------------|
| ACT | Artemisinin derivative- based combination therapy |
| ACTs | Artemisinin-based combination treatments |
| AMA | American Medical Association |
| Amu | Atomic mass unit |
| ASC | All sides closed |
| ASO | All sides open |
| DDT | Dichloro diphenyl trichoroethane |
| DEET | Diethyl-meta-toluamide |
| EM | Electromagnetic |
| EO | Essential oil |
| EPA | Environmental Protection Agency |
| FTIR | Fourier transform infra-red |
| GC-MS | Gas Chromatography-Mass Spectroscopy |
| IR | Infrared |
| IUGR | Intrauterine growth retardation |
| LC | Lethal concentration |
| L. poits | Labiatae points |
| OSO | One side open |
| Ppm | Part per million |
| RDT | Rapid diagnostic test |
| WHO | Who health organization |
| AchE | Acetylcholinesterase |

## 1.0 INTRODUCTION

### 1.1 Backgroundto the study

Plants have always served as food and medicine to man since the beginning of life. Their nutritional and medicinal potentials have been attributed to the phytochemicals and other chemical constituents contained in them. Despite their importance, it has been reported that out of the 250,000 to 500,000 species of existing plants on earth, only about 300 species are utilized in the food, pharmaceutical, cosmetics and perfume industries. Traditionally used medicinal plants produce a variety of compounds of known therapeutic properties (Umedum *et al.*, 2014).

Medicinal plants are used in traditional treatments to cure variety of diseases. In the last few decades there has been an exponential growth in the field of herbal medicine. Natural products have been a source of drugs for centuries (Din*et al.*, 2011). Traditional medicines (plants source) has been used for thousands of years for the treatment of malaria and are the source of two main groups (artemisinin and quinine derivatives) of modern antimalarial drugs (Kazembe *et al*., 2012).

Repellents are substances applied to the skin, which prevent insects from biting such surface (Traoré-Coulibaly*et al.*, 2013).An insect repellent is a substance that causes an organism to move away from the odour source, insects perceive thevolatile repellents by smell (Luts *et al.*, 2014).

Essential oils are volatile natural complex secondarymetabolites characterized by a strong odour and have agenerally lower density than that of water (Arun *et al.*, 2009). They are natural volatile mixtures of hydrocarbons with a diversity of functional groups, and their

repellent activity has been linked to the presence of monoterpenes and sesquiterpenes (Moreira*et al.*, 2010;Chaubey 2012; Traoré-Coulibaly*et al.*, 2013). Essential oils are plant products obtained by hydro-distillation or other methods (Luts *et al.*, 2014). This complex of compounds are produced by plants, giving them their characteristic smell and taste, and are usually composed of 20–80 or more substances. Their main components are monoterpenes (C10) and sesquiterpenes (C15) derived from isoprene (Luts *et al.*, 2014).Several monoterpenes have been reported as insect repellents (Luts *et al.*, 2014).There are 17,500 aromaticplant species among higher plants andapproximately 3,000 essential oils are known out of which300 are commercially important for pharmaceuticals,cosmetics and perfume industries. Apart from insecticidal potential they are lipophilic in nature and interfere with basic metabolic, biochemical, physiological and behavioural functions of insects. They are also used as flavour in food products, odorants in flagrances, pharmaceuticals (antimicrobial) and as insecticides(Moreira*et al.*, 2010).

*Hyptis suaveolens* (L.Poit) is one of the important traditional medicinal plants belonging to family lamiaceae (Umedum *et al.*, 2014). It is commonly called Bush mint, Bush tea, Pignut, or Chan. It is known generally as Vilayati Tulsi in Hindi, Konda Thulasi in Telugu, Bhustrena in Sanskrit, Daddoya-ta-daji ( family name) and specifically Sarakuwan sauro in Hausa, Efiri (family name) in Yoruba, Nchuanwu  (family name) in Ibo, and Tanmotswangi-eba in Nupe (Umedum *et al.*, 2014; Ghafari *et al.*, 2014).

Leaves of *Hyptis suaveolens* have been traditionally used as a stimulant, antisplasmoidic, against colds and diarrhoea (Shaikat *et al.*, 2012). It has been and still in use traditionally to repel mosquitoes by burning the leaves (Singh *et al.*, 2011; Vongsomboth*et al.*, 2012).

The repellent activity of the whole *Hyptis suaveolens* essential oil and single major constituents against adults of *S. granarius* has been evaluated (Benelli *et al.*, 2012). Amongst

the several methods available to evaluate the repellence of natural products, the filter paper tests in Petri dish is one of the most commonly used bioassays (Benelli *et al.*, 2012). Several authors reported a large variability in the composition of this family due to genetic, geographical and seasonal factors. Since the biological activities of essential oils are composition-dependent, it is apparent that it is very important to fully characterize these mixtures from the chemical point of view (Benelli *et al.*, 2012).

Malaria is a major public health problem with an estimated two million children worldwide dying of it yearly. Regardless of the fact that it is one of the oldest recorded diseases, malaria remains one of the world's most deadly infectious diseases. It is arguably, the greatest menace to modern society in terms of morbidity and mortality. Though preventable, treatable and curable, there is no known vaccine against the disease. This makes it an efficient and unrepentant killer. Several centuries after its discovery, malaria still remains a devastating human infection, resulting in 300-500 million clinical cases and three million deaths every year. Malaria is endemic throughout Nigeria (Olurishe *et al*., 2007; Nigeria Malaria Indicator Survey 2010; Okafor and Oko-Ose 2012; Etusim *et al.*, 2013; Odey *et al.*, 2013).

The management of severe malaria remains challenging, mainly due to the fact that it does not only depend on the use of effective anti-malaria drugs but also the use of effective parenteral anti-malaria drugs. Other factors that make management of malaria challenging are; cost-intensive supportive measures, unavailability of highly skilled personnel, insufficient functional referral systems, lack of blood transfusion services, lack of good infrastructure and inadequate organization of hospital services. Parenteral quinine had been the first-line treatment for severe malaria in Nigeria until June 2011 when the policy was revised to intravenous artesunate as a first-line antimalarial drug (Odey *et al.*, 2013).

Malaria caused by *Plasmodium falciparum* is the most dangerous form of the disease resulting in life threatening complications such as anaemia and cerebral malaria. The pattern of exposure to malaria infection, the type of treatment and the degree of compliance with the anti-malaria regimen, local drug resistance patterns, individual's age and genetic makeup all tend to influence the severity of the disease (Etusim *et al.*, 2013).

A synthetic insecticide is a poisonous chemical or mixture of chemicals that is intended to prevent, repel, or kill any insect or pest. However, synthetic insecticides present hazardous impacts far beyond their intended targets. Insecticides have inherent toxicity because they are designed to kill living organisms that are considered "pests", that is any unwanted insect. Many insecticides are known to pose significant, acknowledged health risks to people— including birth defects, damage to the nervous system, disruption of hormones and endocrine systems, respiratory disorders, skin and eye irritations and various types of cancers (Agency for Toxic Substances and Diseases Registry, 1994).

The synthetic insecticide Dichloro diphenyl trichoroethane (DDT) was widely used in urban aerial sprays to control urban mosquito and other insects. DDT has caused chronic effects on the nervous system, liver, kidneys, immune systems,tremors, increased blood levels of liver-produced enzymes, changes in the cellular chemistry in the central nervous system, hind leg paralysis, convulsions and subtle effects on neurological development and decreased in thyroid function in experimental animals.Teratogenic, mutagenic, carcinogenic effects and sterility were also observed in experimental animals(Agency for Toxic Substances and Diseases Registry, 1994).

Chemical repellents are important in protecting people from blood-feeding insects and may therefore also reduce transmission of arthropod borne diseases. N,N-diethyl-3-methylbenzamide (DEET) is one of the most well-known arthropod repellents. DEET is

generally safe for topical use if applied as recommended, although adverse effects such as serious neurologic effects have been reported. It does not readily degrade by hydrolysis at environmental pHs and has been identified as aubiquitous pollutant in aquatic ecosystems (Abagli and Alavo 2011).

## 1.2 Statement of the Problem

> Paré Toé*et al.,*(2009) have shown that people's motivation to use mosquito nets considerably decreased less than a year after the campaigns and people prefer to spend night without insecticidal nets. Therefore, use of mosquito net is not sufficient to effectively control malaria vectors, especially in the West African countries where urbanization promotes the proliferation of mosquitoes.

> To achieve successful vectors control and reduce substantially the prevalence of malaria and other vector-borne diseases, an integrated management of these vectors must be adopted.

> Insecticides have inherent toxicity and are known to pose significant health risks to people.

> Globally, the malaria situation is serious and still deteriorating, hence the need to search for solution.

> Malaria predominantly affects the poor and underprivileged hence the need to search for affordable means of eliminating the disease.

**1.3 Justification**

➢ Traditionally,*Hyptis suaveolens* has been used and is still in use to repel mosquito but the scientific basis behind this practice has not been validated.

➢ Mosquitoes have become the most important single group of insects well-known for their public health importance (Singh*et al.,* 2011).

➢ Considering the use of the plant to repel mosquitoes, it is worthwhile exploring the repellency properties of the plant essential oil and larvicidal property for a possible mosquito control strategy (Singh *et al.*, 2011).

➢ On the possible eradication of malaria, Arigbabuwo opined that prevention is better than cure (Okafor and Oko-Ose 2012). Hence the search for possible eradication of mosquito right at the larvae stage.

➢ To the best of my knowledge, there are no sufficient data available on the physicochemical properties of essential oil of *Hyptis suaveolens* leaves, hence looking at its physicochemical properties for possible use in making soap, cream or perfume for use to repel the mosquito.

**1.4 Aim and Objectives**

1.4.1 **Aim**

The general aim of this research work is to extract and characterise the insecticidal properties of essential oil of *Hyptis suaveolens* leaves.

1.4.2 **Specific objectives**

The specific objectives of this work are to;

i. Extract essential oil of *Hyptis suaveolens* leaves through hydro-distillation.

ii. Determine the mosquitocidal capability of the essential oil.

iii. Determine the mosquito repellency properties of essential oilof *Hyptis suaveolens* leaves

iv. Determine the larvicidal activities of the essential oil of *Hyptis suaveolens*.

v. Explore the physicochemical properties of the essential oil so as to recommend its possible use as body cream, perfume or toilet soap that can be use on the body to repel mosquitoes.

vi. Characterise the oil by GC-MS and FTIR.

**CHAPTER TWO**

**2.0 LITERATURE REVIEW**

**2.1 Plants**

Plants have always served as food and medicine to man since the beginning of life. Their nutritional and medicinal potentials have been attributed to the phytochemicals and other chemical constituents contained in them. Despite their importance, it has been reported that out of the 250,000 to 500,000 species of existing plants on earth, only about 300 species are utilized in the food, pharmaceutical, cosmetics and perfume industries. Traditionally medicinal plants produce a variety of compounds of known therapeutic properties (Umedum *et al*., 2014).

Many of the herbs and shrubs are found to have promising medicinal properties, mosquito larvicidal and mosquito repellent properties. Plant products can be used either as insecticides for killing larvae or adult mosquitoes or as repellents for protection against mosquito bites, depending on the type of activity they possess. Plants remain as one of the most important sources of several compoundswhich possess potential insecticidal or repellent properties and are free from harmful effects (Din *et al.*, 2011; Singh *et al.*, 2011; Azmathullah *et al.*, 2013). Many plant products have been reported either as insecticides for killing mosquito larvae or adults or as mosquito repellents for adults (Din *et al.*, 2011). Plant products can be obtained either from the whole plant or from a specific part (leaves, stem bark, roots and fruits) by extraction with different types of solvents such as ethanol, methanol, hexane, petroleum ether, chloroform, among other products, depending on the polarity of the phytochemicals (Singh *et al.*, 2011).

### 2.1.1 **Mechanisms employed by plants to repel mosquitoes (insects)**

Plants are endowed with a potential to produce a wide range of allele-chemicals that protect the plants from insect-pests (Ramya *et al.*, 2008). Mechanisms were developed to repel phytophagous pests, i.e. protective hairy or waxy surfaces, hardened cuticles or tissues or the cellular production of repellent and toxic chemicals. In most cases these protective devices are sufficient to repel generalists among the phytophagous insects (Kiprop *et al.*, 2005; Din *etal.*, 2011).

Medicinal plants are used in traditional treatments to cure variety of diseases. In the last few decades there has been an exponential growth in the field of herbal medicine. Natural products have been a source of drugs for centuries (Din *et al.*, 2011). Thus, one of the approaches for control of mosquito borne diseases is the interruption of the disease transmission by killing or preventing mosquito bites. Herbal products with proven potential as insecticides or repellents can play an important role in the interruption of transmission of mosquito borne diseases both at the individual and community level.

### 2.1.2 **Advantages of plants origin (non-synthetic) insecticides**

They are biodegradable in nature and relatively safer to human beings and to non-target organisms of the environment (Alouani *et al.*, 2009; Rajamma*et al.*, 2011).Plant based insecticides are a promising tool for targeting mosquitoes in their larval stages and adult mosquito control (Carvalho 2003; Din 2011). Aromatic plants are among the most efficient insecticides of botanical origin, and essential oils (EOs) often constitute the bioactive fraction (Carvalho 2003; Benelli*et al.*, 2012).Plants are rich source of bioactive organic chemicals and offer an advantage over synthetic insecticides as these are less toxic, less prone to development of resistance, and easily biodegradable. Plant derived insecticides are eco-friendly, non-toxic to non-target organisms, non-persistent in nature, besides they are less

known to promote drug resistance. Application of bio-pesticides has been reported to have positive impacts on bollworm population management. Therefore, researchers world over are engaged in a mission to hunt for novel phytochemicals that could potentially be used in the management of insect-pests (Tiwarya*etal.*, 2007; Ramya *et al*., 2008; Bhat and Khanal 2009; Ramathilaga *et al*., 2012; Ravi *et al.*, 2012; Dua *et al.*, 2013; Azmathullah *et al.*, 2013).

2.1.3 **Role of medicinal plants in the treatment of malaria**

Medicinal plants may be define as those plants that are commonly used in treating and preventing specific ailments and diseases and that are generally considered to be harmful humans (Nwachukwu *et al*., 2010). They have been used since times immemorial virtually in all cultures as a source of medicine (Ibrahim *et al*., 2012).Traditional medicines (plants source) has been used for thousands of years for the treatment of malaria and are the source of two main groups (artemisinin and quinine derivatives) of modern antimalarial drugs (Kazembe *et al*., 2012). Medicinal plants contain principles which are responsible for their medicinal properties and herbal preparations accounts for 30-50% of the total medicine consumption. British explorers were cured of malaria by Southern Africans using traditional (plants) medicines long before colonization. In Zimbabwe elderly people 50 years and above were interviewed between year 2005 to 2009 about the plants they used to treat and cure malaria for the purpose of developing new drugs and vaccines for the treatment, management, prevention and control of malaria or mosquitocides to destroy the mosquitoes or repellents to ward off the vectors (Kazembe *et al*., 2012: Talkmore *et al*., 2014).The Portuguese discovered that slaves in South America use the bark of the stem of cinchona plant to cure malaria and fever and subsequently quinine was commercially obtained from the bark and root of the plant. In Zimbabwe medicinal plants such as *Adansonia digitate, Albizia amara, Dalbergia nitidula, Lania discolour* and *Vangueria infausta* and many others are source of antimalarial agents (*Kazembe et al*., 2012). In Nigeria over 50 medicinal plants are

used for malaria therapy, the following parts could be used bark, root, stem, bulb, rhizomes, leaves, fruits or whole plants (Tolu *et al*., 2007; Ibrahim *et al*., 2012; Nwachukwu *et al*., 2010)some are used alone (not in combination) for malaria therapy these could be taken as tincture, decoction, infusion, and concoction, some are used in combination with others while some are used as adjuncts included in malaria therapy (Tolu *et al*., 2007).

## 2.2Mosquito: Causativevector for Malaria

Mosquitoes (Diptera: Culicidae) are the oldest human enemy and represent a significant threat to human health because of their ability as vector pathogens that cause diseases that afflict millions of people worldwide more than any other anthropods (Carvalho 2003; Kumar and Maneemegalai 2008; Arivoliand Samuel 2011). About 3500 species of mosquitoes have been described worldwide. Relatively few of them are significant vectors of human diseases. (Taha*et al*, 2011). WHO has declared mosquito as "Public enemy number one" since they are responsible for the transmission of various dreadful diseases (Arivoliand Samuel 2011). Several mosquito species belonging to genera *Anopheles*, *Culex* and *Aedes* are vectors for the pathogens of various diseases like malaria, filariasis, Japanese encephalitis, dengue, dengue haemorrhagic fever and yellow fever(Carvalho 2003;Thakur *et al.*, 2004; Tiwarya*et al.*, 2007; Kumar and Maneemegalai 2008; Bhat and Khanal 2009; Bhat and Kempraj 2009; Alouani *et al.*, 2009; Mgbemena 2010; Din *et al.*, 2011; Singh *et al.*, 2011; Arivoliand Samuel 2011; Rajamma *et al.*, 2011; Taha*et al.*, 2011; Ramathilaga *et al.*, 2012; Vongsombath*et al.*, 2012; Ravi*et al.,* 2012; Dua *et al.*, 2013; Azmathullah *et al.*, 2013).Mosquitoes are some of the most adaptable and successful insects on earth. Mosquito-borne diseases are a major problem in almost all tropical and subtropical countries and currently there are no successful vaccines against most of the mosquito borne diseases (Ramathilaga *et al.*, 2012; Din*et al.*,2011). *Aedesalbopictus* (Skuse) also known as "The tiger mosquito" has become a major threat in many parts of the world due to its vector competency.This species is an aggressive daytime

biter, acting as a vector in transmission of arboviruses causing Dengue, Yellow Fever, several encephalitis and chikungunya (Bhat and Kempraj 2009).Vector control is primordial and very essential means for controlling transmission of filariasis, malaria, Japanese encephalitis and dengue in human society (Ramathilaga *et al*, 2012).

There are five common species of Plasmodium responsible for the transmission of malaria in endemic region of the world, these are: *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale, Plasmodium malariae*and*Plasmodium knowlesi. Plasmodium falciparum* is the most virulent; it is responsible for over 90% of all causes of malaria in sub-Saharan Africa(Enato and Okhamafe, 2005). This is followed by *Plasmodium vivax*while *Plasmodium ovale*and*Plasmodium malariae*cause a generally milder form of malaria that is rarely fatal. The zoonotic species *Plasmodium knowlesi*, prevalent in Southeast Asia, causes malaria in macaques but can also cause severe infections in humans (Enato and Okhamafe 2005).

### 2.2.1Life cycle of mosquito

Mosquito goes through four separate and distinct stages of its life cycle: Egg, Larva, Pupa, and Adult. Each of these stages can be easily recognized by its special appearance (Miller 2012; American Mosquito Control Association 2014).

Egg: Eggs are laid one at a time or attached together to form "rafts." They float on the surface of the water. In the case of *Culex* and *Culiseta* species, the eggs are stuck together in rafts of up to 200. *Anopheles*, *Ochlerotatus* and *Aedes*, as well as many other genera, do not make egg rafts, but lay their eggs singly. *Culex*, *Culiseta*, and *Anopheles* lay their eggs on the water surfacewhile many *Aedes* and *Ochlerotatus* lay their eggs on damp soil that will be flooded by water (American Mosquito Control Association, 2014). Their eggs can survive long periods of drought or cold, before rain or snow-melt comes and washes them into water or

raises water levels to where they are. *Aedes* eggs may require certain conditions before they will hatch, such as a certain day length, a period of freezing, or being wetted and dried several times. This prevents them from hatching in midwinter or too soon in spring, before conditions are right. These kinds of eggs can betransported hundreds of miles before they hatch, and in fact, that is how the Asian tiger mosquito came to the United States from the other side of the globe (Miller 2012).Mosquito eggs are tiny, less than a millimetre long. They begin white, but darken to black, brown or reddish brown within the first day. Most eggs hatch into larvae within 48 hours; others might withstand sub-zero winters before hatching. Water is a necessary part of their habitat (Miller 2012; American Mosquito Control Association 2014).

Larva:The larva (plural - larvae) lives in the water and comes to the surface to breathe. Larvae shed (molt) their skins four times, growing larger after each molt, in between each molt they are called instar hence there are 1st, 2nd, 3rd and 4th instars. They arecommonly called wigglers; because they move in the water by wiggling. Most larvae have siphon tubes for breathing and hang upside down from the water surface. *Anopheles* larvae do not have a siphon and lie parallel to the water surface to get a supply of oxygen through a breathing opening. *Coquillettidia* and *Mansonia* larvae attach to plants to obtain their air supply. The larvae feed on microorganisms and organic matter in the water. During the fourth instar, the larva changes into a pupa; the larvae typically go from egg to pupa in less than a week, though some take a week or more(Miller 2012; American Mosquito Control Association 2014).

Pupa:The pupal stage is a resting, non-feeding stage of development, but pupae are mobile, responding to light changes and moving (tumble) with a flip of their tails towards the bottom or protective areas. This is the time the mosquito changes into an adult. This process is

similar to the metamorphosis seen in butterflies when the butterfly develops, while in the cocoon stage, from a caterpillar into an adult butterfly. For *Culex* species in the southern United States this takes about two days in the summer. When development is complete, the pupal skin splits and the adult mosquito (imago) emerges (American Mosquito Control Association, 2014).

Adult:The newly emerged adult rests on the surface of the water for a short time to allow itself to dry and all its body parts harden. The wings have to spread out and dry properly before it can fly. Blood feeding and mating does not occur for a couple of days after the adults emerge (American Mosquito Control Association, 2014).How long each stage lasts depends on both temperature and species characteristics. For instance, *Culex tarsalis*, a common California (USA) mosquito, might go through its life cycle in 14 days at 70° F and take only 10 days at 80° F. On the other hand, some species have naturally adapted to go through their entire life cycle in as little as four days or as long as one month (American Mosquito Control Association, 2014).

Egg



Larvae

Pupa

Pupae

Adult mosquito

Figure1. Life Cycle of Mosquito(Dawn 1992: Biology of Anophelles Mosquitoes-General Medical Entomology for Students, 4th Edition 33-51)

## 2.3 Malaria: Disease caused by Mosquito bite

Malaria is a potentially lethal disease transmitted by the female Anopheles mosquito (Bello 2005). It is a major public health problem. Malaria is a febrile illness characterized by fever

and related symptoms. It is a parasitic mosquito-borne infection with both acute and chronic phases. It has been one of the most prevalent human diseases affecting particularly the populations of tropical regions and in the past those of temperate climates. Malaria is a complex disease that varies in epidemiology and clinical manifestation in different parts of the world. This variability occurs as a result of several factors such as: the species of malaria parasites that are found in a given area, the distribution and efficiency of mosquito vectors, climate and other environmental conditions, the susceptibility of malarial parasites to commonly used or available antimalarial drugs and the level of acquired immunity by the exposed human population (Enato and Okhamafe 2005; Navy and Marine Corps Public Health Centre 2011).

Regardless of the fact that it is one of the oldest recorded diseases, malaria remains one of the world's most deadly infectious diseases. It is arguably, the greatest menace to modern society in terms of morbidity and mortality. Though preventable, treatable and curable, there is no known immunity. This makes it an efficient and unrepentant killer (Katsayal*et al.*, 2009). Globally, the malaria situation is serious and still deteriorating. It predominantly affects the poor and underprivileged (Singh *et al.*, 2011). Several centuries after its discovery, malaria still remains a devastating human infection, resulting globally in 300-500 million clinical cases with 90% in tropical Africa and three million deaths every year (Enato and Okhamafe 2005). World Health Organization (WHO) reported in 2000 that half of over 2400 million of the world's population (as at that time) were at risk of malaria which was mainly in Africa (Katsayal*et al.*,2009). Olurishe *et al.*,(2007)cited other earlier researchers and stated that annually about 1.2-2.7 million deaths occur as a result of Malaria. Katsayal*et al.,*(2009)reported that more than 500 million people were affected with Malaria and about 2.5million death were reported each year. Despite the combined efforts by 102 countries to eradicate malaria, it remains the major disease in the world today in terms of lives lost and

economic burden. Progress has been made however in some countries. In countries such as United States, eradication of endemic malaria is complete (Etusim *et al.*, 2013). Over 75% of the estimated 2.5 million deaths each year occur in children living in Sub-Saharan Africa. It is a negative factor in the socio-economic development in sub-Saharan Africa. Nigeria bears up to 25% of the malaria disease burden in Africa. In Nigeria over 100 million people are at risk of malaria every year out of which over 300,000 cases lead to death annually, 60 per cent of outpatient visits to health facilities, 30 per cent of childhood deaths and 11 per cent of maternal death. It also aggravates anaemia and malnutrition in children and pregnant women. It is estimated that about 50 percent of the adult population in Nigeria experience at least one episode of the disease yearly, while children of under five years old have up to 2 to 4 attacks of the disease annually, it is    endemic throughout Nigeria (Enato and Okhamafe 2005; Olurishe *et al.*, 2007; Katsayal*et al.*, 2009; Nigeria Malaria Indicator Survey 2010; Okafor and Oko-Ose 2012; Etusim *et al.*, 2013; Odey *et al.*, 2013; Musoke *et al.*, 2013). Malaria continues to ravage much of Africa despite attempts to find a lasting solution to the dreadful and deadly parasitic disease, the majority in sub-Saharan Africa. The governments of African nations have taken the disease condition with a firm grip and have launched various initiatives such as "Roll Back Malaria Program" all in an effort to eradicate the disease which has no viable vaccines. The World Health Organization (WHO) has recommended that combination therapy consisting of different drugs that possess different mechanisms of action be used in areas identified to have high levels of multi- drug resistance. These will usually result in better cure rates and will also delay the development of resistance (Olurishe *et al.*, 2007).

The recent increases in malaria mortality rates in Africa are probably due to increasing resistance to insecticides and antimalarial drugs, breakdown in public-health infrastructure,

and land-use changes such as dam building, irrigation and deforestation (Enato and Okhamafe 2005; Navy and Marine Corps Public Health Center 2011).

Artemisinin-based combination treatments (ACTs) are preferred because artemisinin compounds have rapid parasite and fever clearance effects and also reduce gametocyte rate with the potential to reduce transmission. Artemisinin based combination therapy (ACT) has been advocated as the therapy of choice to handle widespread drug resistance in Plasmodium.In children less than 5 years, a delay in accessing treatment for uncomplicated malaria beyond 48 hours from the onset of symptoms increases fatality hence, the Roll Back Malaria initiative recommends early diagnosis and prompts treatment with an artemisinin-based combination therapy (ACT) within 24 hours of the onset of symptoms. The management of severe malaria remains challenging, mainly due to the fact that it does not only depend on the use of effective anti-malaria drugs but also the use of effective parenteral anti-malaria drugs, it also depends on relatively cost-intensive supportive measures, the availability of highly skilled personnel, functional referral systems, blood transfusion services, good infrastructure, and adequate organization of hospital services. Parenteral quinine had been the first-line treatment for severe malaria in Nigeria until June 2011 when the policy was revised to intravenous artesunate as a first-line antimalarial. The current national treatment guidelines require that the diagnosis of malaria be confirmed parasitologically either by light microscopy or rapid diagnostic test (RDT) in settings where light microscopy is not feasible before treatment with parenteral artesunate (alternatives are either parenteral quinine or artemether), which is to be followed with a full course of the recommended ACT. The Cross River State government in collaboration with development partners has made consistent efforts in the control of malaria through scaling up preventive interventions and case management. Between 2008 and 2010, the proportion of Nigerian children under the age of 5 years with suspected malaria who received treatment within 24

hours with an ACT increased from 7% to 12%. However, a recent report from the study area suggested that several factors were militating against the uptake of the recommended ACTs by children with malaria in the study area. There is limited information on the management practices for severe malaria in children less than 5 years. To understand the current practice, an audit of the management of severe malaria in children under 5 was conducted in public and private facilities in Cross River State, south-south Nigeria (Odey *et al.*, 2013).

### 2.3.1 **The malaria parasite**

Malaria is caused by protozoa of the genus Plasmodium. There are over 150 species of the malaria parasite that infect many species of vertebrates. Each type of protozoa tends to remain within one type of host, such as bird or mammal. Four species infect and rely on humans to sustain malaria transmission: *Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae and Plasmodium ovale.* The protozoa are transmitted to humans by the bite of the female anopheles mosquitoes. *Plasmodia* are also transmitted to humans by direct inoculation of infected red blood cells via transfusion, needles, and congenitally. *Plasmodium knowlesi* has recently been described as a human pathogen. It is known that *Plasmodium knowlesi* differs from the four species of human malaria in that it is a zoonotic infection of long- tailed macaques in Southeast Asia (Katsayal*et al.*, 2009).*Falciparum malaria* is the most dangerous form of the disease resulting in life threatening complication such as anaemia and cerebral malaria. The pattern of exposure to malaria infection, the type of treatment and the degree of compliance with the anti-malaria regimen, local drug resistance patterns, and an individual's age and genetic makeup all tend to influence the severity of the disease (Etusim *et al.*, 2013).

Malaria affects the health and wealth of nations and individuals alike. In Africa today, malaria is understood to be both a disease of the poor and a cause of poverty. Malaria has

significant measurable direct and indirect costs, and has been shown to be a major constraint to economic development. What this mean is that the gap in prosperity between countries with malaria and countries without malaria has become wider every single year. Economic growth has increased substantially where malaria has been eliminated over the following five years. Countries with intensive malaria lagged behind in growth by 1.3% per person per year compared to neighbouring countries; a 10% decrease in malaria incidence was associated with a 0.3% increase in annual economic growth (Enato and Okhamafe 2005). About 300 to 500 million clinical cases of malaria occur each year with 90% of these occurring in tropical Africa - mostly caused by *Plasmodium falciparum*. In Africa, a visit to the paediatric ward or to the general outpatient department of most hospitals is all that is needed to convince a visitor that malaria remains a major cause of mortality and morbidity among African children (Enato and Okhamafe 2005).

Pregnant women are also at high risk of malaria, and malaria is responsible for a substantial number of maternal morbidity, and perinatal morbidity and mortality. In pregnancy, the consequences of malaria are severe both to the mother and her unborn child. Malaria in pregnancy can result in a number of maternal, foetal and infant complications. Important examples are: severe maternal illness, hypoglycaemia, acute pulmonary oedema and anaemia; foetal distress, premature labour, intrauterine growth retardation (IUGR), prematurity, low birth weight infants and stillbirths (Enato and Okhamafe 2005). Malaria contributes to an estimated 8 to 36% of prematurity and to an additional 13 to 70% of intrauterine growth retardation, depending on level of malaria risk and 3 to 85 of all infant deaths have shown that maternal malaria infection accounts for almost 30% of all the causes of low birth weight that can be prevented during pregnancy through antenatal interventions. Maternal parasitized red blood cells and perivillous fibrin deposition were found to be associated independently with increased risk of premature delivery (Etusim *et al.*, 2013).The parasite attacks and

destroys the red blood cell and may affect vital body organs like brain, liver, etc. Splenomegaly occurs in all forms of malaria with repeated attack causing a greatly enlarged spleen. Parasitized cells also lose their deformability and are rapidly phagocytosed and destroyed in the spleen. The production of red blood cells in the bone marrow is also reduced and an immune destruction of the red cells may occur. The epidemicity of malaria is measured by the rate of splenic enlargement and parasite count (Etusim *et al.,* 2013).

### 2.3.2 **Requirement for the prevention of Malaria**

1. Awareness of the risk; there is need to create awareness of the risk of being bitten by Mosquito and to also know that the type of Malaria disease transmitted varies depending on the location and time of the year (Easmon 2013).

2. Bite avoidance; there is the need to use insecticides impregnated mosquito net round beddings or gauze to cover windows and doors. Spray the rooms with insecticides to kill mosquitoes that may be in the room. Proper dresses to protect against mosquito bites are also needful (Easmon 2013).

3. Use Mosquito repellent cream; Mosquito repellent creams are very useful (Easmon 2013).

4. Insecticide-treated mosquito nets; this should be used also when sleeping outdoors or in unscreened rooms, this significantly reduces the risk of bites from Mosquitoes (Easmon 2013).

5. Preventive medicines; Taking medicines to prevent malaria is essential, drugs such as Chloroquine, Doxycycline and Mefloquine. Children, breast feeding babies and mothers are to follow doctor's advice on the right medicine to take to prevent malaria (Easmon 2013).

**2.4 Synthetic Insecticides**

A synthetic insecticide is a poisonous chemical or mixture of chemicals or biological substances that are intended to prevent, repel, or kill targeted organisms (insect). In many cases they are designed to impact the immune, reproductive, or nervous system of insects (Stahl*et al.*, 2002; Abagli and Alavo 2011).

2.4.1**Types of synthetic insecticides**

Four insecticides are commonly used for mosquito control which are:

i. Scourge

ii. Anvil

iii. Permethrin and

iv. Malathion.

Scourge, Anvil and Permethrin are pyrethroid (synthetic) insecticides while Malathion is an organophosphate insecticide (Stahl *et al.*, 2002).

2.4.2 **Side effects of synthetic insecticides**

Scourge (active ingredient: Resmethrin),affect, the nervous system. They have been linked with liver and thyroid problems and they can also interfere with the immune and endocrine systems. Scourge pipernyl butoxide, has been classified by US Environmental Protection Agency (EPA) as a possible human carcinogen (Stahl *et al.*, 2002). Anvil (active ingredient: Sumithrin), this affect the central nervous system (Stahl *et al.*, 2002). Permethrin is a neurotoxin. It is more acutely toxic to children than to adults. EPA has classified it as a human carcinogen and it has been shown to cause immune system damage as well as birth defects (Stahl *et al.*, 2002). Malathion is an organophosphate insecticide that can cause acute and long-term neurological health problems. It is toxic to fish and highly toxic to aquatic invertebrates and amphibians. Malathion affects the nervous system by inhibiting the enzyme

acetylcholinesterase (AChE) which breaks down acetylcholine a chemical essential in transmitting nerve impulses across junctions between nerves. Without functioning AchE, acetylcholine accumulates to produce rapid twitching of voluntary muscles, incoordination, convulsions, paralysis and ultimately death. Acute toxicity reactions in humans include headaches, nausea, blurred vision and pupil constriction, slowed heartbeat, respiratory depression, paralysis, coma as well as muscular damage (after inhalation). Birth defects, reproductive problems and genetic damage have been associated with Malathion exposure in humans and animals. Furthermore, Malathion has the potential to contaminate ground and surface water (Stahl *et al.*, 2002).

2.4.3 **Toxicity of synthetic insecticides**

Chemical repellents are important in protecting people from blood-feeding insects, ticks, mites, and other arthropods and may therefore also reduce transmission of arthropod-borne diseases. N,N-diethyl-3-methylbenzamide (DEET) is one of the most well-known arthropod repellents and has been in the market for almost half a century (Abagli and Alavo 2011). Insecticides (or pesticides as the case may be in agriculture) are used to kill insects, weeds, or fungi. However, synthetic insecticides present hazardous impacts far beyond their intended targets. The continuous use of synthetic insecticides causes side effects to non-target organisms and insecticide resistance against mosquitoes (Stahl *et al*, 2002; Alouani *et al.*, 2009; Arivoli and Samuel 2011; Dua *et al.*, 2013).Insecticides have inherent toxicity because they are designed to kill living organisms that are considered "pests", that is any unwanted insect. Many insecticides are known to pose significant, acknowledged health risks to people—including birth defects, damage to the nervous system; disruption of hormones and endocrine systems; respiratory disorders; skin and eye irritations; immune system suppression, reproductive damage (testicular cancer and low sperm counts in men) and various types of cancers(Dua *et al.*, 2013).

The synthetic insecticide DDT was widely used in urban aerial sprays to control urban mosquito, gypsy moth, Japanese beetle and other insects in the 1940's. By 1972, due to widespread development to DDT, it was banned in the United State there was also evidence that it was increasing preterm births and also harming the environment. It was found to cause behavioural anomalies and eggshell thinning in populations of bald eagles and peregrine falcons. Although the use of DDT was banned in the US and many other countries, it continues to be manufactured and applied in underdeveloped nations where some of the US food supply is grown.

DEET is effective against many different blood-sucking arthropods. The protection efficacy depends on the type of formulation, application pattern, species, and feeding behaviour of the arthropod. DEET is generally safe for topical use if applied as recommended, although adverse effects such as serious neurologic effects have been reported. Many people consider that DEET and related compounds are a health and environmental hazard. DEET does not readily degrade by hydrolysis at environmental pHs and has been identified as an ubiquitous pollutant in aquatic ecosystems. Concern about the deleterious effects associated with synthetic chemicals has revived interest to explore plants as a source of natural insecticides, acaricides, and repellents for medical, veterinary and crop protection use (Abagli and Alavo 2011). During the last 50 years, worldwide use of synthetic insecticides to control insect pests has led to both insecticide resistance and environmental persistence. Total dependence on synthetic insecticides for vector control is being reviewed due to this increasing tendency towards insecticide resistance by vectors and environmental side effects (Thakur *et al.*, 2004; Kumar and Maneemegalai 2008; Ramya*et al.*, 2008; Bhat and Khanal 2009; Mgbemena 2010; Din 2011; Taha*et al.*, 2011; Ramathilaga *et al.*, 2012; Dua *et al.*, 2013). The mishandling of synthetic chemicals in public health programs has caused many problems to human beings as well as the environment (Din *et al.*, 2011; Ravi*et al.*, 2012).

2.4.4 **Remedy**

To eliminate the problems of synthetic insecticides, major consideration has been on the use of natural plant based products as larvicides which can provide an alternative to synthetic insecticides (Din *et al.*, 2011). Synthetic insecticides suffer disadvantages of residual toxicity, contamination of foods and development of resistance by pest species as enumerated under toxicity (Rajamma*et al.*, 2010). In Africa, most of our agricultural produce is produced by poor resource farmers who cannot easily afford the cost of safer synthetic insecticides. As stated earlier, the use of synthetic insecticides against mosquitoes has a lot of problems. It has therefore become necessary to search for other alternatives such as inert dusts and botanical insecticides, which are environmentally friendly and cost effective that can eradicate or reduce cases of malaria (Obeng-Ofori 2010). Therefore, in recent years there has been a revival of interest in plant derived insecticides (Rajamma*et al.*, 2010).

**2.5Larvicides**

Larviciding is a successful way of reducingmosquitodensities in their breeding places before they emerge into adults. Larviciding largely depends on the use of synthetic chemical insecticides—organophosphates (e.g. temephos, fenthion), insect growth regulators (e.g. diflubenzuron, methoprene), etc. Although effective, their repeated use has disrupted natural biological control systems and sometimes resulting in the widespread development of resistance (Tiwarya*et al.*, 2007).

**2.6Biological(Non-synthetic) Insecticides**

Biological control is a method which uses biotic agents that are toxic or lethal to target insects. In view of the increasing resistance of mosquitoes to chemical insecticides and the lack of new alternative methods to control mosquitoes, insecticides of plant origin are gaining more popularity in this context, particularly due to their environmental feasibility. Biological control agents can adapt to mosquito breeding habitats and pose no danger to people (Ramathilaga*et al.*, 2012). The increasing resistance of malaria parasites and mosquitoes to available drugs and insecticides respectively has created a growing interest in herbal remedies in the hope of identifying new leads for antimalarial and repellent drug development.

Plants are known to produce secondary metabolites that are found to be physiologically active and have been used for medicinal purposes for centuries (Traoré-Coulibaly*et al.*, 2013). Botanical insecticides have long been touted as alternatives to synthetic chemical insecticides for pest management because they pose little threat to the environment and human health (Umedum *et al.*, 2012).

### 2.6.1*Hyptis suaveolens* **(Labiatae Poits)**

In developing countries, Lamiaceae have traditionally been used for their insecticidal and repellent properties against several insects' species. Most of them belong to the *Hyptis* genus that includes more than 400 species that grow in the tropical regions of the world, mainly in Africa and America and are highly aromatic plants (Shenoy *et al.*, 2009; Benelli *et al.*, 2012).

*Hyptis suaveolens* (L.Poit) is one of the aromatic and odoriferous important traditional plants belonging to the family lamiaceae, an ethno-botanically important medicinal plant. The plant has been considered as an obnoxious weed. It is a brushy erect plant with fragrant hairy cordate(Gavani and Paarakh 2008;Shenoy *et al.*, 2009;Moreira *et al.*, 2010; Singh *et al.*, 2011; Malar*et al.*, 2012; Agarwal *et al.*, 2013; Noudogbessi *et al.*, 2013; Islam and Noguchi 2013; Islam *et al.*, 2014; Umedum *et al.*, 2014). *Hyptis suaveolens* is a medium aromatic

shrub found in the tropics and subtropics, and distributed as the aggressive annual weedy species in the northern part of Thailand.*Hyptis suaveolens* popularly known in Brazil for "alfazema"is a fast-growing perennial herb found in dense clumps along roadsides, in over-grazed pastures and around stockyards in the tropics. The plant gives off a characteristic minty smell when crushed. The plant is native to tropical America but now distributed throughout the whole world from tropical to subtropical regions and, therefore, the plant is sometimes regarded as pan-tropical, it is a soft suffrutescent and ruderal weed that normally grows along the roadsides and the wet margins of ponds(Gavani and Paarakh 2008;Shenoy *et al.*, 2009;Moreira *et al.*, 2010; Singh *et al.*, 2011; Malar*et al.*, 2012; Agarwal and Varma 2013; Noudogbessi *et al.*, 2013; Islam and Noguchi 2013; Islam *et al.*, 2014; Umedum *et al*., 2014). It is found in French, Guiana, Brazil, Venezuela, Ecuador in Southern America; United States in North America; Bangladesh, China and North EastIndia in Asia; Deccan Peninsula, Andaman and Nicobar Island, Philippines, Benin, Kenya, Nigeria, Sudan, and Cameroon in Africa.The stems of the plant are four-angled, velvety, having long hairs and gland dots and can reach a height of 2 m. The leaves are opposite and ovateor obovate, about 2.5 to 10cm longand 2-4 cm widewith serrulate margins and a long petiole up to 3 cm long. Leaves are often purple tinged particularly on the margin, the lower surface bears hairs. Flowering starts in it at an early age of two to three months. It produces copious blue flowers in small cymes along branch that ends with reduced leaves. Calyx is hairy in nature and is nearly 5 mm long in flower while it enlarges to 10 mm long in fruit and become ribbed. The flowers are auxiliary with long stalk, hairy calyx and about 4mm long. They are often dark purple and glandular. The corolla is two-lipped, mauve with dark purple lines at the base of the broad two-lobed upper lip. The seeds are flat and mucilaginous (Raizada, 2006; Mandal*et al.*, 2007; Gavani and Paarakh 2008;Shenoy *et al.*, 2009;Moreira *et al.*, 2010; Singh *et al.*, 2011; Shaikat *et al*., 2012; Iwalokun*et al.*, 2012; Malar*et al.,* 2012;Agarwal and Varma 2013;

Umedum *et al*., 2014; Islam *etal.*, 2014). It is commonly called Bush mint, Bush tea, Pignut, or Chan. It is known generally as vilayati tulsi in hindi; konda thulasi in Telugu; bhustrena in Sanskrit; daddoya-ta-daji (family name) and specifically sarakuwan soro in Hausa; efiri (family name) in Yoruba; nchuanwu (family name) in Ibo; and tanmotswangi-eba in Nupe (Mandal *et al.*, 2007; Shenoy *et al.*, 2009;Malar*et al.*, 2012; Agarwal and Varma 2013; Umedum *et al*., 2014; Ghafari *et al*., 2014).

In several studies *Hyptis suaveolens*essential oil (EO) has shown useful insecticidal properties against many foodstuff pests. *Hyptis suaveolens* EO has shown a toxic activity against *Plutella xylostella* (L.) (*Lepidoptera Plutellidae*) larvae and *Callosobruchus maculatus* (F.) (*Coleoptera Bruchidae*) adults. In recent studies, it was reported that *Hyptis suaveolens*EO had a marked toxic and repellent activity against adults of both*S. granarius* and *S. zeamais* (Benelli *et al.*, 2012). Research conducted the use of *Hyptis suaveolens*for protection against mosquito bites has shown that it is as effective as DEET (N, N-dimethyl-3-methyl benzamide), one of the well-known arthropod repellents. The ability of *Hyptis suaveolens* to act as an effective insecticide or pesticide has been attributed to its essential oils. However, it is advised that in cases where it has been employed by method of mixed cropping, caution should be applied since *Hyptis suaveolens* is a fairly prolific plant and may compete with crops for space, water and nutrients (Umedum *et al.*, 2012).

### 2.6.1.1*Composition ofHyptis suaveolens*

The major compositions of *Hyptissuaveolens*are 1, 8- cineole, sabinene (34%), β-caryophyllene (11.2%), limonene (5.8%), 4-terpineol (2.5%), terpinolene (10.7%), β-pinene (8.2%), and β-phellandrene depending on the geographic factors of plant collection (Okonogi*et al.,* 2005, Singh *et al.*, 2011; Benelli *et al.*, 2012). Noudogbessi *et al.*, 2013 reported that essential oil from *Hyptis suaveolens* contains 1, 8- cineole (32.0%) and β-

caryophyllene (29.0%) in Australia (1997), same authors reported that in Nigeria (1999) the constituent of *Hyptis suaveolens* essential oil are; sabinene (16.5%), trans-α-bergamortene (13.6%), terpinen-4-ol (9.6%), α-pinene (8.5%), β-caryophyllene (6.2%), caryophyllene oxide (4.5%). Same authors reported that in Cameroon (2005) high presences of sabinene (20.6%), β-caryophyllene (17.5%)and bergamotol (10.9%) were found (Noudogbessi,*et. al*., 2013). In Thailand the following were observed; sabinene (25.4%), terpinolene (13.48%), β-caryophyllene (1.69%), 1, 8- cineole (9.94%), β-pinene (6.72%), limonene 5.89(%) (Noudogbessi *et al.*, 2013). In Ivory Coast; germacrene-D (25.4%), benzyl benzoate (13.6 to 30.2%), diterpene hydrocarbon (7.9 to 16.8%), palustrol (8 to 16.2%), 1, 8- cineole (11.9%) were obtained (Noudogbessi *et al.*, 2013). In 2012 from Senegal to Dakar; β-caryophyllene (17.4%), sabinene (10.6%), sclarlene (9.7%), terpinolene (9.4%) and β-pinene (6.5%) are the composition (Noudogbessi *et al.*, 2013). In Brazil Martins *et al.*, 2007 identified large proportion of sparthulenol (8.43 to 24.70%), globulol (6.89 to 14.55%), dehydroabietol (3.84 to 11.80%), α-cadinol (4.25 to 8.05%)and β-phellandrene (2.99 to 4.40%) (Noudogbessi *et al*, 2013). According to Benelli *et al.,* 2012, Monoterpene hydrocarbons were the most represented chemical class amounting to 67.4%, followed by sesquiterpene hydrocarbons which represent 16.4%. Oxygenated terpenes were lesser represented, reaching only 10.0%, that is, 6.7% and 3.3% for monoterpenes and sesquiterpenes, respectively (Benelli *et al.,* 2012). The leaves of the plant have been shown to contain alkaloids, terpenes and volatile oils (Shaikat *et al*., 2012). *Hyptis suaveolens* has been reported to contain basic food nutrients (chemical components) such as: protein (10.00-14.22%), carbohydrates (66.61-75.05%), fats (2.00-4.46%) and fibre (5.15-9.04%). The high content of carbohydrate show that it is a good source of energy and can help in the oxidation of fats. A diet rich in fibre is desirable because fibre has a physiological effect on the gastrointestinal function. It also has a biochemical effect on the absorption and re-absorption of bile acids and consequently the absorption of

dietary fats and cholesterol. Thus it can serve as source of nutritional dietary supplements. Analysis of the protein composition of the seeds showed the presence of globulins (39%), glutelins (36%), albumins (24%) and prolamins (1%). The content of branched amino acids is higher in *Hyptis suaveolens*than in maize and other cereals. Thus, it could provide a good supply of almost all the essential amino acids for different age groups. This medicinal plant therefore, has great potential for benefitting people in countries suffering from poverty and malnutrition. Though there has not been any report on the extensive use of this plant as food, its use in Asian food recipes as an appetizer due to the presence of its essential oil has been reported. It therefore serves as an edible aromatic flavouring for food. *Hyptis suaveolens*has phytonutrients such as alkaloids, tannins, saponins, volatile oils, flavonoids and terpenoids these are responsible for its medicinal properties (Shenoy *et al.*, 2009; Shaikat *et al*., 2012; Umedum *et al*., 2014; Ghafari *et al*., 2014). The plant is also rich in some mineral elements like potassium (K), calcium (Ca), magnesium (Mg), nitrogen (N), sodium (Na) and phosphorus (P) (Umedum *et al*., 2014). Due to the presence of these chemical substances, the plant has been reported to possess antioxidant, anti-inflammatory, antimicrobial, anti-diarrhoeal, anthelmintic, anti-diabetic, anti- cancerous, antinociceptive, antiulcer, antibacterial activities wound-healing and insecticidal properties. So far, there has not been any review from literature on the efficacy of this plant in all dimensions (Proc. WOCMAP 111. 2005; Shaikat *et al.*, 2012; Umedum *et al*., 2014; Ghafari *et al*., 2014). It has been observed that the chemical composition of essential oil extracted from *Hyptis suaveolens* fresh leaves showed several differences with respect to previous studies (Benelli *et al*., 2012).

2.6.1.2.*Uses ofHyptis suaveolens*

The genus *Hyptis* is known to be used for traditional medicine as an anticancer and antifertility agent in females and for the treatment of gastrointestinal infections, cramp, and pain such as stomach ache, as well as in the treatment of skin infection (Okonogi*et al.*, 2005; Raizada 2006; Mandal *et al.*, 2007; Shenoy *et al.*, 2009;Moreira *et al.*, 2010; Malar*et al.*, 2012; Noudogbessi *et al.*, 2013; Islam *et al.*, 2014). It is applied as appetizer in Asian food recipe because of its essential oil flavour (Nantitanon *et al.*, 2007). Some previous works has shown the compositions, antifungal, antibacterial and anticonvulsant activities of *Hyptissuaveolens* leaf oil (Okonogi*et al.*, 2005), in addition to the general traditional medicinal use of the genus *Hyptis*. It is also popularly used in the treatment of respiratory, indigestion, wounds, sudorific, galactogogue, infection of uterus, parasitic skin diseases catarrhal condition,colds andfever. The aqueous extract of the leaves has showed an antinociceptive effect and acute toxicity (Mandal*et al.*, 2007; Gavani and Paarakh 2008;Shenoy *et al.*, 2009;Moreira *et al.*, 2010; Malar*et al.*, 2012). Different parts of the plant have been used by traditional healers in the treatment of various ailments and disease conditions. In the northern part of Nigeria, a decoction of the leaves is used for treating boils, eczema and diabetes mellitus. Crushed leaves are applied on the forehead to treat headaches. Infusion made from the leaves and the inflorescence is used as stimulant, carminative, diuretic and antipyretic drugs. A decoction of the whole plant is also used to alleviate diarrhoea and various kidney ailments(Umedum*et al.*, 2014). In southern Nigeria particularly Lagos it is used for the treatment of fever, catarrh and cold, stomach ache, worm problem, cramps, convulsion, diabetes and skin diseases (Nantitanon *et al.*, 2007; Iwalokun*et al.,* 2012).Leaves of *Hyptis suaveolens* have been traditionally used as a stimulant, antispasmodic and against colds and diarrhoea (Shaikat *et al.*, 2012).*Hyptis suaveolens* leaves has been and still in use traditionally to repel mosquitoes by burning the leaves or placing branches or whole plants inside houses (Singh *et al.*, 2011; Vongsombath*et al.*, 2012)though not

scientifically proven. In West Africa farmers traditionally introduce *Hyptis suaveolens*leaves in their granaries for the protection of cowpea seeds against bruchids damages (Benelli *et al.*, 2012).

In Benin, *Hyptis suaveolens* leaves is in use traditionally to control certain diseases such as jaundice, hyperthermia, breast abscesses, haemorrhoids, anal and oral candidiasis and generalised edema (Noudogbessi *et al.*, 2013).

Leaves and seeds of *Hyptis suaveolens* have been shown to contain mineral elements which are very important in human nutrition. These minerals which include calcium, potassium, magnesium, nitrogen, and sodium, are required for repair of worn out cells, strong bones and teeth in humans, building of red blood cells and for body mechanisms. The presence of these minerals shows that the plant can be used as supplement in diet for calcium and potassium. Also, *Hyptis suaveolens* has been shown to contain other metals like zinc, copper and iron. Zinc plays a vital role in growth, aids the catalytic and regulatory action of more than 300 enzymes and helps to maintain a healthy immune system. Copper plays an important role in a wide range of physiological processes in the body which include iron utilization, elimination of free radicals, development of bone, and production of the skin and hair pigment called melanin. Iron is used at the active site of many redox enzymes associated with cellular respiration, oxidation and reduction in plants and animals, and also plays a vital role in forming complexes with molecular oxygen in haemoglobin and myoglobin (Umedum*et al.*, 2014).Several laboratory studies have also been carried out on *Hyptis suaveolens* and the leaf extracts of plant have been found to display anti-malarial, anti-bacterial, larvicidal, insecticidal, nematocidal, antioxidant, anticonvulsant and fungi-toxic activity (Iwalokun*et al.*, 2012).

## 2.7 Essential Oils

Essential oils are volatile natural complex secondarymetabolites characterized by a strong odour and have agenerally lower density than that of water (Arun *et al.*, 2009). They are natural volatile mixtures of hydrocarbons with a diversity of functional groups, and their repellent activity has been linked to the presence of monoterpenes and sesquiterpenes (Moreira*et al.*, 2010; Chaubey 2012; Traoré-Coulibaly*et al.*, 2013). Essential oils are plant products obtained by hydro-distillation or other methods (Luts *et al.*, 2014). This complex of compounds are produced by plants, giving them their characteristic smell and taste, and are usually composed of 20–80 or more substances. Their main components are monoterpenes (C10) and sesquiterpenes (C15) derived from isoprene. Although there are exceptions, most monoterpenes present in essential oils have very low toxicity to mammals and are rapidly degraded in the environment. These characteristics and the discovery of the insecticide and repellent effects of some monoterpenes have awoken great interest in the possibility of using these compounds as alternative tools for insect control and generally pest control. Several monoterpenes have been reported as insect repellents (Luts *et al.*, 2014). There are 17,500 aromaticplant species among higher plants andapproximately 3,000 essential oils are known out of which300 are commercially important for pharmaceuticals,cosmetics and perfume industries. Apart from insecticidal potential they are lipophilic in nature and interfere with basicmetabolic, biochemical, physiological and behavioural functions of insects. Genera capable ofelaborating the compounds that constitute essential oilsare distributed in a limited number of families, such asMyrtaceae, Lauraceae, Rutaceae, Lamiaceae,Asteraceae, Apiaceae, Cupressaceae, Poaceae,Zingiberaceae and Piperaceae.Essential oil compounds and their derivatives are considered to be an alternative means of controllingmany harmful insects and their rapid degradation in theenvironment have increased specificity that favoursbeneficial insects (Arun *et al.*, 2009). The activity of aromatic repellent plants is due

to essential oils present in the plant material.They are used as flavour in food products, odorants in flagrances, pharmaceuticals (antimicrobial,) and as insecticides(Moreira*et al.*, 2010; Chaubey 2012; Traoré-Coulibaly*et al*., 2013). Essential oils of various plants have been identified as potential sources of insecticides which can be evolved into commercial formulations (Bhat and Khanal 2009; Ramathilaga *et al*., 2012).A large number of essential oils are known to behave as insecticides/ovicides, attractants/repellents, anti-feedants, juvenile hormone analogues, etc. This has necessitated the need for research and development of environmentally safe, biodegradable, low cost, indigenous methods for vector control, which can be used with minimum care by individual and communities in specific situations (Rajamma*et al.*, 2011). Overall, among natural products essential oils are known to exhibit low toxicity to mammals, and the most terpenoids and phenols found in plant essential oils have been approved as flavouring compound in food (Benelli *et al.*, 2012). Essential oils have received much attention as potentially useful bioactive compounds against insects showing a broad spectrum of activity against insects, low mammalian toxicity and degrading rapidly in the environment (Tiwarya*et al.*, 2007)

## 2.8Monoterpenes

Monoterpenes are compounds of two isoprene units, which are ten carbon atoms and sixteen hydrogen atoms per molecule, molecular weight 136 amu. There are an estimated 2,000 varieties of monoterpenes. Monoterpenes are found in most essential oils: Galbanum (80%), Angelica (73%), Hyssop ((70%), Rose of Sharon (54%), Peppermint (45%), Juniper (42%), Frankincense (40%), Spruce (38%), Pine (30%), Cypress (28%), and Myrtle (25%). While offering a variety of healing properties, the most important ability of the monoterpenes is that they can reprogram miswritten information in the cellular memory. With improper coding in the DNA, cells malfunction and diseases result, including lethal ones such as cancer(Park *et al*., 2005). Monoterpenes may interfere with basic behavioural functions of

insects. Other exhibit acute toxicity while others repellent, anti-feedant or disrupt on growth and developments or reproduction, and or interfere with physiological or biochemical processes (Ibrahim*et al*., 2010).

## 2.9Sesquiterpenes

Sesquiterpenes are compounds of three isoprene units, with fifteen carbons and twenty-four hydrogens per molecule, molecular weight 204 amu. There are more than 10,000 kinds of sesquiterpenes. Sesquiterpenes are the principal constituents of Cedar-wood (98%), Vetiver (97%), Spikenard (93%), Sandalwood (Aloes) 90%, Black Pepper (74%), Patchouli (71%), Myrrh (62%), and Ginger (59%). They are also found in Galbanum, Onycha, and Frankincense (8%). Sesquiterpene molecules deliver oxygen molecules to cells, like haemoglobin does in the blood. Sesquiterpenes can also erase or deprogram miswritten codes in the DNA. Sesquiterpenes are thought to be especially effective in fighting cancer because the root problem with a cancer cell is that it contains misinformation, and sesquiterpenes can erase that garbled information. At the same time the oxygen carried by sesquiterpene molecules creates an environment where cancer cells can't reproduce. Hence, sesquiterpenes deliver cancer cells a double punch-one that disables their coded misbehaviour and a second that stops their growth. The American Medical Association (AMA) has said that if they could find an agent that would pass the blood-brain barrier, they would be able to find cures for ailments such as Lou Gehrig's disease, multiple sclerosis, Alzheimer's disease, and Parkinson's disease. Such agents already exist and have been available since Biblical times. The agents, of course, are essential oils-particularly those containing the brain oxygenating molecules of sesquiterpenes(Park *et al.*, 2005).

## 2.10 Repellents

Repellents are substances applied to the skin, which prevent insects from biting such surface (Traoré-Coulibaly*et al*., 2013).An insect repellent is a substance that causes an organism to move away from the odour source, insects perceive thevolatile repellents by smell (Luts *et al.*, 2014). A mosquito repellent is a substance applied to skin, clothing, or other surfaces which discourages insects from landing or climbing on that surface (Singh *et al.*, 2011).Diethyl benzamine (DEET) which is the gold standard is in use for more than 50 years. In the case of malaria, a safe and effective way of preventing Anopheles female bites is needed (Traoré-Coulibaly*et al*., 2013).The repellent activity of the whole *Hyptis suaveolens* essential oil and single major constituents against adults of *S. granarius* were evaluated. Amongst the several methods available to evaluate the repellence of natural products, the filter paper tests in Petri dish is one of the most commonly used bioassays (Benelli *et al*., 2012). It is well acknowledged that the *Hyptis suaveolens*essential oil chemical composition and biological activity change as a function of the origin and collecting period of the plants. This is a common feature among secondary metabolites and from essential oils of Lamiaceae plants in particular. Several authors reported a large variability in the composition of this family due to genetic, geographical and seasonal factors. Since the biological activities of essential oils are composition-dependent, it is apparent that it is very important to fully characterize these mixtures from the chemical point of view.At the lowest dosage, the following component has ability to repel mosquitoes and other arthropods; sabinene, terpinolene, 4-terpineol, α-pinene and β-pinene (Benelli *et al.*, 2012).It is well acknowledged that insecticidal constituents of many plant extracts and EOs against stored-product insects are mainly monoterpenoids, such as limonene, linalool, terpineol, carvacrol, myrcene and terpinolene. The toxicity of this latter constituent was recognized for several stored product insect pests such as *S. zeamais*, *Tri- bolium castaneum* (Herbs) *(Coleoptera Tenebrionidae),*

*Callosobruchus chinensis L. (Col- eoptera Bruchidae)* and *Sitophilus oryzae L. (Coleoptera Dryophthoridae)*. Moreover, terpinolene possess anti-feeding activity against *Hylobius pales* (Herbst) *(Coleoptera Curculionidae)*and larval growth-inhibiting effects against *Choristoneura occidentalis Freeman (Lepidoptera Tortrici-dae)* (Benelli *et al.*, 2012).

**2.11Mechanism employed by Insectsagainstrepellents(essential oil components).**

If insects are exposed to repellents and other odours, the response of neurons subjected to stimulation depends on the chemical structure of the stimulus, its duration and intensity, previous neuronal experience, and the capacity to adapt to new situations produced by environmental changes or the individual's development. Sensory systems have the ability to adjust their sensitivity to the different intensities of a stimulus. It implies a decrease in the capacity to perceive a stimulus, moving the perception threshold to higher concentrations, or producing a shift in the stimulus–response curve to higher concentrations.A decrease in olfactory responses after exposure to specific odours has been described in moths, exposed to their respective sexual pheromones. It has also been reported for *Drosophila melanogaster* (Meigen) larvae exposed to alcohols, acids, and acetates benz-aldehyde or iso-amyl acetate. In olfactory receptor neurons, the chemical interaction between the odour molecule and the receptor is dynamic and reversible. In a saturated system, molecules are continuously interacting with the receptor. The result of this interaction is that a high amount of calciumenterstheneuron,andthisincreaseof intracellular calcium triggers an alternative signalling pathway that results in proportional responsiveness of the neuron to higher concentrations of the odour. In this way, the neuron (and the whole sensory system) adjusts its responsiveness tohigher intensities of a stimulus that innormal conditions would be indistinguishable. This phenomenoniscalledsensoryadaptation (Luts *et al.*, 2014).

## 2.12 Gas Chromatography-Mass Spectrometry (GC-MS)

GC-MS machine containscolumns. Helium is normally used as carrier gas, temperature programming is set. The sample is injected and the mass spectraare recorded. Total running time taken is recorded as well. Compounds are detected in electronic signal through the column as they separate. The compounds are identified by comparing the retention times of chromatographic peaks using their mass spectra with NIST library to relative retention (NARICT Zaria).A knowledge of the chemical constituents of plants is desirable not only for the discovery of therapeutic agents, but also because such information may be of great value in disclosing new sources of economic phyto-compounds for the synthesis of complex chemical substances and for discovering the actual significance of folkloric remedies (Purushoth*et al.*, 2013). Mass spectrometry, coupled with chromatographic separations such as Gas chromatography (GC/MS) is normally used for direct analysis of components existing in traditional medicines and medicinal plants (Purushoth*et al.*, 2013).In the last few years gas- chromatography mass-spectrometry has become firmly established as a key technological platform for metabolite profiling in plant, which has proven to be a valuable method for the analysis of non-polar components and volatile essential oil, fatty acids and lipids (Sen and Batra 2012; Purushoth*et al.*, 2013). GCMS based metabolome analysis has profound applications in discovering the mode of action of drugs or herbicides (Sen and Batra 2012). It has been observed that the chemical composition of EO extracted from *Hyptis suaveolens* fresh leaves showed several differences with respect to previous studies. It is well acknowledged that the *Hyptis suaveolens* EO chemical composition and biological activity change as a function of the origin and collecting period of the plants. This is a common feature among secondary metabolites and from essential oils of Lamiaceae plants in particular.Several authors reported a large variability in the composition of this family due to genetic, geographical and seasonal factors (Benelli *et al.*, 2012).

## 2.13 Fourier Transform Infra-Red Spectroscopy (FTIR)

The FTIR measuring principle is a measurement with infrared (IR) light. The scan area of the IR wave length by use of the FTIR measuring principle is large. The principle of FTIR is that the gas to be analysed is led through a cuvette with an IR light source at one end that is sending out scattered IR light, and a modulator that cuts the red IR light into different wave lengths. At the other end of the cuvette is a detector measuring the amount of IR light passing through the cuvette. It is the absorption of light at different wave lengths that is an expression of the concentration of gases to be analysed (Doyle 1991).

The total internal energy of a molecule in a first approximation can be resolved into the sum of rotational, vibrational and electronic energy levels. Infrared (IR) spectroscopy is the study of interactions between matter and electromagnetic (EM) fields in the IR region, in this spectral region, the EM waves mainly couple with the molecular vibrations. In other words, a molecule can be excited to a higher vibrational state by absorbing IR radiation. The probability of a particular IR frequency being absorbed depends on the actual interaction between this frequency and the molecule. In general, a frequency will be strongly absorbed if its photon energy coincides with the vibrational energy levels of the molecule. IR spectroscopy is therefore a very powerful technique which provides fingerprint information on the chemical composition of the sample. FTIR spectrometer is found in most analytical laboratories (Doyle 1991).

# CHAPTER THREE

## 3.0 MATERIALS AND METHODS

### 3.1 Materials

#### 3.1.1 **Plant material from Samaru, Zaria.**

Fresh *Hyptis suaveolens* leaves were used in the experiment for the extraction of essential oil.

#### 3.1.2 **Other materials**

Anopheles' mosquito larvae were identified (by an expert) and gotten from drainage in Samaru along Sokoto Road, some of the larvae were allowed to pupateand transform to the adult mosquito.

#### 3.1.3 **Chemicals and reagents**

All chemicals and reagents used were of analytical grade

#### 3.1.4 **Equipment**

Round bottom flask, water condenser, Erlenmeyer flasks, separating funnel, glass bottles with tight cover, paper tape, Cardboard papers, Aluminium cage with wire gauge, Cage made of Mosquito net with wooden support, plastic containers and petri dishes.

### 3.2Methods

#### 3.2.1Plant material collection and identification

The plants were collected from Samaru area of Zaria, Kaduna State. They were then taken to the herbarium, Department of Biological Science for identification. Voucher specimen number 2020 wasdeposited there. More of the plant leaves were then plucked and taken to Pharmacognosy laboratory for further use (extraction).

### 3.2.2 **Extraction of essential oil from *Hyptis suaveolens* leaves**

*Hyptis suaveolens* (1kg) fresh leaves were washed and placed in round bottom flasks of the hydro-distillation set up with 1liter distilled water. The apparatus was then placed on a heating mantle for 2 hrs. The essential oil was collected as distillate (mixture of essential oil and water). This was transferred to a glass separating funnel and the essential oil separated from the water based on density.Percentage yield of the essential oil extracted was calculated using this formula; $\% \text{yield} = \frac{\text{mass of product}}{\text{Mass of sarting material}} \times 100$

### 3.2.3 **Mosquito breeding**

Some of the collected mosquito larvae were taken to the laboratory washed three to four times and introduced into a clean transparent glass container; the container was placed in a mosquito cage that was prepared for the experiment. Cream cracker biscuit (Beloxxi) powder was sprinkled into the glass container which serves as food for the larvae. The $3^{rd}$ and $4^{th}$ instar larvae were collected and used according to WHO (2005) guidelines for the larvicidal bioassay. For mosquito repellency determination,the larvae were left to pupate, transform into adults stage and remain in that same cage for at least 24 hours before introducing them into another cage for repellency experiment to be carried out. They were left for 24 hours prior to the commencement of the experiment so as to be strong and be sure that whatever behaviour that will be observed will not be due to their fragility.

### 3.2.4 **Determination of mosquitocidal capability of the essential oil**

In this experiment 25 adult mosquitoes each were introduced into a separate mosquito cages and tagged 1-6.Group 1 had no essential oil and therefore served as control, group 2 had 0.2g (equivalent to1 ml), group 3 had 0.4g (equivalent to 2 mls), group 4 had 0.6g (equivalent to 3 mls), group 5 had 0.8g (equivalent to 4 mls)while group 6 had 1.0g (equivalent to 5

mls)essential oil dropped into a container in the cages according to the method described by Bulugahapitiya and Arachchige (2007) for repellency.The mosquitocidal effects of the essential oil were observed and records were taken at the end of 90 minutes in all the groups.

### 3.2.5 Determination of mosquito repellency effect of essential oil from *Hyptis suaveolens* leaves

Experiment one (1)

This was carried out by the modified method of (Bulugahapitiya and Arachchige 2007). Large cages were prepared by introducing 0.02g (1ml) of the essential oil into each cage containing 25 adult mosquitoes. The behaviour of the mosquitoes were then observed and recorded at 30, 40, 60 and 90 minutesrespectively. The control group was set up with pure acetone and no essential oil. The test groups were three; group one was carried out in mosquito cage that was left uncover- all sides open (ASO), group two one side cover with cardboard paper (OSO) and group three all sides covered with cardboard paper (ASC).

All the above methods were repeated three times.

### 3.2.6 Determination of mosquito repellency effect of essential oil of*Hyptis suaveolens* leaves on rats.

Experiment two (2)

Mosquitoes (25 adults) were introduced into each of the three separate cages grouped I, II and III. Restrained rats (3)were also introduced into each of the cages. The rats had their back hairs shaved off and essential oil (0.1g equivalent to 0.5 ml) was applied on the bare skin of group one (test rats). The other twogroups served as control. Group two (II)had glycerine applied to the shaved skins. Group three (III) had no essential oil or glycerine applied to their

bare skins. The numbers of mosquito bites were counted at the end of 90 minutes by observing red spot on the shaved area.

### 3.2.7 Test for larvicidal effect of the essential oil (WHO, 2005) guidelines

Essential oil concentrations of 6.25, 12.50, 25, 50, 100, and 1000ppm were prepared from1% stock solution (see appendix 1). For each of the above concentrations using 25 larvae in their $3^{rd}$ and $4^{th}$ instar, numbers of dead larvae were counted after 6, 12and 24hours. A control group was set up for the same timing without the essential oil (WHO/CDS/WHOPES/GCDPP/2005.13).

### 3.2.8 Characterization of essential oil

#### 3.2.8.1 *Gas Chromatography-Mass Spectroscopy (GC-MS).*

The essential oil was subjected to GC-MS analysis on a GC-MS instrument with Elite – DB-5M column and the GC-MS solution version 2.53 software. Initially oven temperature was maintained at $60^{o}C$ for 3.50 minutes, and the temperature was gradually increased up to $250^{o}C$ at 10.0/24.0 min and 4.0 µl of sample was injected for analysis. Helium gas 99.995% of purity was used as a carrier gas as well as an eluent. The flow rate of helium gas was set to 0.99 ml/min. The sample injector temperature was maintained at 250º C and the split ratio is 40 throughout the period of experiment. The ionization mass spectroscopic analysis was done with 70 eV. The mass spectra were recorded for the mass range 50-600 m/z for about 24 minutes. Identification of components was based on comparison of their mass spectra with that of the library. As the compounds separated on elution through thecolumn they were detected in electronic signals. As individual compounds eluted from the Gas chromatographic column, they entered the electron ionization detector where they were bombarded with a stream of electrons causing them to break apart into fragments. The fragments were actually

charged ions with a certain mass. The m/z ratio obtained was calibrated from the graph

obtained which was called as the mass spectrum graph which is the fingerprint of the

molecule. The identification of compounds was based on the comparisons of their mass

spectra with NIST Library 2008 WILEY8, FAME. Quantitative determinations were made by

relating respective peak areas to TIC areas from the GC-MS(Purushoth*et al*., 2013;

Mahadkar*et al*., 2013; GCMS analysis, NARICT Zaria; Usman Dan Fodiyo University,

Sokoto).

3.2.8.2 *Fourier Transform Infra-Red (FTIR) Spectroscopic Analysis.*

FTIR relies on the fact that the most molecules absorb light in the infra-red region of the

electromagnetic spectrum. This absorption corresponds specifically to the bonds present in

the molecule. The frequency ranges are measured as wave numbers typically over the range

$4000 - 600$ cm$^{-1}$. The background emission spectrum of the IR source is first recorded,

followed by the emission spectrum of the IR source with the sample in place. The ratio of the

sample spectrum to the background spectrum is directly related to the sample's absorption

spectrum. The resultant absorption spectrum from the bond natural vibration frequencies

indicates the presence of various chemical bonds and functional groups present in the sample.

3.2.8.3 *Determination of Physicochemical Properties*

i. Determination of Iodine Value

For the determination of Iodine Value, Wij's method (Firestone 1994) was used. The

essential oil (0.35g) was weighed accurately into a 250 ml Erlenmeyer flask and 20 ml carbon

tetrachloride ($CCL_4$) solution was added. Another 20 ml of carbon tetrachloride was placed

into another flask to serve as blank. Wij's (25 ml) reagent was added to both flasks. The two

flasks were stoppered; the contents were mixed well by swirling and stored in a dark place at

$25 \pm 5$ °C for 30 minutes. At the end of 30 minutes, 10 ml of 30% potassium iodide solution

and 100 ml of purified water were added to both sample solutions. These were titrated immediately with standard 0.1 N thiosulfate solutions until the yellow colour almost disappears. One(1) ml of starch solution which served as indicator was added and titrated drop-wise with vigorous swirling till the disappearance of the blue starch-iodine colour.The blank was titrated in the same manner.

The equation below was used to calculate the iodine value.

Iodine value $= \frac{(Blt - St) \text{ x M x } 12.69}{\text{weight of sample}}$

12.69 is a constant in the formula, Blt = result obtained from the blank, St = result gotten from the test and M = molarity.

ii.     Determination of Saponification value

Saponification Value determinationwas carried out according to the method described in Lubrizol (2010).One (1)gm of the essential oil was weighed and transferred into a round bottom flask. To the content of the round bottom flasks,20 ml of 0.5 N alcoholic KOH solutions was added. Another flask was set up without the essential oil which served as blank. The two round bottomed flasks were refluxed for 1 hour. After the refluxing, both the round bottomed flasks were allowed to cool. Titration was carried out using 0.5 N HCl with phenolphthalein indicator till the disappearance of pink colour, which indicated the end point. The saponification value was calculated using the formula below.

Saponification value $= \frac{(B - S) \text{ x } 280.5 \text{ x } 0.1}{weight\ of\ sample}$

This was done in triplet.

B = end point value for the blank, S = end point value for test, 280.5 = a constant in the formula and 0.1 = molarity.

iii.  Determination of Acid Value

This was done using the method described in Lubrizol(2010). The test substance, which is the essential oil from the leaves of *Hyptis suaveolens*(0.5 grams),was taken into a 250 ml Erlenmeyer flask.Ethanol (50 ml)was added to the Erlenmeyer flask, the solution was then mixed by gently heating the flask in a water bath until all the essential oil was dissolved. Few drops of phenolphthalein indicator were added to a 20 ml 0.1N KOH aqueous solution in a beaker. This mixture (phenolphthalein indicator and 0.1N KOH) was then titrated against the solution in the Erlenmeyer flask till the colourless solution turns to pink. The amount of KOH used was recorded as the acid value. The above was repeated three times and average result taken.

The equation, Acid value $= \frac{56.10 \ x \ St \ x \ M}{sample \ weight}$ was used to calculate the acid value.

M stands for molarity, St for test result and 56.10 a constant in the formula.

iv.  Determination of Peroxide Value

Peroxide value determination was done as described in Lubrizol (2006). The essential oil (2.00g) was measured into a 100 ml glass stoppered Erlenmeyer flask. Acetic acid - chloroform solution (12 ml) was measured and added to the essential oil. The flask was swirled until the sample was completely dissolved; this was done carefully by warming the flask on a hot plate. Saturated potassium iodide solution (0.2 ml) having a concentration of 0.01mol was then added to the flask, stoppered and contents swirled for exactly 1 minute. Twelve (12) mls of deionized water was added immediately and shaken vigorously, iodine formed in the chloroform layer was liberated.One (1) mL starch solution (as an indicator) was added to 50 mL 0.1N sodium thiosulfate solution in a beaker; this mixture was emptied into a burette and titrated against the contents of the Erlenmeyer flask until the disappearance of

blue gray colour that was in the aqueous upper layer.The whole procedure was repeated without the essential oil which served as the blank for the experiment.The above was done in triplet.Calculation was done using the formula;

$$\text{Peroxide value} = \frac{(V1 - V0) \text{ x M x } 1000}{weight\ of\ sample}$$

V1 is the end point from test, v0 is end point from blank, M is molarity and 1000 a constant.

v.    Determination of Ester Value

This is described in Lubrizol 2010 as, Ester value = saponification value - acids value.

vi.    Determination of Free Fatty Acids Value

The determination of free fatty acids value was done according to the method described in Lubrizol (2010).

$$\text{Free Fatty Acids value } (\%) \quad = \frac{Acid\ value}{1.99}$$

The above formula was used in calculating the free fatty acid value in percentage where 1.99 is a constant.

3.2.8.4 *Statistical analysis*

Standard data analyses were done using Probit analysis and analysis of variance (ANOVA) of statistical package for social sciences (SPSS) version 20.0.Results of difference between groups were expressed as Mean±S.D except otherwise stated. The difference between various treatments groups were compared using the Duncan multiple range test.P values less or equal to 0.05 (p≤0.05) were taken as significant.

# CHAPTER FOUR

## 4.0 RESULTS

### 4.1 Percentage yield of Essential oil from *Hyptis suaveolens* leaves.

The quantity of essential oil obtained from three (3) independent extractions using 1 kg in each case of leaves of *Hyptis suaveolens* is presented in Table 4.1. An average quantity of 0.4-0.5 mls was extracted and the average yield was 0.05%. The oil is a clear liquid with physical appearance of pale yellow.

### 4.2 Mosquitocidal capability of Essential oil from *Hyptis suaveolens* leaves

Essential oil from *Hyptis suaveolens*leaves showed mosquitocidal activity against adult mosquitoes. At the end of 90 minutes observation of mosquitocidal effect, all the mosquitoes in different groups except those in control group were not active. In the first cage, that is, the group with 0.20g (1 ml) essential oil 16% were knockdown (dead) while the remaining 84% were seen resting on the wall, all far from the petri dish where the essential oil was placed. In the group with 0.40g (2 mls) essential oil, 72% were inactive far away from the essential oil while 28% were knockdown (dead). As the concentration increases the percentage of dead mosquitos'increases and the inactive ones in all cases were seen far away from the essential oil. At 0.60g (3mls) 36% were knockdown (dead) and 64% weakened while 40% were knockdown (dead) and 60% weakened mosquitoes were recorded at0.80g (4mls). At 1.00g (5mls), 44% were knockdown (dead) while 56% were weakened (not active). Table 4.2 summarises the mosquitocidal potential of the essential oil.

Table 4.1 Extracted Essential oil from *Hyptis suaveolens* leaves

| Weight of leaves (kg) | Quantity of oil (mls) |
| --- | --- |
| 1 | 0.5 |
| 1 | 0.6 |
| 1 | 0.4 |
| Average quantity extracted (Average yield) | 0.5 |

The percentage yield of essential oil extracted was 0.05.

Table4.2Mosquitocidal potentialsof the essential oil of*Hyptis suaveolens*leaves

| Groups | Essential oil Conc.(g/mls) | Time (mins) | Percentage dead (%) | Percentage inactive (%) |
|--------|----------------------------|-------------|---------------------|-------------------------|
| 1 | 0.00 | 90 | 0 | 0 |
| 2 | 0.20 | 90 | 16 | 84 |
| 3 | 0.40 | 90 | 28 | 72 |
| 4 | 0.60 | 90 | 36 | 64 |
| 5 | 0.80 | 90 | 40 | 60 |
| 6 | 1.00 | 90 | 44 | 56 |

$N = 25$.   $LC_{50} = 6.20$ and $LC_{90} = 21.28$ppm

**4.3 Repellency effect of Essential oil from *Hyptis suaveolens* leaves**

4.3.1 **Mosquito repellent potential of *Hyptis suaveolens* essential oil. Experiment one**

The mosquito repellency effect of *Hyptis suaveolens* essential oil done without covering any side of the mosquito cage with cardboard paper (ASO) this serve as group 1 and is presented in table 4.3. Essential oil from *Hyptis suaveolens* leaves had strong repellency effect against mosquitoes. In this study,0.20g (1 ml) of the essential oil repelled 65.32% of 25 adult mosquitoes within a period of 30 minutes. At 40 minutes 73.32% of the mosquitoes were repelled, at 1 hour post application, 77.32% of the mosquitoes were completely repelled from the source of the essential oil not only were they repelled but were weakenedas they were observed to be resting on the wall of the net far away from the source of the essential oil.

Table 4.5 shows the result of group two (2) conducted with one side (OSO) of the mosquito cage left uncovered with a cardboard paper, at 30 minutes after application, 73.32% of 25 adult mosquitoes were repelled from the source of the oil. The increase in percentage repellency within the same time frame may be due to level of aeration that is less in thissecond group which builds up the concentration of the essential oil considering its volatility.

A third grouprecorded inTable 4.7 was done in a mosquito cage with all side closed (ASC) the repellency effect was higher in this group. At 30 minutes post application 85.32% of the mosquitoes were repelled from the essential oil, at 40 minutes 97.32% were repelled, at 60 minutes, there was 100% (88% repelled and 12% dead) repellency and at the end of 90 minutes, 100% repellency (84% repelled and 16% dead) was recorded also. In all groups, the repelled mosquitoes were weak, unable to fly and resting on the wall of the cage far away from the essential oil.

Control groups

Control were set up for each of the three groups without essential oil but conditions remain unchanged (ASO, OSO and ASC)and in all cases the mosquitoes were actively flying through out, the results are shown in Tables 4.4, 4.6 and 4.8.

Table 4.3Repellency effects of Hyptis suaveolens essential oil for all sides open (ASO).

| Essential oil (g) | Time(Mins) | Active Mean/SD | Percentage Active (%) | Inactive Mean/SD | Percentage Inactive(%) | Mortality (%) |
|---|---|---|---|---|---|---|
| 0.20 | 30 | $8.67\pm1.53^{b}$ | 34.68 | $16.33\pm1.53^{a}$ | 65.32 | 0 |
| 0.20 | 40 | $6.67\pm1.53^{ab}$ | 26.68 | $18.33\pm1.53^{ab}$ | 73.32 | 0 |
| 0.20 | 60 | $5.67\pm1.53^{a}$ | 22.68 | $19.33\pm1.53^{b}$ | 77.32 | 0 |
| 0.20 | 90 | $7.33\pm1.15^{ab}$ | 29.32 | $17.67\pm1.15^{ab}$ | 70.64 | 0 |

Group one (1), N=25.$LC_{50}$ = 0.061 and $LC_{90}$ = 183.078ppm. Means with different letters are statistically different (p≤0.05)

Table 4.4Repellency effects of *Hyptis suaveolens*essential oil of the control for all sides open (ASO).

| Essential oil (g). | Time (Mins.) | Percentage Repellency (%) | Percentage Mortality (%) |
|---|---|---|---|
| 0 | 30 | 0 | 0 |
| 0 | 40 | 0 | 0 |
| 0 | 60 | 0 | 0 |
| 0 | 90 | 0 | 0 |

N= 25

Table 4.5Repellency effect of *Hyptis suaveolens*essential oil for one side left open (OSO).

| Essential oil (g) | Time (Mins) | Active Mean/SD | Percentage Active (%) | Inactive Mean/SD | Percentage Inactive (%) | Mortality Mean/SD | Percentage Mortality (%) |
|---|---|---|---|---|---|---|---|
| 0.20 | 30 | $6.67\pm2.52^b$ | 26.68 | $18.33\pm2.52^a$ | 73.32 | 0 | 0 |
| 0.20 | 40 | $3.00\pm2.65^a$ | 12.00 | $22.00\pm2.65^b$ | 88.00 | 0 | 0 |
| 0.20 | 60 | $0.33\pm0.58^a$ | 1.32 | $23.33\pm0.58^b$ | 93.32 | $1.33\pm0.47$ | 5.32 |
| 0.20 | 90 | $0.00\pm0.00^a$ | 0.00 | $22.67\pm1.15^b$ | 90.68 | $2.33\pm1.33$ | 9.32 |

Group two (2), N =25. $LC_{50}$ = 0.572 and $LC_{90}$ = 2.005ppm.Means with different letters are statistically different $(p\leq0.05)$.

Table 4.6Repellency effect of *Hyptis suaveolens* essential oil of the control for one side open (OSO).

| Essential oil (g). | Time (Mins) | Percentage Repellency (%) | Percentage Mortality (%) |
|---|---|---|---|
| 0 | 30 | 0 | 0 |
| 0 | 40 | 0 | 0 |
| 0 | 60 | 0 | 0 |
| 0 | 90 | 0 | 0 |

N=25

Table 4.7Repellency effect of *Hyptis suaveolens* essential oil for the group with all side closed (ASC).

| Essential oil(g) | Time (Mins) | Active M/SD | Percentage Active (%) | Inactive M/SD | Percentage Inactive (%) | Mortality Mean/SD | Percentage Mortality (%) |
|---|---|---|---|---|---|---|---|
| 0.20 | 30 | $3.67\pm0.58^b$ | 14.68 | $21.33\pm0.58^{ab}$ | 85.32 | 0.00 | 0.00 |
| 0.20 | 40 | $0.67\pm0.58^a$ | 2.68 | $24.33\pm0.58^c$ | 97.32 | 0.00 | 0.00 |
| 0.20 | 60 | $0.00\pm0.00^a$ | 0.00 | $22.00\pm0.00^b$ | 88.00 | $3.00\pm0.00$ | 12.00 |
| 0.20 | 90 | $0.00\pm0.00^a$ | 0.00 | $21.00\pm0.00^a$ | 84.00 | $4.00\pm0.00$ | 16.00 |

Group three (3), N =25.LC50 = 0.511 and LC90 = 1.178ppm. Means with different letters are statistically different ($p \leq 0.05$)

Table 4.8Repellency effect of *Hyptis suaveolens* Essential oil of the control for all sides

closed (ASC).

| Essential oil (g). | Time (mins.) | Percentage Repellency (%) | Percentage Mortality (%) |
|---|---|---|---|
| 0 | 30 | 0 | 0 |
| 0 | 40 | 0 | 0 |
| 0 | 60 | 0 | 0 |
| 0 | 90 | 0 | 0 |

N = 25

4.3.2 **Mosquito repellent potential of *Hyptis suaveolens* essential oil.**

Experiment Two

At the end of 90 minutes there was 100% repellency in the first group where 0.10g (0.5mls) essential oils was rubbed on the shaven dorsal part of the rats, this has been proven by zero number of biteson shaven dorsal part of rats. In the second group with 0.10g (0.5 mls) glycerine on the bare skin there was 46.67% of bites at the end of 90 minutes. In the third group with nothing applied on the bare skin of the rats, 45.33% bites were recorded at the end of 90 minutes. Table 4.9 shows the summary of this experiment.

**4.4 Larvicidal effect of Essential oil from *Hyptis suaveolens*leaves.**

*Hyptis suaveolens* essential oil has shown very strong larvicidal properties against mosquito larvae. There is low mortality rate at lower dosage and not significantly different from each other. At a concentration of 6.25ppm the mortality rate was 4% over a period of 6 hours, same concentration at 12 and 24 hours gave mortality rate of 11%. The $LC_{50}$ and $LC_{90}$ at this concentration were 25.21 and 302.67 respectively and a regression equation of $Y= 818.6x—161.36$. At a concentration of 12.50ppm a mortality rate of 4% was also recorded at 6 hours, at 12 and 24 hours 13% mortality were recorded, same $LC_{50}$, $LC_{90}$ and regression equation with 6.25ppm. At concentration of 25.00ppm, 20%, mortality rate was recorded for 6 hours and 30% for both 12 and 24 hours, the $LC_{50}$, $LC_{90}$ and regression equation are 9.575, 352.458 and $Y=0.7264x+0.2465$ respectively. There was no significant differences at 50, 100 and 1000ppm, the mortality rate were 100% for the period of 6, 12 and 24 hours with $LC_{50}$, $LC_{90}$ and a regression equation of 0.549, 0.853 and $Y=0.7264x+0.2465$ respectively. There was no

record of death in the control even after 24 hours. The summary of the larvicidal properties are recorded in Table 4.10.

Table 4.9Repellency effect of *Hyptis suaveolens* essential oil against mosquito bites

| Substance applied  (g) | Bites | Percentage Bites (%) |
|---|---|---|
| 0.01 essential oil | $0.00\pm0.00^b$ | 0.00 |
| 0.01 glycerine | $11.67\pm1.53^a$ | 46.67 |
| 0.00 essential oil | $11.33\pm2.08^a$ | 45.33 |

Means with different letters are statistically different (p≤0.05)

Table 4.10: Larvicidal effect of the essential oil from *Hyptis suaveolens* leaves.

| Concentrations (ppm). | Time (hours) | Mortality | Percentage Mortality | $LC_{50}$ | $LC_{90}$ | Regression Equation |
|---|---|---|---|---|---|---|
| 6.25 | 6 | $2.33\pm0.52^c$ | 4 | 25.206 | 302.669 | Y = 818.6x—161.36 |
| | 12 | | 11 | | | |
| | 24 | | 11 | | | |
| 12.50 | 6 | $2.33\pm0.52^c$ | 4 | 25.206 | 302.669 | Y = 818.6x—161.36 |
| | 12 | | 13 | | | |
| | 24 | | 13 | | | |
| 25 | 6 | $7.00\pm0.52^b$ | 20 | 9.575 | 352.458 | Y = 1695.8x—360.07 |
| | 12 | | 30 | | | |
| | 24 | | 30 | | | |
| 50 | 6 | $25.00\pm0.52^a$ | 100 | 0.549 | 0.853 | Y=0.7264x+0.2465 |
| | 12 | | 100 | | | |
| | 24 | | 100 | | | |
| 100 | 6 | $25.00\pm0.52^a$ | 100 | 0.549 | 0.853 | Y=0.7264x+0.2465 |
| | 12 | | 100 | | | |
| | 24 | | 100 | | | |
| 1000 | 6 | $25.00\pm0.52^a$ | 100 | 0.549 | 0.853 | Y=0.7264x+0.2465 |
| | 12 | | 100 | | | |
| | 24 | | 100 | | | |
| Control | 6 | $0.00\pm0.00^c$ | 0 | | | |
| | 12 | | 0 | | | |

| 24 | 0 |
|----|---|

Means with different letters are statistically different ($p \leq 0.05$). Larvicidal overall $LC_{50} = 3.57$, $LC_{90} = 13.828$, Overall Regression equation, $y = 0.2868e^{0.1402x}$; Overall $R^2 = 0.9959$

## 4.5 Physicochemical properties of essential oil from *Hyptis suaveolens* leaves.

Essential oil *of Hyptis suaveolens* leaves can be identified using its physicochemical properties which are shown below

   i.   Iodine value

The iodine value is $23.59 \pm 0.12$g/100g as recorded in Table4.11.

   ii.   Saponification value

The saponification value is $100.18 \pm 0.8$mgKOH/g, as seen in Table 4.11

   iii.   Acid value

The acid value is $3.37 \pm 0.01$mgKOH/g, as shownin Table 4.11.

   iv.   Peroxide value

The peroxide value is $40 \pm 0.02$meq/kg, as shown in Table 4.11

   v.   Ester value is $34.30 \pm 1.00$mg/g, as seen in Table 4.11

   vi.   Free Fatty Acid value (%) is $0.15 \pm 0.57$, as seen in Table 4.11

Table 4.11: Physicochemical analysis of the essential oil of *Hyptis suaveolens* leaves

| Physicochemical tests | Composition |
|---|---|
| Iodine Value (g/100g) | 23.59$\pm$0.12 |
| Saponification Value (mgKOH/g) | 100.18$\pm$0.8 |
| Acid Value (mgKOH/g) | 3.37$\pm$0.01 |
| Peroxide Value (meq/kg) | 40.00$\pm$0.02 |
| Ester (mg/g) | 34.30$\pm$1.00 |
| Free Fatty Acid Value (%) | 0.15$\pm$0.57 |

N=3.

**4.6 Gas Chromatography- Mass Spectroscopy (GC-MS)**

4.6.1 **Characterization of the essential oil from *Hyptis suaveolens leaves* by GC-MS**

From the mass spectra of *Hyptis suaveolens* obtained by GC-MS analysis it revealed that the essential oil from the leaves of this plant contains twenty (20) compounds (see appendix 16). The peaks of the compounds can be seen in Figure 4.1.

The most abundant compound is Eucalyptol with peak area of 31.12% and retention time of 7.626. This is followed by Bicyclo[7.2.0]undec-4-ene4,11,11-trimethyl-8-methylene, with a peak area of 17.30% and retention time of 13.483, while 1,3,6,10-Cyclotetradecatetraene, 3,7,11-trimethyl-14-(1-methylethyl) is the least abundant compound with a peak of 0.58% and a retention time of 22.966. From the entire compounds identified by GC-MS the percentage similarity to that in the mass spectra data base was found ranging between 80-97% (see appendix 16).

Table 4.12 showed the break-down of the components in the essential oil from *Hyptis suaveolens*. Terpenes has the highest percentage (46.97) followed by alcohol with 43.41% and other hydrocarbons 9.61%. Terpenes are known to have high insecticidal properties from several literatures.
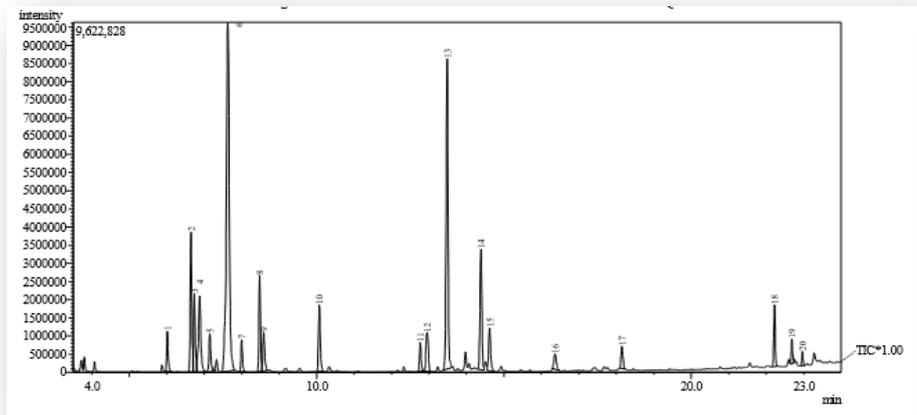
**Figure 2:GC-MS Chromatogram**

Table 4.12Components of the essential oil from *Hyptis suaveolens*leaves by GC-MS

| Compounds | Amount in percentage (%) |
|---|---|
| Terpenes | 46.97 |
| Alcohol | 43.41 |
| Other hydrocarbons | 9.61 |

**4.7 Fourier Transform Infra-Red Spectroscopy (FTIR)**

**4.7.1 Characterization of the larvicidal compound(s) in the bioactive fraction by FTIR**

Characterization of the bioactive fraction of essential oils from *Hyptis suaveolens* (L. Poits) was carried out to determine the types of functional group(s) in the essential oils of *Hyptis suaveolens* fraction. The spectra revealed the presence of fifteen (15) functional groups as shown in Fig. 3.

The peak at 448.46, indicate the presence of a C-I stretch bond Alkyl halides of strong intensity and its derivatives group present. The peak at 1078.24, indicate the presence of a C-O stretch bond Alcohols of medium strong intensity and its derivatives. The peak at 1160.22 and 1299.1, indicate the presence of O=C-O-C stretch bond esters (aliphatic and aromatic compounds respectively) both having strong, very strong intensity. At the peak 1231.59, there is the presence of =C-O-C stretch bond ether of medium strong intensity.

C-H bend is present at the peak 1450.52, Alkenes and Alkyls of strong intensity. At peak 1644.37, an indication of C=O stretch Amides is present with strong broad intensity. At the peaks 2943.47 and 3431.48, an indication of O-H stretch Carboxylic Acids are present with strong, broad intensity. At peaks 3736.24 and 3841.36, an indication of N-H symmetric bond of weak intensity is present.
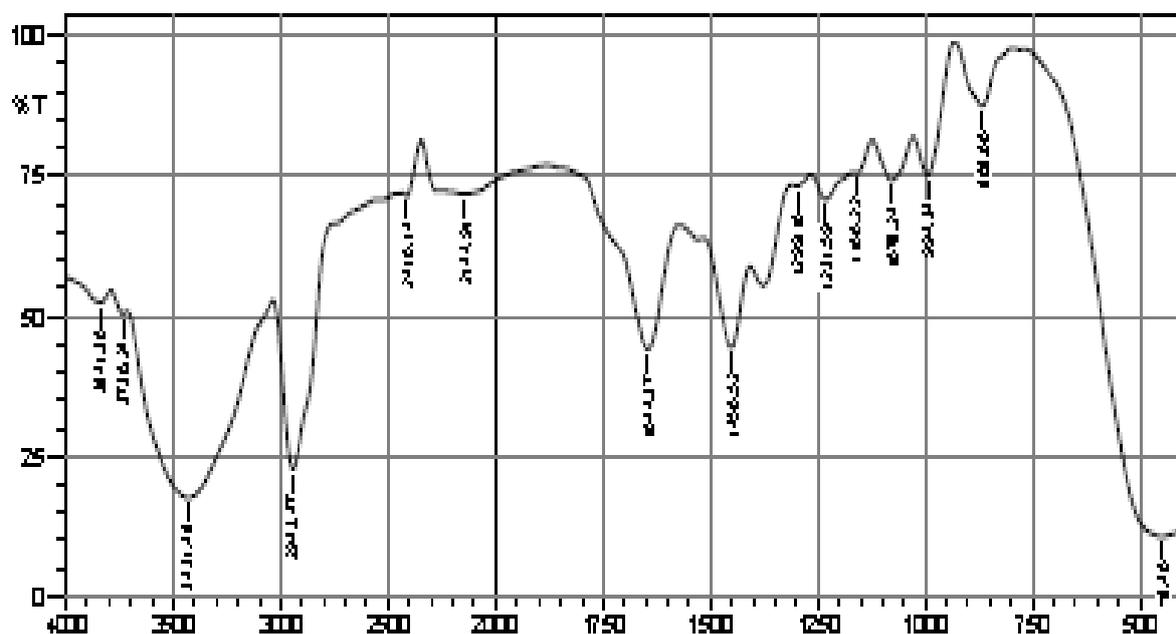
**Figure 3: FTIR chromatogram obtained from essential oil of *Hyptis suaveolens* leaves**

Table 4.13;Functional groups of the components of essential oil from *Hyptis suaveolens* by

FTIR

| PEAK NUM. | ABSORBANCE(nm) | CLASS OF COMPOUND | FUNCTIONAL GROUP | INTENSITY |
|---|---|---|---|---|
| 1 | 448.46 | Alkyl halides (R-I) | C-I stretch | S |
| 4 | 1078.24 | Alcohols(R-CH2- OH) ☐ | C-O Stretch | m-s |
| 5 | 1160.22 | Esters(aliphatic) | O=C-O-C stretch | s, vs |
| 6 | 1231.59 | Ethers(Ar-O-R) | =C-O-C stretch | m-s |
| 7 | 1299.1 | Esters(aromatic) | O=C-O-C stretch | s, vs |
| 8 | 1450.52 | Alkenes and Alkyls | C-H bend | S |
| 9 | 1644.37 | Amides(R-C(O)-NH- R) | C=O stretch | s, broad |
| 12 | 2943.47 | Carboxylic, Acids(R- C(O)-OH) | O-H stretch | s, broad |
| 13 | 3431.48 | Carboxylic Acids(R- | O-H stretch | s, broad |
| 14 | 3736.24 | Amines(R-NH$_2$) | N-H symmetric | W |
| 15 | 3841.36 | Amines(R-NH$_2$) | N-H symmetric | W |

vs-very strong, s- strong, m-medium, w-weak.

**CHAPTER FIVE**

## 5.0 DISCUSSION

The clear liquid oil with physical appearance of pale yellow is same with all previous work.Essential oil yield of about 0.4-0.6ml/kg (equivalent to 0.05%) obtained in this studiesis approximately equivalent with the work of Raizada(2006) whoshowed a percentage yield of 0.1 from the same plant, never the less, it is low compared with the work of the following researchers; Iwalokun *et al.*(2012)with a percentage yield of 0.31 and Okonogi *et al.*(2005)with a percentage yield of 0.21. The difference in percentage yield could be due to the moisture level of the leaves and the chemotypic profile of the *Hyptis suaveolens* strains analysed (Iwalokun *et al.*, 2012). Latitude, altitude, soil composition, climate and genetic composition are factors that have been implicated for chemotype variations in *Hyptis suaveolens* and other species of *Hyptis* as well as other aromatic herbs belonging to the Lamiaceae family (Iwalokun *et al.*, 2012). Various bioactive compounds were recovered from the essential oil samples of this chemotypes with variations in yield, composition and pharmacological effects (Iwalokun *et al.,* 2012).Mandal *et al.* (2007) obtained the following percentage yield from the same plant using several solvent which are steam distillation (yield: 0.24%), petroleum ether extract (yield: 1.6%) and ethanol extract (yield: 2.64%); Bachheti *et al.* (2013) indicated a percentage yield of 17.44 from seed oil and not leaves; Shenoy *et al.* (2009) reported percentage yield of 4.78% for Petroleum ether, 8.52% for Solvent ether, 3.30% for Chloroform and 5.48% for Alcohol; Gavani and Paarakh (2008) also reported a percentage yield of 4.86% for methanol extract. The difference in these studies and the present work could be due to the different solvents used and in one case different part of the plant, apart from the above mentioned factors.

Mosquitocidal capability against adult mosquitoes could be due to the presence of terpenes in the essential oilas revealed by the GC-MS and FTIR. Terpenes are generally known to have insecticidalproperties. Earlier work done by Olotuah (2013)showed that essential oil from *Hyptis suaveolens* leaves has insecticidal activities against several insects. Adda *et al.*(2011) also showed that essential oil from *Hyptis suaveolens* leaves has ovicidal activity against some insects;all these may be due to terpenes found in the plant which has the highest percentage as seen in Table 4.12.

The strong repellent activity against mosquitoes as seen in this work is still connected to the presence of terpenes. This agrees with the work of Benelli *et al.* (2012) where the essential oil repels some insects such as *Sitophilus granarius* which kept distance from source of the essential oil that was applied on whatman filter paper. As expected the repellency effect is concentration dependant, this goes in line with the work of Abagli and Alavo (2011) which showed that essential oil from *Hyptis suaveolens* has strong repellency towards mosquitoes, the higher the concentration the higher the repellency, in their work in a field studies 97% of mosquitoes were repelled within a period of 15 minutes which demonstrated same effectiveness as DEET. Abagli and Alavo (2011)work showed higher repellency effect than this studies, this may be due to the time of the year the leaves were plucked, the location and the solvent used for the extraction(Iwalokun *et al.*, 2012). Its effect persists up to 5 hours post application, similar to this work which shows persistency even 4 hours after application. The overall lethal concentration at 50% ($LC_{50}$) is 3.34ppm and $LC_{90}$ is 0.88ppm. A significant ($\leq$ 0.05) repellent activity with differences in repellency rates is a function of both concentration and observation time, this is in perfect agreement with the work of Conti *et al.*(2011), these authors showed that differences in repellency rates, were as a function of both concentration and observation time.Singh *et al.* (2011) also showed that *Hyptis suaveolens* has strong ability to repel mosquitoes, these authors proved that plant placement, smouldering ( dry and

fresh leaves, which gives 90% repellency), spraying (essential volatile oil) and sticks formation of *Hyptis suaveolens* leaves repels mosquitoes strongly due to the presence of intense pungency of the leaves. In their study they were able to prove that *Hyptis suaveolens* is an effective plant to repel mosquito as well as various insects.

Strong larvicidal properties against mosquito larvae due to the presence of terpenes are concentration dependant. Lower concentrations gave lower mortality rates and not significantly ($\leq 0.05$) different from each other, at higher concentrations mortality rates was higher and there were no significant ($\leq 0.05$) differences between higher concentrations. These are further confirmed by giving same $LC_{50}$ and $LC_{90}$ for lower concentration (25.21and 302.67 respectively for both 6.25 and 12.50ppm) and same $LC_{50}$ and $LC_{90}$ for higher concentrations(0.549 and 0.853 respectively for 50-1000ppm). The work of Conti *et al.*(2011) also showed that there are no significant differences between lower concentrations, at dosages ranging from 250 to 350 ppm; mortality rates were lower and not significantly different from each other.Arivoli and Samuel(2011) also showed that essential oil of *Hyptis suaveolens* has larvicidal activities against mosquito larvae, which is concentration dependant as was seen in this research work. There was no record of death in the control even after 24 hours.

Iodine value is determine by the degree of unsaturation of oils and fats and hence tendency of a fatty oil to absorboxygen. The higher the iodine value the higher the degree of unsaturation and the likelihood for the oil to go rancid. Oils with low iodine value are good for soap-makingthey are referred to as non-drying oil (STANDS4 LLC, 2015).The higher the iodine value the more reactive, less stable, softer, and more susceptible to oxidation and rancidification is the oil, fat, or wax. Iodine value of 23.59$\pm$0.12g/100gobtained from this

study indicates that the oil can be used for soap-making. Oils with higher iodine values are not good for soap-making.

Saponification values are highly significant to consider in soap making. It is important that the saponification value is high enough (not too much alkali) but to contain sufficient soapiness, low saponification value means small fatty acid salts which may not be sufficient enough to remove or saponify the fat or oil due to less soapiness.Saponification value of $100.18\pm0.8$mgKOH/g was obtained from this study which is high enough (good) for soap-making. The result is similar to that of Umedum *et al.* (2014) and Bachheti*et al.*(2013) who both obtained saponification value of over 100mgKOH/g.

Acid value is the amount of KOH or NaOH required in milligram to neutralize one gram of oil. Oils with low acid value are better in making soap. The acid value of $3.37\pm0.01$mgKOH/g obtained from this study make it good oil that can be used on the skin. This value corresponds with the work of Umedum *et al.* (2014) who recorded $3.3\pm0.01$mgKOH/g and Bachheti *et al.* (2013) who also recorded an acid value of 3.3mgKOH/g.

Peroxide value shows how fats and oil can undergo autoxidation; it shows the extent of the spoilage of oil. Peroxides are intermediates in autoxidation of oil; this shows that high peroxide value shows the extent of oil spoilage. A peroxide value of $40\pm0.02$ meq/kg shows that the oil is good for use either on the body or for consumption.

Free Fatty Acids value (%) is a shelf life indicator test for hydrolytic rancidity, free fatty acid value (%)of $0.15\pm0.57$is ideal for use either on body or consumption.

Esterswithlowmolecularweights,suchasethylacetate,areusuallyvolatilefragrantliquids;fatsareso lidesterswithpleasantaromas (The American Heritage Science Dictionary, 2002).An ester

value of 34.30±1.0mg/g was obtained in this study. To the best of my knowledge there is no much information on ester value.

Looking at the physicochemical properties of the essential oil from *Hyptis suaveolens* leaves, the oil can be made into a stick or any other form (essential oil, wood flour and starch as a binder) to be burnt as shown by Singh et al.(2011) which gave 60% repellency to mosquito. The oil can also be formulated into cream (Okonogi *et al*., 2005) or perfumes to be used to keep mosquitoes away.

The result of GC-MS revealed that terpenes are the most abundant component in the essential oil with the highest percentage of 46.97%, this is followed by alcohol which consituent 43.41% other hydrocarbons were 9.61%(see Table 4.12). Terpenes are known to repel insects (Werner 1995; Gracia2005; Park *et al.,* 2005; Choi *et al.*, 2002).

The presence of the compounds found in this work corresponds with the works of Moreira *et al.* (2010) which showed that the following are present in the essential oil of *Hyptis suaveolens*; β-pinene (6.55%), 1-octen-3-ol (0.28%), eucaliptol (47.64)%, gama-ellemene (8.15%), etc and Umedum*et al.*, (2014) also showed that, Cyclohexane (0.47%), α-pinene (2.04%), β-pinene (6.72%), 1-Octen-3-ol (2.42%), β-Elemene (0.6%), Bergamotol (0.64%), Phenanthrene (0.72%), etc are all present in the leaves of *H. suaveolens*. All the above compounds are terpenes. Werner (1995) showed that different terpenes cause toxicity to some insects. In his work, he showed that at different concentrations different terpenes have differing toxicity to insects which causes mortality. García2005 also showed that terpenes are highly toxic (causes mortality) and has high ability to repel insects. In their research they showed how terpenes repel insects effectively. Park *et al*.(2005) on their part selected five terpenes (monoterpenes) which all repel mosquitoes more effectively than known synthetic repellents.Choi *et al*.(2002) did an extensive work which revealed that monoterpenes are

effective mosquito repellents and can be used in place of DEET which is a well-known mosquito repellent. All these confirm that terpenes are actually responsible for the insecticidal properties of the essential oil obtained from the leaves of *Hyptis suaveolens*.

The most abundant compound of the essential oil in this work is Eucalyptol with peak area of 31.12% and retention time of 7.626, This corresponds with the work of Moreira *at al.*(2010),these authors revealed that Eucalyptol, gama-ellemene and β-pinene are among the most abundant components (47.64 %, 8.15% and 6.55 % respectively). The work of Franz *et al.* (2005) shows that Phellandrene is abundant in the essential oil of *Hyptis suaveolens* which is also present in this study. Vongsombath *et al.*(2012) showed that α-pinene is a major component of *Hyptis suaveolens* which correspond with this study. The work of Noudogbessi *et al.* (2013) is also in perfect agreement with this study. Little differences in the percentages obtained may be due to the solvents used for the extraction,moisture level of the leaves and the chemotypic profile of the *Hyptis suaveolens* strains analysed (Iwalokun *et al.*, 2012).

The next abundant component of the essential oil in this study is Bicyclo[7.2.0]undec-4-ene4,11,11-trimethyl-8-methylenewith a peak area of 17.30% and retention time of 13.483, while 1,3,6,10-Cyclotetradecatetraene, 3,7,11-trimethyl-14-(1-methylethyl) is the least abundant compound with a peak of 0.58% and a retention time of 22.966. From the entire compound identified by GC-MS the percentage similarity to that in the mass spectra data base was found to be ranging from 80% and 97%.

Characterization of the bioactive fraction of essential oils from *Hyptis suaveolens* (L. Poits) was carried out to determine the types of functional group(s) in the essential oils of *Hyptis suaveolens* fraction. The spectra revealed the presence of fifteen (15) functional groups as shown in Table 4.13, which shows the presence of the following class of compounds; esters, ethers, alkenes, alkyls, alcohols, amines, amides, alkyl halides and carboxylic acids. Esters

are used for making surfactants such as soap, detergent etc. They are also used for making perfumes because of their sweet smell, unsaturated esters have stronger odour than the saturated ones. Ethers have pleasant smell that makes them suitable for use in the preparation of perfume, soap, anaesthesia etc.

## CHAPTER SIX

## 6.0 CONCLUSION, SUMMARY AND RECOMMENDATIONS

### 6.1 Conclusion and Summary

❖ Percentage yield of 0.05. This is averagely sufficient using hydro-distillation

❖ Essential oil from *Hyptis suaveolens* leaves has shown mosquitocidal activity

❖ It has strong repellency effect against adult mosquitoes

❖ It also has very strong larvicidal properties.

❖ Characterization of the essential oil done using GC-MS revealed the presence of terpenes as the most abundant component. A lot of work has been done which revealed that terpenes have high ability to repel and or kill insects generally and in particular mosquitoes.

❖ Since terpenes have been revealed to be present in the essential oil from the leaves of this plant (*Hyptis suaveolens*) and the oil has successfully killedand repelled adult mosquitoes and at the same time eliminated mosquito larvae, it then suggest that the activities of the essential oil is as a result of the terpenes contained in the oil.

❖ FTIR analysis revealed the presence of functional groups such as esters, ethers, carboxylic acids etc all of which are useful in the making of soap, perfume and several other things.

❖ Characterization of the oil through determination of physicochemical properties revealed that the iodine, acid, saponification, peroxide, ester and free fatty acids values suggest that the oil can be formulated into cream or perfume to be used directly on the skin to repel mosquitoes considering its friendly nature both to the skin and the environment.

## 6.2 **Recommendation**

Considering the insecticidal properties of essential oil from *Hyptis suaveolens* leaves, we therefore recommend that the essential oil be used in subsequent work to make soap, perfume and or mosquito repellent popularly known as mosquito coil (right concentration of the essential oil mixed with saw dust or any other dust that can be used).

Further studies could be done by extracting the components of the essential oil and test each one of them to know the particular compound(s) that are responsible for the mosquitocidal, larvicidal and repellency properties. This information could help in determining the possibility of using the oil to make insecticides or use it as a component of fumigant to eliminate all unwanted target insects.

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## APPENDICES

1) 1% Stock Solution of the Essential Oil

The stock solution was prepared by introducing 200mg of the essential oil which is equivalent to 1 ml essential oil into a test tube containing 20 ml distilled water, 1 ml of DMSO was added to aid the mixing of the oil with the water (WHO/CDS/WHOPES/GCDPP/2005.13).

2) GC-MS Machine type used

SHIMADZU, JAPAN QP2010

3) Percentage yield

$$\text{Yield} = \frac{Mass\ of\ product\ (oil\ yield)}{Mass\ of\ starting\ material}\ X\ 100 = 0.05.$$

(0.5mLs equivalent to 100mg)

One (1) ml of the essential oil weighs 200mg, that is to say 0.5 ml volume of the oil gives an average weight of 100 mg, hence the above percentage yield.

4Mosquitocidal capability of essential oil from *Hyptissuaveolens* leaves

**Mosquitocidal**

$y = 0.620e^{0.127x}$
$R^2 = 0.995$

- Mosquitocidal
- Expon. (Mosquitocidal)
- Expon. (Mosquitocidal)

5Repellency effect of the essential oil from *Hyptis suaveolens* leaves

| Conc. | $LC_{50}$ | $LC_{90}$ |
|---|---|---|
| Overall | 3.336 | 0.878 |

6 Graphical expression of ASO repellency: Estimate against Probit

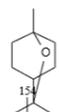7 Graphical expression of OSOrepellency: Estimate against Probit

**OSO**

One side open Repellency

y = 0.119x - 0.938
R² = 0.776

♦ OSO

—— Linear (OSO)

Probit

8 Graphical expression of ASCrepellency: Estimate against Probit

9 Graphical expression of overall repellency: Estimateagainst Probit

**Repellency**

$y = -0.783x + 21.77$
$R^2 = 0.755$

Legend:
◆ Repellency
— Linear (Repellency)

Axis labels: Overall Repellency (y-axis), Probit (x-axis)

10 Graph of all sides open (ASO) repellency with control

The chart shows:
- Y-axis: All sides open Repellency (0 to 25)
- X-axis: Time in minutes (0 to 5)
- Equation: $y = 16.63e^{0.028x}$
- $R^2 = 0.275$
- Legend: All sides open (ASO); All sides open control; Expon. (All sides open (ASO))

11 Graph of one side open (OSO)repellency with control

y = 2.134x + 16.83
R² = 0.943

12Graph of all sides closed (ASC) repellency with control

The chart shows:
- Y-axis: All sides closed Repellency (0 to 30)
- X-axis: Time in minutes (0 to 5)
- $y = 1.168x + 20.99$
- $R^2 = 0.740$

Legend:
- ◆ All sides closed (ASC
- ■ All sides closed control
- —— Linear ( All sides closed (ASC)

13larvicidal effect of essential oil from *Hyptis suaveolens*(Overall)

14 Larvicidal effect of essential oil from *Hyptis suaveolens*

Concs 6.25 and 12.5ppm



15 Larvicidal effect of essential oil from *Hyptis suaveolens*

Conc 25ppm



16 Larvicidal effect of essential oil from *Hyptis suaveolens*

Conc 50-1000ppm
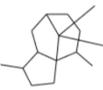


**conc**

y = 0.726x + 0.246
R² = 0.932

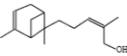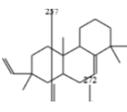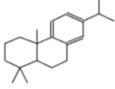◆ conc

—— Linear (conc)

Probit

Conc. in ppm

17Graph of mosquitocidal, repellency and larvicidal activities of essential oil from *Hyptis suaveolens* leaves

18 Components of the essential oil from *Hyptis suaveolens* by GC-MS

| Peak Num. | Retention time. | Area (%) | Compound Name | Molecular weight | Molecular formulae | Structure | S. I |
|---|---|---|---|---|---|---|---|
| 1 | 6.014 | 1.81 | 1S-alpha-Pinene | 136 | $C_{10}H_{16}$ | | 97 |
| 2 | 6.646 | 6.82 | Bicyclo[3.1.0]hexane | 136 | $C_{10}H_{16}$ | | 93 |
| 3 | 6.735 | 3.73 | Beta-Pinene | 136 | $C_{10}H_{16}$ | | 94 |
| 4 | 6.877 | 5.36 | Octen-3-ol | 128 | $C_8H_{10}0$ | | 95 |
| 5 | 7.147 | 2.03 | Alpha- Phellandrene | 136 | $C_{10}H_{10}$ | | 93 |
| 6 | 7.626 | 31.12 | Eucalyptol | 156 | $C_{10}H_{18}0$ | | 93 |
| 7 | 7.997 | 1.47 | Bicyclo[3.1.1]hept-2-ene | 136 | $C_{10}H_{16}$ | | 93 |
| 8 | 8.478 | 4.52 | Cyclohexene, 4-methyl-3 -(1-methylethylidene | 136 | $C_{10}H_{16}$ | | 93 |
| 9 | 8.586 | 2.08 | Bicyclo[2.2.1]heptan-2-one, 1,3,3-trimethyl | 152 | $C_{10}H_{16}0$ | | 95 |
| 10 | 10.071 | 3.55 | 3-Cyclohexen-1-ol,4-methyl-1-(1-methylethyl) | 154 | $C_{10}H_{10}0$ | | 93 |

106

| 11 | 12.766 | 1.32 | Copaene | 204 | $C_{15}H_{24}$ |  | 87 |
|---|---|---|---|---|---|---|---|
| 12 | 12.592 | 2.79 | Cyclohexane,1-ethenyl-1-methyl-2,4-bis(1-methylethenyl) | 204 | $C_{10}H_{15}$ |  | 89 |
| 13 | 13.483 | 17.30 | Bicyclo[7.2.0]undec-4-ene4,11,11-trimethyl-8-methylene,[IR(IR*,4Z,9S*)] | 204 | $C_{15}H_{24}$ |  | 90 |
| 14 | 14.386 | 6.81 | 1,6-Cyclodecadiene,1-methyl-5-methylene-8-(1-methylethyl),[s-(E,E)] | 204 | $C_{15}H_{24}$ |  | 87 |
| 15 | 14.617 | 2.57 | gamma-Elemene | 204 | $C_{15}H_{24}$ | | 91 |
| 16 | 16.362 | 1.13 | 1H-3a,7 Methanoazulene, octahydro-1,4,9,9-tetramthyl | 206 | $C_{15}H_{26}$ |  | 87 |
| 17 | 18.149 | 1.30 | Bergamotol,Z-alpha-trans | 220 | $C_{24}H_{24}O$ |  | 91 |
| 18 | 22.223 | 2.68 | Phenanthrene,7-ethenyl-1,2,3,4,4a,4b,5,6,7,8,8a,9-dodecahydro-1,1,4b, tetramethyl-,[4aS-(4a.alpha.,4b. beta.,7.alpha.,8a.alpha.)] | 272 | $C_{20}H_{32}$ |  | 79 |

| 19 | 22.684 | 1.02 | 7-Isopropyl-1,1,4a trimethyl1,2,3,4,4a,9,10,10a-0ctahydrophenanthrene | 270 | $C_{20}H_{30}$ |  | 76 |
| 20 | 22.966 | 0.58 | 1,3,6,10-Cyclotetradecatetraene, 3,7,11-trimethyl-14-(1-methylethyl) | 272 | $C_{20}H_{32}$ |  | 80 |

19Physicochemical properties

iDetermination of Iodine Value

$$\frac{(Blt - St) \text{ x M x } 12.69}{weight\ of\ sample}$$

Where 12.69 is a constant used for the calculation, Blt is the value obtained from the blank, St the value gotten from the test, Mis molarity which is 0.1 in this experiment and the weight of the sample is 0.35 grams.

ii Determination of Saponification Value

$$\frac{(B - S) \text{ x } 280.5 \text{ x } 1}{weight\ of\ sample}$$

B=End point value for the blank, S=End point value for test, 280.5 is a constant for the calculation, 0.1 is the molarity and weight of sample here is 1 gram.

iiiDetermination of Acid Value

$$\frac{56.10\ x\ St\ x\ M}{sample\ weight}$$

St is the value of KOH used, M the molarity of the solution which is 0.1, 56.1 is a constant used for the calculation and sample weight used in this experiment is 0.5 grams.

ivDetermination of Peroxide Value

$$\frac{(v1 - vo)x\ M\ x\ 1000}{sample\ weight}$$

$v1$ is the value obtained from the burette, $vo$ is value from burette of the blank, M is molarity which is 0.1 in this experiment and the sample weight is 1 gram.

v Determination of Ester Value = Saponification − Acids value

vi Determination of Free Fatty Acids value

$$\frac{Acids\ value}{1.99}$$

12 ml of acetic acid-chloroform solution was made of a mixture of 7.2ml Acetic Acid and 4.8ml Chloroform.