BIOCHEMICAL EXAMINATION AND IDENTIFICATION OF BOTANICALS ACTIVE AGAINST ADULT *ANOPHELES GAMBIAE* FROM THE COASTAL REGION OF KENYA

BY

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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DEDICATION

With love to my late dad ***John Odalo Kachimba***, my mum ***Jeniffer Ayugi Odalo*** and my dear wife ***Beatrice Akinyi Ochieng***; without their inspiring efforts this publication would not have been a reality. My daughters: ***Jeniffer***, ***Jeanet*** and ***Emelda*** who withstood the absence of a dearest dad.

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Not to be forgotten are uncles Jasper Okoth, David Oriwo, Martin Ondego and aunt Christine Ondego who stood by the family when things went the wrong way. Most thanks to my dear brother, Francis Omondi Odalo for his ever willing financial support during the studies.

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LIST OF ABBREVIATIONS

CO ---- Coinjection

CM ----- *Croton menyharthii*

CP ----- *Croton pseudopulchellus*

DDE ----- 1,1-Dichloro-2,2-bis-(*p*-chlorophenyl)ethene

DDT ----- 1,1,1-Trichloro-2,2-bis-(*p*- chlorophenyl)ethane

DEET ----- N, N-diethyl-*m*-toluamide

DMP ----- Dimethyl phthalate

ET ----- *Endostemon tereticaulis*

FID ----- Flame Ionization Detector

GC ----- Gas Chromatography

GLV ----- Green Leaf Volatiles

GM ----- Genetically Modified

GoK ----- Government of Kenya

HNO_3 ----- Nitric acid

HP ----- Hewlett Packard

MIM ----- Multilateral Initiative on Malaria

MF ----- *Mkilua fragrans*

MS ----- Mass Spectrometer/spectra

OFI ----- *Ocimum fischeri*

OFO ----- *Ocimum forskolei*

PC ----- *Plectranthus cyneus*

PL ----- *Plectranthus longipes*

RC_{50} ----- Concentration which is able to repel 50% 0f test insect population

RT_{90} ----- Time at which 90% of insect test population is repelled

TDR ----- Tropical Diseases Research

UoN ----- University of Nairobi

VH ----- *Vernonia hildebrandtii*

ABSTRACT

Malaria still remains one of the most important tropical parasitic and vector-borne diseases in terms of geographical distribution, incidence, the extent of the morbidity and mortality. It is endemic in about 100 countries inhabited by >40% of the world's population with at least 500 million clinical cases reported each year. More than 90% of these cases occur in Africa, where it is one of the most important causes of morbidity and mortality among infants, young children, pregnant women, non-immune travelers, refugees, displaced persons and labourers entering endemic zones. Management of malaria has become more difficult due to the development of resistance by the vectors to a number of once "golden" insecticides and repellents and the spread of resistance to anti-malarial drugs in *Plasmodia* species. The search for effective vaccines against malaria is still in progress. Personal potection against mosquito bites using repellents and bed nets occupies a central position in the fight against malaria. Human toxicity and allergic reactions have been reported for some commercial available repellents like N, N-diethyl-3-methylbenzamide (DEET). The need for alternative repellents is more urgent than ever before.

Plants and their metabolites have been shown to be effective against mosquitoes. Some of the leading repellents and insecticides like pyrethrins are plant-derived or modeled. This justifies further bio-prospecting for anti-mosquito products from plants. In the search for new and potent mosquitocides and repellents, we carried out bio-evaluation of the Kenyan coastal flora. Essential oils and solvent extracts from aerial parts of 24 plants were evaluated for their repellent and insecticidal activities. Essential oils of 7 plants (*Croton pseudopulchellus*, *Croton menyharthii*, *Endostemon tereticaulis*, *Mkilua fragrans*, *Ocimum fischeri*, *Ocimum forskolei* and *Plecranthus longipes*) showed good repellent activity (RC_{50} = 1.515-9.583 x 10^{-5} mg/cm^2) with 3 (*M. fragrans*, *O. forskolei* and *P. longipes*) also exhibiting significant insecticidal activity (LC_{50} = 3.044-5.106 x 10^{-3} mg/cm^3). Four repellent (carvacrol, 4-isopropylbenzenemethanol, phytol and thymol) and one insecticidal (carvacrol) principles are reported.

CONTENTS

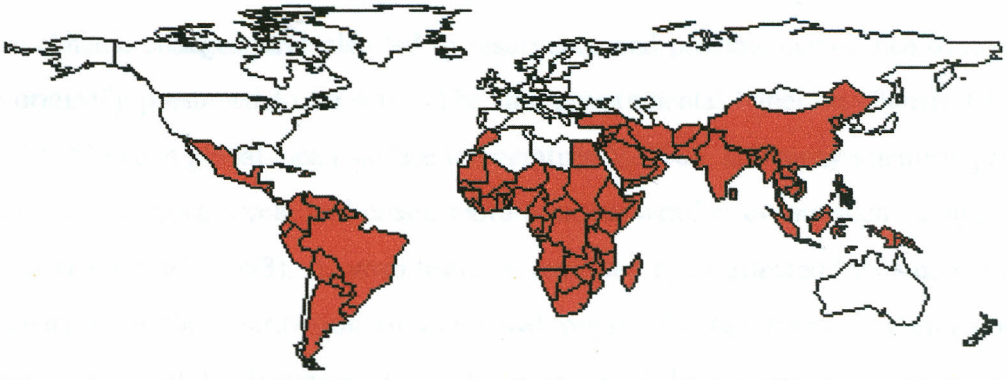
CHAPTER 1

INTRODUCTION

1.0 Background

Malaria is by far the most serious and widespread parasitic disease occurring in man. It is the world's most important tropical disease and kills more people than any other communicable disease (Clements, 1992; Trigg and Kondrachine, 1998). It is an old disease which was common in the marshy areas around Rome hence the name, from the Italian word, *mal-aria* or 'bad air.' It was also known as Roman fever (Kreier, 1980ab). Malaria is endemic in 101 countries and territories (Fig. 1) inhabited by 2.4 billion people (40% of the global population). Clinical cases of malaria reported each year exceed 500 million with >90% of the cases in sub-Saharan Africa where the disease contributes substantially to underdevelopment and places severe strain on the limited health care facilities. The remaining cases of malaria are largely confined to South and South East Asia and parts of South America (Trigg and Kondrachine, 1998).

Figure 1: Global distribution of malaria (http://www-micro.msb.le.ac.uk/224/Bradley/History.html)



Regions which were originally considered safe, like highlands of western Kenya, are now affected by highland malaria (Bouma *et al.*, 1996; Dhingra *et al.*, 1998). Malaria has encroached into the highland communities as a result of wide-scale population settlement linked to transport and agricultural development and epidemics have now become frequent. Some authors have labelled these resurgences as new typology variant, "highland malaria," demanding special attention in the new global

commitment to Roll Back Malaria (Fontenielle *et al.*, 1990). The highlands of Kenya constitute a densely populated, politically significant area, which serves as a major source of revenue and foreign exchange from agricultural exports. The Government of Kenya (GOK) has recently defined 15 districts in the highlands, as being prone to epidemics with the worst affected districts being Kericho, Buret, Nyamira, Kisii, Gucha and Nandi (Hay *et al.*, 2002).

Mortality due to malaria is estimated at 1.5-3 million deaths each year with the majority occurring among young children in Africa (WHO, 1998; Trigg and Kondrachine, 1998). Other high-risk groups include pregnant women, non-immune travellers, refugees, displaced persons and labourers entering endemic areas. Some 20 million Kenyans are regularly exposed to the disease and >8 million succumb to malaria each year. Over 30,000 who do not get rapid or proper treatment die (Anon, 2001a).

The seriousness of the malaria problem has been greatly exacerbated in recent years as a result of several factors. The disease has spread into new areas as a result of changing land utilisation, especially irrigation and plantation agriculture. This has led to epidemics in regions previously free of malaria, especially in the highland areas of East Africa (Trigg and Kondrachine, 1998). Failure of malaria control measures in regions of conflict in Africa and South East Asia has also contributed to an upsurge of malaria in areas where it was previously managed (Trigg and Kondrachine, 1998). Continuous climatic changes have also led to resurgence and possible occurrence of malaria in areas that were originally presumed to be safe. The Intergovernmental Panel on Climate Change (IPCC) predicts 1-3.5 °C rise in global mean surface temperature by 2100, and many scientists predict that the effects on the hydrological cycle will create more extreme weather events such as hurricanes, floods and droughts (Patz *et al.*, 1998). Human health is expected to be affected by climatic variations as a result of changes in the distribution of biological organisms that transmit vector-borne diseases (McMichael *et al.*, 1996). Because of the dependence of the vectors and pathogens on climatic factors, these diseases are expected to change in distribution and intensity. Malaria is one of the vector-borne diseases that are expected to be most sensitive to long-term environmental change (WHO, 1999b). In the global distribution of malaria, small climatic changes can have considerable effect on the transmission of malaria, and historic changes in the epidemiology of the disease appear to be related to changes in global temperature (De Zulueta, 1987). Current concern about increasing global temperature has resulted in predictions of widening gap in geographic distribution of malaria in

temperate climates (Kalkstein and Smoyer, 1993). The growth rate of the vector population is dependent on temperature. Higher temperatures shorten the mosquito generation time, and thus may result in higher vector densities that may increase the likelihood of transmission (Bouma *et al.*, 1996). Temperature also affects the development of the parasite in mosquito vector. The duration of the sporogony is related to ambient temperature. Below a given threshold (variously estimated as 15-19 ^{0}C), parasite development cannot be completed (Bouma *et al.*, 1996). This could explain the rapid decrease in the prevalence of *P. falciparum* in sub-tropical temperate zones (Bouma *et al.*, 1996), and its increase in the tropics. Other climatic parameters such as rainfall and humidity influence the transmission conditions mainly through their effect on breeding (density) and longevity of the vector population, respectively (Onori and Grab, 1980). Finally, the spread of drug resistant strains of parasite has seriously reduced the efficacy of most anti-malarial drugs.

1.1 Malaria parasite

Malaria is a protozoan disease transmitted by the anopheline mosquitoes. It is caused by parasitic protozoa of the genus *Plasmodium*, which infect human and insect hosts alternatively. There are four species of *Plasmodia*, which cause malaria in man: *Plasmodium vivax*, *P. ovale*, *P. malariae* and *P. falciparum* (Ridley, 1997) with the latter species being the most virulent (Khaemba *et al.*, 1994; Ridley, 1997).

The *Plasmodium* species have a life cycle, which is split between the vertebrate host and the insect vector. The parasite undergoes a developmental stage in the female mosquito. The infected female *Anopheles* injects materials, which contain primitive malarial parasites called sporozoites, from her salivary glands before feeding on the host blood. Once in the human body, the parasite starts the asexual phase (schizogony) of its cycle. The sporozites circulate in the blood for one hour and then settle in the liver where they enter the parenchyma cells and multiply producing vast numbers of merozoites (pre-erythrocytic schizogony). After 9-16 days, the infected cells rupture releasing the merozoites into the blood stream where they penetrate, multiply and destroy the red blood cells resulting in bouts of fever (Gutsevich *et al.*, 1974).

In the red blood cells, the parasites develop into two forms: asexual and sexual cycle. The sexual cycle produces male and female gametocytes, which circulate in the blood and are taken up by a female mosquito when taking a blood meal. The male and female gametocytes fuse to form oocysts

on the wall of the mosquito stomach. These oocysts develop and produce large numbers of sprozites, which migrate to the salivary glands ready to be injected into the host when the mosquito takes its next blood meal.

1.2 The disease

P. vivax and *P. ovale* cause benign tertian malaria. It is the mildest form of malaria and takes 10-17 days from the time of infection to appearance of symptoms. *P. malariae* cause quartan malaria, which takes 28-30 days from the time of infection to appearance of symptoms. *P. falciparum* cause jungle fever, also known as malignant tertian malaria, which takes 6-12 days from infection to appearance of symptoms (Stedman, 1990; Ridley, 1997). Although the four species of malaria parasites can infect humans and cause illness, only *P. falciparum* malaria is potentially life threatening (Nevill *et al.*, 1994). Symptoms include fever, shivering, joint pains, headache, repeated vomiting, generalised convulsions and coma. If not treated, the disease particularly that caused by *P. falciparum*, progresses to severe malaria and death. Severe anaemia is often the cause of death in children in areas with intense malaria transmission. In its severe form (celebral malaria), the blood vessels to the brain get blocked, hence inducing delirium, coma and death. The paroxysms (caused by the release of merozoites from infected cells) recur every 48 hours in tertian (*vivax* or *ovale*), 72 hours in quartan (*malariae*) and indefinite but frequent intervals (usually about 48 hours) in malignant tertian (*falciparum*) malaria (Stedman, 1990).

1.3 Mosquitoes

Mosquitoes belong to the family Culcidae which consists of sub-families: Toxorhynchitinae, Anophelinae and Culicinae. The genera of the sub-families are: *Toxorhynchites*, *Anopheles*, *Culex*, *Aedes* and *Harpagomyia*. There are about 3500 species of mosquitoes which are found all over the world, except in the polar regions (Clements, 1992).

1.3.1 Life cycle

Mosquitoes require an environment of standing water to lay eggs. They have adapted to complete their life cycle in diverse aquatic habitats including fresh water; salt-water mashes, brackish water or water found in containers, old tyres, tree holes, hoof prints or leaf axials. The life cycle has four stages (Plate 1). The female lays her eggs (up to several hundred at a time) on the surface of water or in an

area subject to flooding. Unhatched eggs of some species can withstand weeks to months of desiccation, remaining viable until the right conditions for hatching are available (Clements, 1992). The eggs of most species hatch in 2-3 days. The larvae feed on the "particulate" matter in water, which include aquatic micro-organisms (bacteria, diatoms, algae or particles derived from decayed plant tissues). Anopheline larvae typically feed at the water surface, in the particle-rich layer just below the surface for 7 days until they change into pupae (Clements, 1992). The pupae remain aquatic organisms and float at the air/water interface for 2 to 3 days before emerging into adult mosquitoes. The life span of adult mosquitoes ranges from a few days to several weeks in tropical regions, while it may be longer in temperate regions, especially in species that overwinter (Clements, 1992). Understanding the lifecyle of the vector may be useful in designing vector control strategies that may be useful in reduction of malaria.

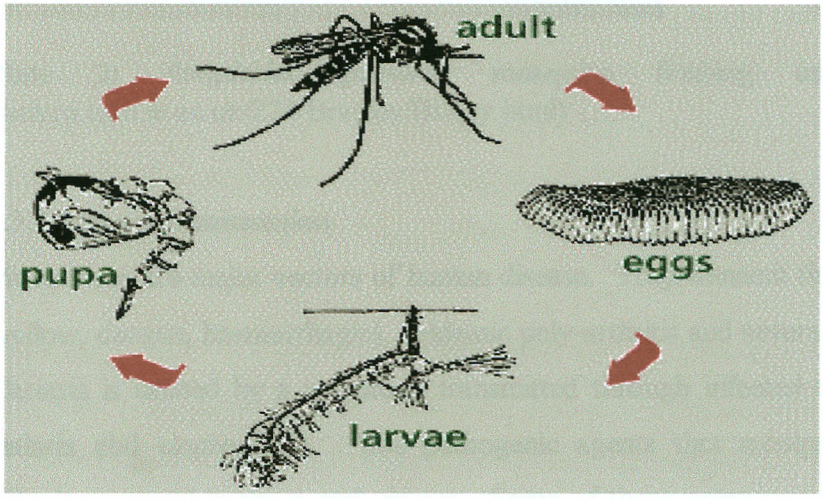


Plate 1: Life cycle of a mosquito (http://www.ento.okstate.edu/mosquito/lifecycle.html)

1.3.2 Feeding

Mosquitoes feed on nectar, rotting fruits, honeydew and blood after locating the host from a distance. Two modes of feeding have been distinguished in male mosquitoes and three in females; water ingestion, nectar feeding (male and female) and blood feeding (females only) (Plate 2) (Clements, 1963; Franke *et al.*, 1978). The anopheline and culicine females feed every 3 to 4 days on vertebrate blood to obtain protein for the development of eggs. Certain species of mosquitoes prefer to feed at twilight or night while others bite during the day. The timing is not governed directly by light or darkness but by endogenous (circadian) rhythms, which are reset daily by the change from light to

dark at sunset. Some mosquito species are zoophilic (prefer animal blood) and others are anthrophilic (showing preference for human blood). Knowledge of mosquito feeding habits is necessary for designing malaria control strategies since it is during feeding that the parasite is transmitted between hosts.

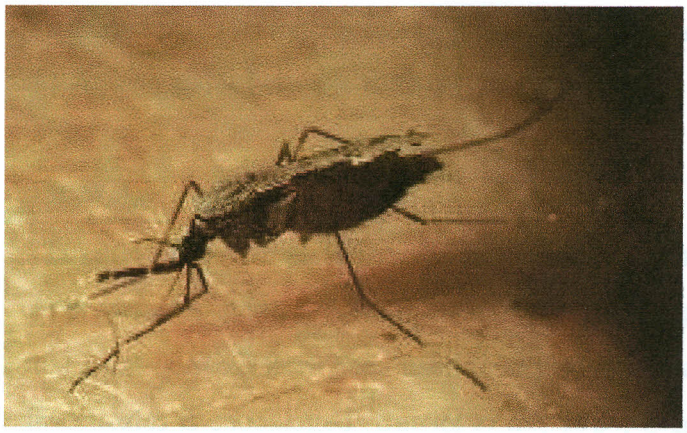


Plate 2: *Anopheles gambiae* mosquito feeding on human host (http://www-micicro.msb.le.ac.uk/224/Bradley/Bilogy.html)

1.3.3 Disease transmission

Mosquitoes are major vectors of human disease. They transmit the arboviruses responsible for fevers (yellow, dengue, haemorrhagic), epidemic poly-arthritis and several forms of encephalitis. Bancroftian filariasis is caused by a nematode transmitted through infected mosquito bite. They also transmit malaria and elephantiasis. The pathogenic agents that mosquitoes transmit include *Plasmodia*, *Filariae*, several bacteria and viruses. Some of these organisms alternate between a parasitic phase and a free-living phase; others are entirely parasitic and many alternate between the mosquito and other hosts. The blood sucking habit render adult female mosquitoes prone to acquiring pathogens and parasites from one vertebrate host and transmitting them to another. Mosquitoes are protected against infective organisms by structural barriers and their immune system (Clements, 1992). Malaria is transmitted by bite of infected female anopheline mosquitoes. Only 60 out of the 380 species of the anopheline mosquitoes can transmit malaria. In some areas of sub-Sahara Africa, *An. gambiae*, *An. funestus* and *An. arabiensis* are the efficient vectors due to their longevity, association with man and difficulty in their eradication.

In several tropical countries the impact of malaria is increasing, particularly at the frontiers of economic development where deforestation, mining and increased irrigation for agricultural purposes

lead to the migration of large numbers on non-immune workers. The need to control malaria is more urgent than ever before. Malaria still remains one of the great scourges of the world especially in Africa. The death toll due to malaria must be viewed not only in terms of the physical, financial and emotional pain it inflicts on individual families but also by its macro-economic impact. In Kenya, it is estimated that 170 million working days are lost due to the disease each year and the cost of malaria in 31 African states between 1980-1995 is estimated at US $ 74 billion (Anon, 2001a).

1.4 Malaria control strategies

The prevalence of malaria in endemic regions has been minimised by use of various methods. Approaches to malaria control include protection against infection or disease. Insecticides (synthetic and plant-derived) have been used to control malaria vector. Use of mosquito nets impregnated with insecticides is encouraged (Darriet *et al.*, 1984; Charlwood and Graves, 1987). Insecticide aerosols, mosquito coils or vapourizing mats containing pyrethrin and other mosquito repellents have also been employed (Curtis, 1990). Vector resistance has been reported for some of these insecticides such as DDT (Hutson and Robert, 1985). The most effective synthetic repellent so far used is N, N-diethyl-3-methylbenzamide (DEET) (Trigg, 1996).

Measures, which protect against disease but not infection, include chemoprophylaxis. However, drug resistance has been reported with some strains of malaria parasites (Trigg and Kondrachine, 1998). The recent resurgence of malaria as a major global disease has been attributed to difficulties in mosquito control programmes coupled with the development of vector and parasite resistance to insecticides and anti-malarial drugs, respectively (Khaemba *et al.*, 1994). Changing human activities have contributed to the abundance of the major vector, *An. gambiae*, through the creation of numerous breeding habitats (Khaemba *et al.*, 1994). Other environmentally friendly, cost effective and sustainable methods for vector control, chemoprophylaxis and chemotherapy must therefore be sought.

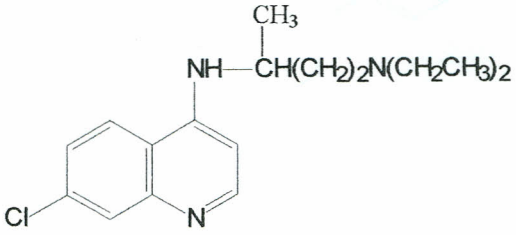
Prevention of malaria involves a variety of measures that may protect against infection or the development of the disease in infected individuals. Today, control of *Anopheles* is attempted through environmental management, insecticides and repellents depending on the biology of the vector species

and affordability. Measures that protect against infection are directed against the mosquito vector through environmental management, insecticides or repellents and biological control agents.

Development of malaria vaccine has been initiated with more than a dozen candidate vaccines, some of which are undergoing clinical trials (WHO, 1998.). The three main types of vaccines being developed are anti-sporozite (Franke *et al.*, 1999), anti-asexual blood stage and transmission-blocking vaccines (WHO, 1998). The vaccines are designed to prevent infection, reduce severe and complicated manifestation of the disease and arrest the development of the parasite in the mosquito, respectively (WHO, 1998). Chemically synthesized vaccine, SPf66 has been tested giving insignificant protection and serious side effects (Alonso *et al.*, 1994; D'Alessandro *et al.*, 1995; Nosten *et al.*, 1996; Migasena *et al.*, 1997). Peptide based vaccines have been successfully employed but face the challenge of toxicity (BenMohamed *et al.*, 2002). Other candidate trial vaccines include nucleic acids that target asexual stage (Doolan and Hoffman, 2002) and pre-erythrocytic stage (Ballou *et al.*, 2002). Besides vaccines, chemoprophylaxis and chemotherapy have been used to minimise disease prevalence.

1.4.1 Single therapy

Individual drugs were initially used for treatment of malaria but drug resistance has been reported in some strains of the parasite. Drug-resistant strains of *P. falciparum* are now common in South East Asia and Africa (WHO, 1998). Resistance to chloroquine (**1**) is now widespread (Sharma, 1996; Trigg and Kondrachine, 1998; WHO, 2001). Chloroquine is cheap, safe for use in pregnancy and was previously highly efficacious (Winstanley and Breckenridge, 1987). The loss of this drug has been a major setback to the effective treatment and control of malaria. Furthermore, resistance to other anti-malarials such as mefloquine (**2**) dapsone (**3**) and pyrimethamine (**4**) has also been reported (WHO, 1998; WHO, 2001). Resistance to the other available drugs like quinine (**5**) (Price *et al.*, 1999), halofantrine (**6**) and amodiaquine (**7**) (Bray *et al.*, 1996) has also been observed or demonstrated in *vitro*. Halofantrine (**6**) exhibits serious toxicity at dosages required for treatment of resistant strains (Ter Kuile *et al.*, 1993). Artemisinin (**8**) derivatives like artesunate (**9**) and artemether (**10**) have been known to show no cross-resistance with known anti-malarials but high rate of treatment failures have been reported (WHO, 1998; WHO, 2001). A limited number of single drugs for treatment of malaria including atovaquone (**11**) and proguanil (Paludrine®) (**12**) are still available today. However, as resistance is being reported against these, double therapy seems to be the solution in the treatment of drug resistant malaria.



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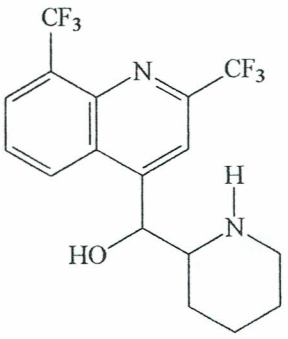

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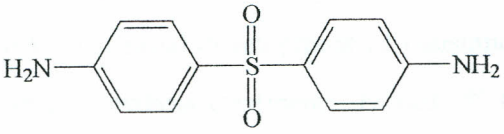

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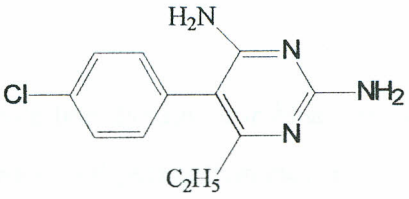

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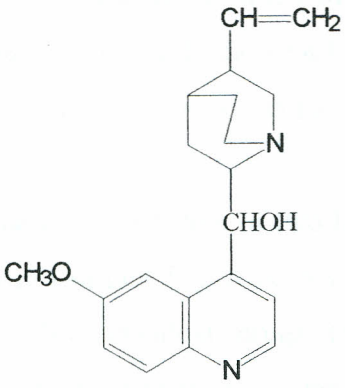

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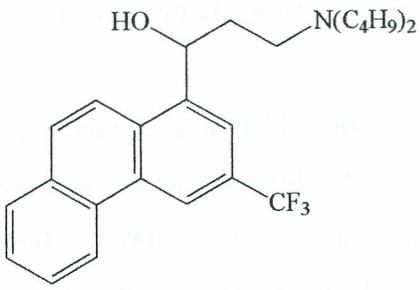

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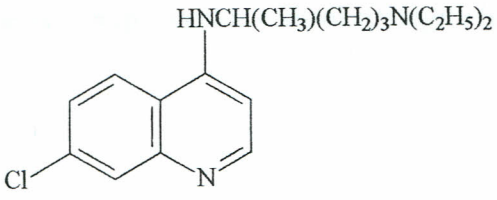

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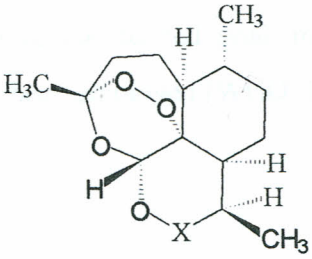

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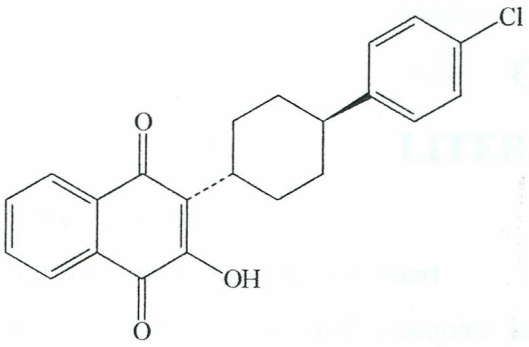

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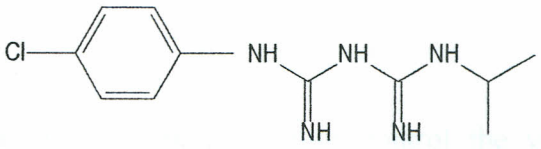

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1.4.2 Combination therapy

Following rapid development of resistance to single line therapy, combinations of drugs have proved useful in malaria treatment. Fansidar® (sulfadoxine and pyrimethamine), malarone® (proguanil and atovaquone) and co-artemether® (artemether and lumefantrine) are available today (Kamya *et al.*, 2001). The combination of an oral artemisinin derivative (usually artesunate) and mefloquine has become standard treatment for multidrug-resistant *falciparum* malaria (Angus *et al*, 2002). Resistance is already being reported for combinations like Fansidar® (Kamya *et al.*, 2001).

The looming failure of two-line combination leaves triple therapy as a viable approach to fighting the drug resistant *P. falciparum* malaria. Triple-line combination slows down development of resistance to the individual drugs (McIntosh and Greenwood, 1998). Chloroquine plus sulfadoxine-pyrimethamine (SP) and amodiaquine in combination with SP (Winstanley *et al.*, 2002) have been used. Large parts of the world may soon be without any effective anti-malarial drug and therefore vulnerable to devastating malaria epidemics. Other alternative malaria control strategies need to be developed since chemotherapy is failing to achieve the desired goal; malaria eradication. Besides chemotherapy, vector control strategies have also been employed (WHO, 1998).

CHAPTER 2

LITERATURE REVIEW

2.1 Vector Control

2.1.1 Environmental management

Interfering with the natural mosquito larval habitat has also been used to control the vector populations. Clearing of bushes, drainage of water marshes, covering essential water sources and safe disposal of used containers and tyres have been advocated. This method has several drawbacks as it interferes with the natural ecosystems, the cost and labour involved in the drainage of large water marshes is enormous (Kreier, 1980a).

2.1.2 Biological control

Biological control as a component of integrated vector management (IVM) enlists the use of natural enemies of a vector as the "bio-rational" option to control it without having to resort to use of chemicals. For mosquitoes, biological control employs the use of predators, parasites or entomopathogens. Some of nature's best mosquito destroyers including the mosquito fishes *Gambusia affins* (Meisch, 1985) and *Poecilia reticulata* (Dua *et al.*, 1997) have been used to feed on the mosquito larvae. Previously, other fishes such as Amargosa pupfish (*Cyprinoden neradensis amargosae*) and guppies (*Poecillia retimilata*) were used. *Tilapia* have also been bred to control mosquito larvae as in Namanjala Sub-location in Kitale, Kenya (Anon, 2001a).

Many species of bacterial, fungal and other microbial pathogens of mosquito are known. Many of these potential biological control agents are still in the developmental stages and only a few have been registered for use as mosquito control agents. Toxins produced by some *Bacillus* species have been used to control mosquito larvae. The species used include *Bacillus thuringiensis* H-4 and *Bacillus sphaericus* (WHO, 1996; Dua *et al.*, 1997). Commercially available bacteria-derived larvicides include Vectobec® or Bactoculicide® and Vectolex® or Spherix® with *Bacillus thuringiensis* var. *israelensis* and *Bacillus sphaericus*, respectively, as the active agents.

In the rice fields where mosquitoes are a great menace, use has been made of a mermathid nematode (*Romanomermis culicivorax*) and a fungus (*Lagenidium giganteum*) with little or no adverse effects

on non-target organisms (Lawrence and Cynthia, 1990). These microbial agents have the disadvantages of a narrow host range, high cost, sensitivity to UV light, pH and heat.

Genetically modified (GM) mosquitoes offer an attractive control strategy (Morel *et al.*, 2002; Miller and Greenwood, 2002). They are yet to be released into the wild, where they are expected to mate with normal mosquitoes, making their offspring unable to transmit the disease (Anon, 2001b). Although this may be an attractive approach, their survival in the competitive environment has not been tested and natural selection pressures may eliminate their offspring.

2.1.3 Chemical insecticides

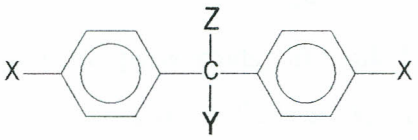
Insecticides are chemicals that are used to control insect populations by poisoning. There are both synthetic and natural insecticides. Inorganic compounds act largely as contact poisons while organic compound of plant origin and synthetic organic compounds may act as contact or stomach poison and are sometimes used as fumigants (Kirk and Othmer, 1981).

2.1.3.1 Synthetic insecticides

These can be classified into organic and inorganic insecticides. Arsenicals and fluorides have been used as inorganic insecticides. The insecticidal activity of the fluorides is related to the fluorine content and their solubility in the digestive juices of the insect. The insecticidal activity of arsenicals is directly related to the percentage of arsenic they contain. The arsenates act by producing regurgitation, torpour and quiescence in insects (Kirk and Othmer, 1981). Other metals like lead or copper, in combination, lead to increased toxicity (Kirk and Othmer, 1981). $Cu(C_2H_3O_2)_2.3Cu(AsO_2)_2$, cuprous cyanide (CuCN) have been used as mosquito larvicides. Other inorganic insecticides that have been used include; borax ($Na_2B_4O_7.10H_2O$), sodium tetraborate ($Na_2B_4O_7$) and zinc phosphide (Zn_3P_2) (Metcalf and Flint, 1962).

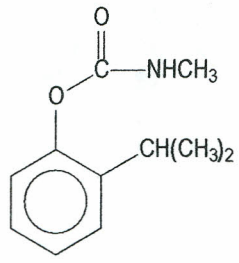
4,6-Dinitro-*o*-cresol (DNOC) was the first synthetic organic insecticide to be used (Kirk-Othmer, 1981). The organothiocyanate, 2-(2-butoxyethoxy)ethyl thiocynate ($C_4H_9OCH_2CH_2SCN$) was the first widely used synthetic organic insecticide (Kirk and Othmer, 1981). The thiocyanate group (-SCN) is responsible for the bio-chemical action. Organochlorine insecticides including DDT (**13**) have been used to control hundreds of species of insects especially vectors of public health significance

(Kirk and Othmer, 1981). However, its efficacy has reduced substantially due to insect resistance. Its high toxicity and non bio-degradability has led to its banning in most countries (Kirk and Othmer, 1981). DDT analogues include TDE (**14**), Perthane (**15**), DDE (**16**), Gix (**17**) and Methoxychlor (**18**) (Metcalf and Flint, 1962). TDE suffers from the same environmental and resistance problems as DDT (Metcalf and Flint, 1962). Perthane is substantially bio-degradable and is applied where low acute and chronic toxicities are desired (Metcalf and Flint, 1962). Methoxychlor gives a more rapid knockdown than DDT and is of much lower acute and chronic toxicity (Kirk-Othmer, 1981). Its bio-accumulation is comparatively low. Generally, DDT and methoxychlor show cross-resistance (Metcalf and Flint, 1962).



	X	Y	Z
13	Cl	CCl_3	H
14	Cl	$CHCl_2$	H
15	C_2H_5	$CHCl_2$	H
16	Cl	$=CCl_2$	--
17	F	CCl_3	H
18	OCH_3	CCl_3	H

Organocarbamate insecticides have also been used for mosquito control (Kirk and Othmer, 1981). 2-Isopropoxyl-N-methylcarbamate (Baygone) (**19**) and 1-naphthyl-N-methycabarmate (**20**) have been used for mosquito control (Kirk and Othmer, 1981). Organocarbonates have been found to be less effective in mosquito control. They are slightly toxic to mammals and not highly selective. However, they are bio-degradable (Kirk and Othmer, 1981).



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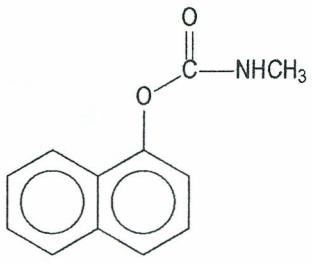

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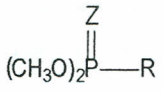
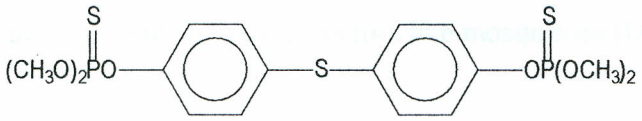
Organophosphorus insecticides are readily degraded and therefore do not present serious problems of bio-magnifications and transfer within the food chain. The organophosphorus insecticides that have been used to control malaria vectors include fenthion (**21**), feninitrothion (**22**), naled (**23**), malathion (**24**), dichlorvos (**23**) and temephos (**24**) (Metcalf and Flint, 1962). Fenthion is useful as a residual insecticide in households for adult mosquitoes and as a larvicide. Temephos is used as a larvicide

(Kirk and Othmer, 1981). Development of resistance to this class of insecticides (synthetic) by mosquitoes has been reported (Bang, 1985). *Anopheles culicifacies s.l.* is reportedly resistant to malathion and DDT (Raghavendra *et al.*, 1991).



	Z	R
21	S	OC_6H_3-*m*-CH_3-*o*-SCH_3
22	S	OC_6H_3-*o*-CH_3-*m*-SCH_3
23	O	$OCHBrCBRCl$
24	O	$SCH(COC_2H_5)CH_2CO_2C_2H_5$
25	O	$OCHCCl_2$



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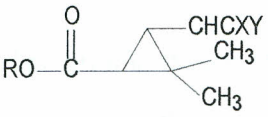
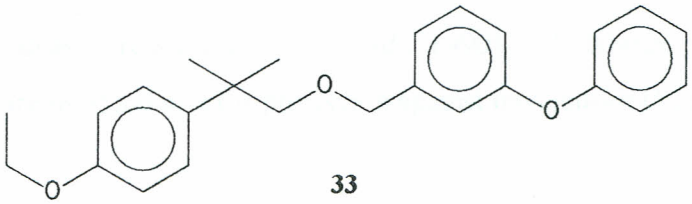
Synthetic pyrethroids, like allethrin (**27**), permethrin (**28**), deltamethrin (**29**), cypermethrin (**30**), cyhalothrin (**31**), cyfluthrin (**32**) and non-ester pyrethroid etofenprox (**33**), designed to mimic natural pyrethrins (Tomlin, 1994), have been used as insecticides to reduce cost (Metcalf and Flint, 1962). Deltamethrin is the most effective synthetic pyrethroid available in the market (Tomlin, 1994). The synthetic pyrethroids offer improved selectivity over other synthetic insecticides and have lower mammalian toxicity (Metcalf and Flint, 1962; Kirk and Othmer, 1981). Pyrethroids are some of the few insecticides with toxicities that increase at lower temperatures (Casida, 1980).



	X	Y	R
27	CH_3	CH_3	3-methyl-2-(1-propenyl)cyclopent-2-enylone
28	Cl	Cl	CH_2-m-$C_6H_4OC_6H_5$
29	Br	Br	$CH(CN)$-m-$C_6H_4OC_6H_5$
30	Cl	Cl	CH_2-m-$C_6H_4OC_6H_5$
31	Cl	CF_3	CH_2-m-$C_6H_4OC_6H_5$
32	Cl	Cl	$CH(CN)$-m-$C_6H_4OC_6H_5$



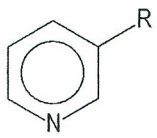
33

The main side effects of pyrethroids include neurotoxicity at high doses and liver hypertrophy (Doria, 1990). Many of the pyrethroids can be mildly to severely irritating to the skin and eyes (Doria, 1990).

The technical grade of a pyrethroid is usually formulated (mixed with carriers, solvents, synergists) for use in commercial pest control. Several inerts (silica, trimethylbenzenes and ethylbenzene) used in pyrethroid formulations are known or suspected carcinogens, or are chemicals like xylenes which depress the central nervous system (Metcalf and Flint, 1962). There are also hazardous contaminants, such as ethylene oxide, benzene and arsenic in several pyrethroids formulations (Metcalf and Flint, 1962). Resistance to synthetic pyrethroids has been reported in several insects including mosquitoes (Miller and Salgado, 1985; WHO, 1996). Resistance to synthetic pyrethroids has been reported for *An. gambiae* (WHO, 1996). The use of pyrethroids in agriculture has led to rapid resistance development in medical vectors like mosquitoes (De Kumar, 1995).

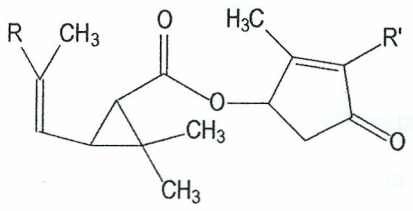
2.1.3.2 Natural insecticides

Insecticides of plant origin have been used for insect pest and vector management. Nicotine (**34**) (the principal alkaloid) from the leaves of *Nicotiana tobacum*, *N. rustica*, *Duboisia hopwoodii*, *Aesclepias syriaca* together with nornicotine (**35**) and anabasine (**36**) are of insecticidal importance (Kirk and Othmer, 1981). Anabasine is the principal alkaloid of *Anabasis aphylla* and in *N. glauca* (Kirk and Othmer, 1981). Several other plants have been shown to have anti-mosquito properties (Deshmukh *et al.*, 1987; Sukumar *et al.*, 1991).



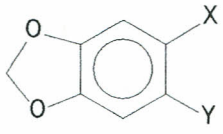
	R
34	N-methylpyrolidyl
35	pyrolidyl
36	piperidyl

The ground flower of *Chrysantherum cinerariaefolium* and *C. coccineum* contain six esters (pyrethrin I (**37**), II (**38**), cinerin I (**39**), II (**40**) and jasmolin I (**41**) and II (**42**)) and has been used as an insecticide (Metcalf and Fint, 1962). Pyrethrum act on insects with phenomenal speed causing immediate knockdown effect (Curtis *et al.*, 1990). However, unless it is formulated with a synergist, most of the knocked insects recover (Curtis *et al.*, 1990). Pyrethrum is incorporated in many mosquito coils, which are used indoors for protection against mosquitoes (Yap *et al.*, 1990).

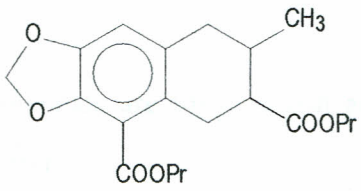


	R	R'
37	CH_3	CH_2=CHCH=CH_2
38	$COOCH_3$	CH_2CH=CHCH=CH_2
39	CH_3	CH_2CH=CHCH_3
40	$COOCH_3$	CH_2CH=CHCH_3
41	CH_3	CH_2CH=CHCH_2CH_3
42	$COOCH_3$	CH_2CH=CHCH_2CH_3

The insecticidal activity of the pyrethrins is enhanced by synergists such as piperonylbutoxide (**43**), 1,2-methylenedioxy-4-(2-(octylsulfinyl)propyl)benzene(sulfoxide) (**44**), sesmax (**45**) and di-*n*-propyl-2-methyl-6,7-methylenedioxy-1,2,3,4-tetrahydronaphthalene-3,4-dicarboxylate (**46**) and MGK-264 (**41**) (Kirk and Othmer, 1981). The synergist enhances the activity of the pyrethroids by inhibiting the detoxification processes. Organophosphorus and carbamate synergists act by deactivating esterases, enzymes that degrade pyrethroids by cleaving the molecule at the ester moiety. Organophosphates have also been shown to increase the inhibition of cholinesterase, an enzyme in the nervous system (Abiola, 1988). Piperonyl butoxide and sulfoxide act by blocking the mixed function oxidase, enzymes which oxidize and detoxify a wide variety of compounds (Casida, 1980; Gaughan *et al.*, 1980).



	X	Y
43	C_3H_7	$CH_2(C_2H_4O)C_4H_9$
44	H	$CH_2CH(CH_3)SC_8H_{17}$ (=O)
45	$OCHO(C_2H_4O)_2C_2H_5$	H



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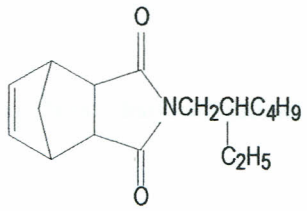
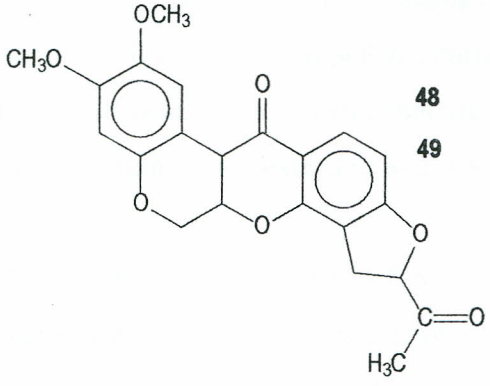


47

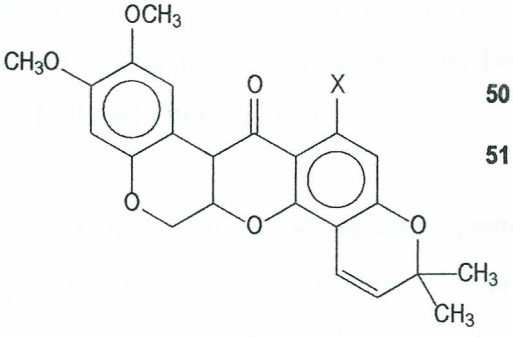
No reports on resistance to the natural pyrethrin have been found. The most common manifestation of pyrethrum poisoning to human beings include rashes on the skin exposed to the chemicals, which may be made worse by exposure to the sun or temperatures high enough to cause sweating (Doria, 1990).

Allergic responses and asthma following exposure to naturally occurring pyrethrins have also been reported (Doria, 1990).

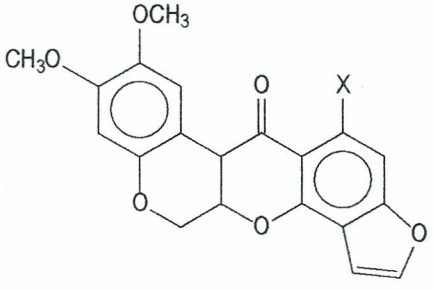
Rotenoids like rotenone (**48**), sumatrol (**49**), degeluen (**50**), L-α-toxicarol (**51**), elliptone (**52**) and malaccol (**53**), which are found in the family Leguminosae, have been used as insecticides. Rotenone (**48**) has been obtained from *Derris elliptica, D. malaccensis*, *Lonchocarpus utilis* and *L. urucu* and is also common in the genera *Tephrosia* and *Milletia* (Metcalf and Flint, 1962). It was also recently isolated from *D. trifolia* (Samita, 2003).



	X
48	H
49	OH



	X
50	H
51	OH



	X
52	H
53	OH

Rotenoids are toxic to fish and have therefore been discouraged as insecticide (Metcalf and Flint, 1962).

Natural insecticides have provided clues to important synthetic analogues such as pyrethrins. Consequently, more such chemicals need to be discovered to avail alternatives for use where resistance has been developed against commercial insecticides.

2.1.4 Repellents

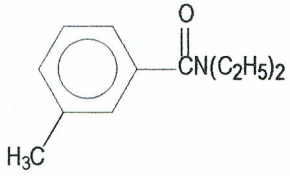
Repellents are substances that are able to protect an organism or derived products from predators or parasites. Repellents are usually employed where it is undesirable to use an insecticide. There are several synthetic and natural insect repellents currently in use. A repellent must show optimal degree of volatility, making it possible for the vapour concentration to be maintained at the protected surface without evaporating so quickly. The efficacy of a repellent depends on the frequency and uniformity of application, the number and species of the organisms attempting to bite, the users inherent attractiveness to blood-sucking arthropods and the overall activity level of the potential host (Schreck, 1995). The overall protection against various pests and vectors is greatly extended by the use of mixtures. The mosquito repellents currently available must be applied to all exposed areas of the skin. The ideal applications of repellents are those that are applied to clothing, gloves and bed nets where the protection time is extended to a week or more (Kirk and Othmer, 1981).

The properties of a good repellent against blood-sucking insects include effective protection of the treated area for several hours (on all types of subjects) under all climatic conditions, complete freedom from toxicity and irritation when regularly applied to human or animal skin, cosmetic acceptability (including freedom from unpleasant odour, taste and touch, harmlessness to clothing), protection against wide variety of biting insects, low cost and availability (Shambaugh *et al.*, 1958). No repellent has all these properties.

N, N-Diethyl-3-methylbenzamide or (N, N-diethyl-*m*-toluamide) (DEET) (**54**) remains the gold standard of commercial insect repellents (Coleman *et al.*, 1993; Gupta and Rutledge, 1994). It is a broad spectrum repellent that is effective against mosquitoes, biting flies, jiggers, fleas and ticks (Fradin, 1998). Fifty years of empirical testing of more than 20,000 other compounds has not resulted in a better product with the duration of protection and broad-spectrum of efficacy as DEET (Schreck, 1995; Quarles, 1996). Commercial products are formulated as aerosol and pump sprays, creams, lotions, solutions, gels, sticks, foams, towelets containing DEET at concentrations ranging between 5-100% (Yap, 1986; Fradin, 1998). DEET has had remarkable safety profile during more than 40 years of use by millions of people worldwide, but it might cause side effects such as cardiovascular disturbances, encephalopathies, allergic and pschotic reactions in isolated cases (Zadikoff, 1979; Robbins and Cherniack, 1986; Edwards and Johnson, 1987; Leach *et al.*, 1988; Qui *et al.*, 1998). It

can damage plastics (watch crystals and eyeglasses frames), rayon, spandex, and other synthetic fibres, leather, painted or varnished surfaces but not natural fibres (cotton, wool) and nylon.

In addition to DEET, other promising structurally similar synthetic chemical repellents include *n*-propyl-N, N-diethylsuccinimate (**55**), *o*-chloro-N, N-diethylbenzamide (**56**) (Metcalf and Flint, 1962), IR 3535 (**57**), KBR 3023 (**58**) and AI3-37220 (**59**) (Walker *et al.*, 1996; Klun *et al.*, 2000; Debboun *et al.*, 2000).



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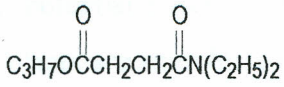

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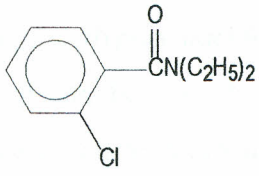

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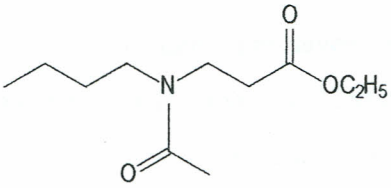

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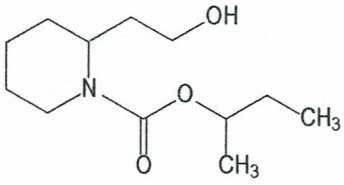

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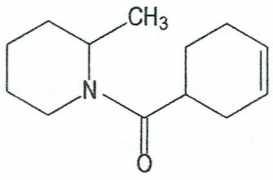

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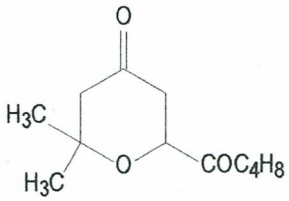
Other synthetic repellents that have been used to control mosquito bites include *n*-butyl-6,6-dimethyl-5, 6-dihydro-1, 4-pyrone-2-carboxylate (**60**), *cis*-dimethylbiyclo [2.2.1] -5-heptane-2, 3-dicarboxylate (**61**), dimethyl phthalate (**62**), 2-ethyl-2-butyl-1, 3-propanediol (**63**) and 2-phenylcyclohexanol (**64**).



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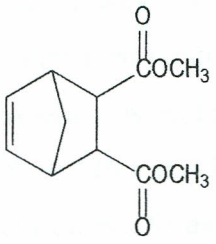

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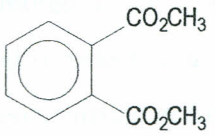

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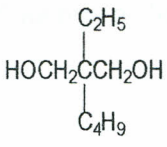

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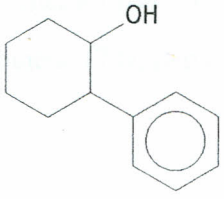

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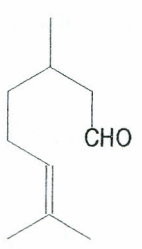
Synthetic repellents may have their disadvantages such as toxicity exhibited by DEET. Consequently, the search for natural repellents must be continued with the aim of discovering safe anti-vector products that can be used in the management of diseases like malaria.

Indigenous rural communities have used plants such as *Ocimum* spp., *Lantana* spp. (Dua *et al.*, 1996), *Azadiracta indica, Ajuga remota* (White, 1973), *Artemisia* spp., *Calamus* spp. (Hwang *et al.*, 1985), *Hyptis suaveolens* Poit, *Tagets minuta* L.(Seyoum *et al.*, 2002a) amongst others to repel mosquitoes. It has also been shown that *Corymbia citriodora* Hook (formerly *Eucalyptus maculata citriodon*) and *Lippia ukambensis* Spreng are potential repellent plants, which can be used by the local people (Seyoum *et al.*, 2002a). These plants are smouldered to produce chemical compounds that repel the mosquitoes (Snow *et al.*, 1987a.). Essential oils extracted from such repellent plants have been used. Plants whose essential oils have been reported to have repellent activity include; citronella, verbena, pennyroyal, geranium, lavender, pine, cajeput, cinnamon, rosemary, basil, thyme, allspice, garlic, and peppermint (Brown and Herbert, 1997). *Conyza newii, Tarchonanthus camphoratus* (Compositae); *Lippia javanica, L. ukambensis* (Verbenaceae); *Plectranthus marruboides* and *Tetradenia riparia* (Labiatae) oils have been reported to have both repellent and mosquitocidal activities against *An. gambiae* (Omolo, 2002). Most of the essential oils tend to give transient protection (<2 hours) (Barnard, 1999). Readily available plant-derived repellents include citronellal (**65**), which is the active ingredient of most natural (herbal) insect repellents. Citronella oil, which contains citronellal (**65**), geraniol (**66**) and citronellol (**67**), was originally extracted from lemon grass (*Cymbopogon nardus)* and shown to be an effective repellent with shorter protection time than DEET-based products (Fradin, 1998). Citronellal-based commercial products include Natrapel®, Avon Skin-So-Soft® and Buzz Away® (Fradin, 1998). Citronella candles have also been promoted as an effective mosquito repellent in the backyard (Fradin, 1998). Bite Blocker®, a plant-based repellent, is a combination of soybean oil, geranium oil and coconut oil in a formulation that has been available in Europe for many years (Lindsay *et al.*, 1996). Other essential oils that have been extracted from some plants with repellent activity include linalool (**68**), 1, 8-cineole (**69**), *p*-menthane-3, 8-diol (**70**), camphor (**71**), 1, 8-menthanediol (**72**), α-pinene (**73**), β-pinene (**74**) (Klocke *et al.*, 1987; Curtis, 1990).



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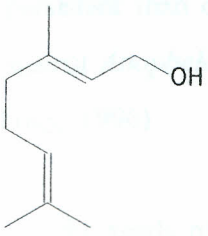

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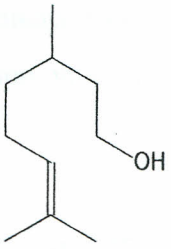

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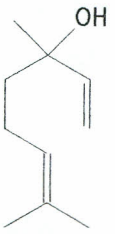

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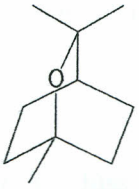

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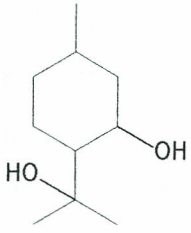

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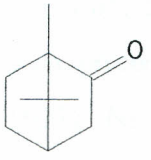

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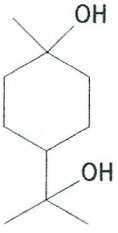

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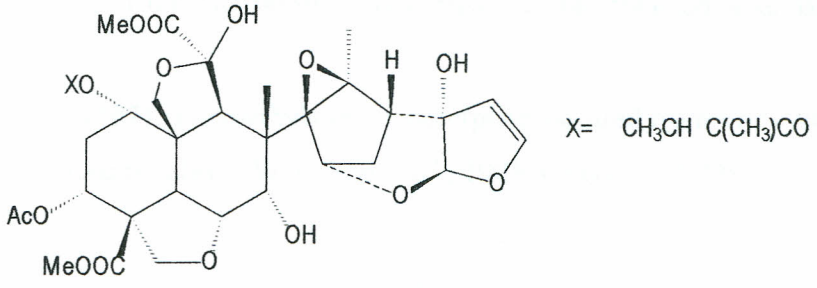
Pyrethrins, which are known for their excito-repellent and insecticide activity, have been used in keeping away mosquitoes (WHO, 1984).

C. citriodora (lemon eucalyptus) has been found to be an effective repellent by both thermal expulsion and direct burning against *An. gambiae* (Seyoum *et al.*, 2002b). It has long been known that its oil can repel mosquitos and that its principle ingredients are citronellal, citronellol, geraniol, isopulegol, δ-pinene and sesquiterpines (Curtis *et al.*, 1991). Although the topical repellency of these ingredients is very low (≤1 hr protection) against *Aedes aegypti* Linn. (Curtis *et al.*, 1991), clothes treated with quwenling (the waste distillate remaining after extraction of essential oil from lemon eucalyptus (Curtis *et al.*, 1990) are more than twice as effective as the same dosage of DEET (Schreck and Leonhardt, 1991). The major ingredient in the repellent waste distillate was reported to be *p*-menthane-3, 8-diol (**70**), with low amounts of other terpene alcohols. The most abundant minor component was dioctyl phthalate, which provided transient protection (23 minutes) from bites of *Ae. aegypti* (Schreck and Leonhardt, 1991).

Quwenling, from the waste distillate of lemon eucalyptus (*Eucalyptus maculata citriodon*) extract is repellent to mosquitoes and has replaced dimethyl phthalate (DMP) in China (Collins *et al.*, 1993). The principal active component is *p*-menthane-3, 8-diol (**70**) (Collins *et al,* 1993). It is used in a formulation, which is principally (50%) **70** with additional isopugenol and citronellol, the repellent

effect of which is more persistent than citronella and nearly as effective as DEET (Trigg and Hill, 1996). Field tests of **70** against *Anopheles* spp. in Tanzania showed 6-7 hours repellency, which was comparible with DEET (Trigg, 1996).

Essential oil extracted from the seeds of the neem tree, *Azidirachta indica* A. Juss (Meliacea) is known for insecticidal and repellent properties (Sharma *et al.*, 1993a; Mulla and Su, 1999). Neem oil formulation (2%) in coconut oil prevented bites by anopheline mosquitoes inside dwellings in villages in India for 12 hours (Sharma *et al.*, 1993b). Using a mixture of neem oil and kerosene in lamps has been shown to provide personal protection from the bites of some anopheline mosquitoes (Sharma and Ansari, 1994; Pates *et al.*, 1997). Neem oil contains several terpenoids, steroids, alkaloids, flavonoids and glycosides (Kumar and Parmar, 1996). Neem extract contains non-volatile insecticidal compounds with the most active component being azadirachtin (**75**). The main disadvantage of neem extracts is their instability to light (Butterworth and Morgan, 1971).



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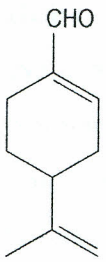
Oxygenated monoterpene essential oils; perillaldehyde (**76**), perill alcohol (**77**), verbenol (**78**), geraniol (**66**), carveol (**79**), and citronellal (**65**) have shown potent repellent activity against *An. gambiae* (Omolo, 2002). Two oxygenated sesquiterpenes, nerolidol (**80**) and caryophyllene oxide (**83**) have also shown promising activity. The compounds are being evaluated for commercial application (Omolo, personal communication).



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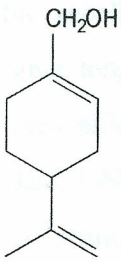

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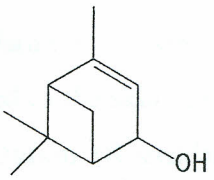

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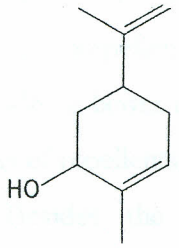

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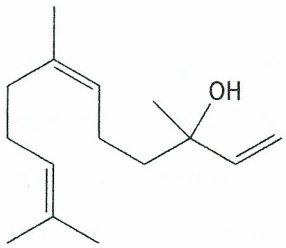

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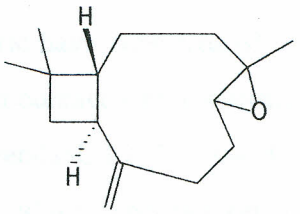

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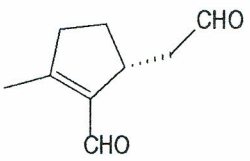
Perillaldehyde (**76**) and perilyl alcohol (**77**) showed remarkable mosquitocidal activity against *An. gambiae* (Omolo, 2002) in the laboratory tests carried out but their commercial value is yet to be established.

The isomers of rotundial (**82**), a demethylated monoterpene, isolated from *Vitex rotundifolia* have also been found to be mosquito repellent (Watanabe *et al.*, 1995; Grayson, 2000).



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The many insecticidal or repellent compounds discovered from plants suggest that plants may be useful sources of safe anti-mosquito products.

2.1.4.1 Repellent or toxicants on fabric

The use of arthropod repellents on the skin and toxicants on fabric are effective strategies for preventing insect attack. Currently, the standard combination approach for the defence against blood-sucking and disease-bearing arthropods is to use DEET on the exposed skin and wear clothing that has been treated with permethrin (Schreck *et al.*, 1984).

DEET or other volatile repellents can be adsorbed into cotton fabric to form a reservoir that evaporates slowly to give long-term repellence with limited skin contact. Repellents applied to clothing usually retain their efficacy longer than on the skin because they adhere better to cotton and synthetic fibres (Rozendaal, 1997). Compared to the skin, there is little loss of repellents from clothes because of abrasion, absorption, or perspiration (Rozendaal, 1997). Besides, the human body temperature is always higher than that of the fabric.

Mosquito nets and other types of fabric have been treated with pyrethroids, such as permethrin, that repel insects at a distance, and when in contact with the treated fabric, the insects are irritated or killed before they can feed on the host (Rozendaal, 1997). Insecticides that are currently recommended for treatment of mosquito nets include alpha-cypermethrin suspension concentrate (SC), cyfluthrin emulsion oil in water (EW), deltamethrin SC and water dispersible tablet (WT), etofenprox EW, lamba-cyhalothrin capsule suspension (CS), and permethrin emulsifiable concentrate (EC) (WHO, 1999a).

The toxicity to non-target organisms and resistance developed against these synthetic insecticides or repellents dictates the need for continuous research for alternatives from plants.

2.2 Plants

2.2.1 Secondary metabolites

Several definations of secondary metabolites have been advanced. These are by-products of metabolism, which are formed by metabolic activities but are no longer used for formation of new cells (Hartmann, 1996). Any importance of these compounds to the inner economy of the plants is yet to be known. Secondary compounds are secondarily utilized for the defense against predators and or ecological interactions with beneficiary insects; they may act as chemical signals in the environment (Visser *at al.*, 1979).

Secondary metabolism can be regarded as the functional level of plant metabolism that is dispensable for growth and development (Table 1) (Hartmann, 1996).

Table 1: Primary and secondary metabolism: Two functional levels with entirely different characteristics (Hartmann, 1996).

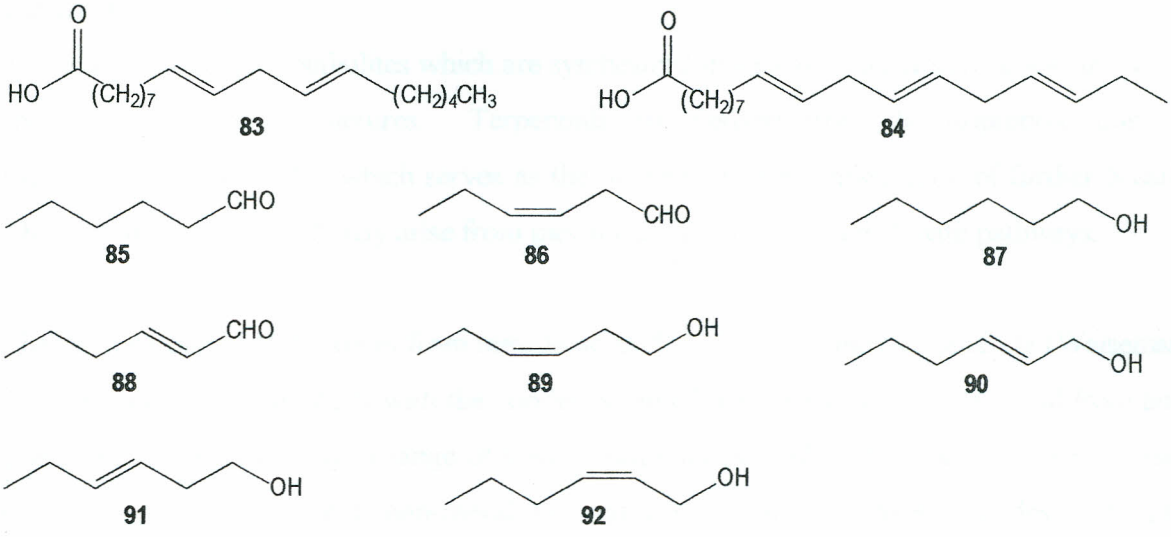
PRIMARY METABOLISM	SECONDARY METABOLISM
Growth and development for individual • Indispensable • Uniform • Universal • Conservative	Interaction of the individual with its environment • Dispensable for growth and development • Indispensable for survival of a population • Unique • Diverse • Adaptive

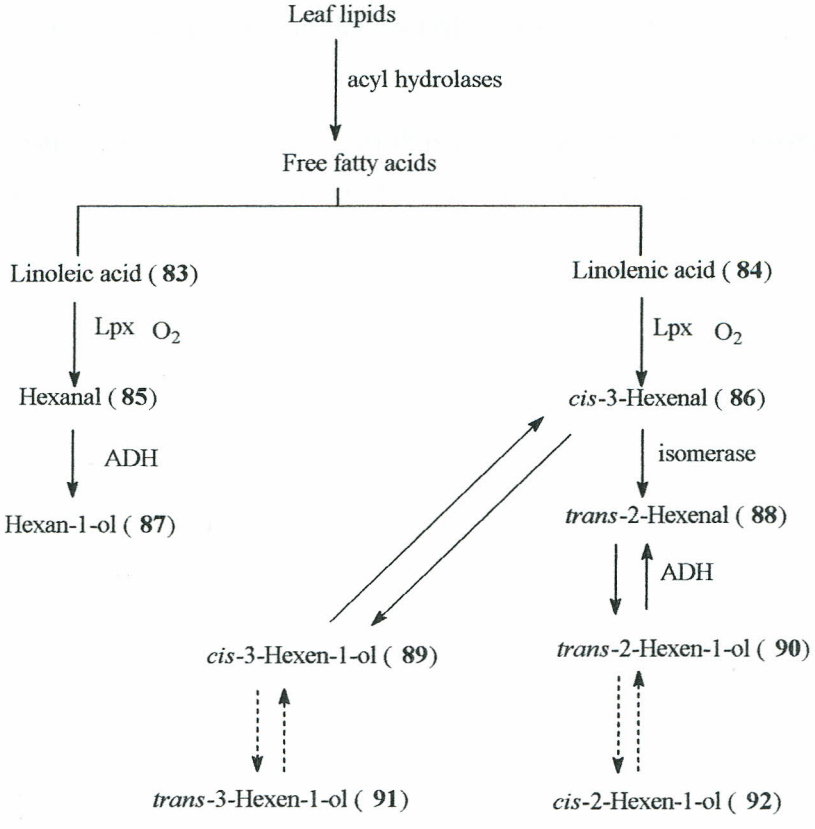
As uses for what was originally regarded as secondary metabolites are discovered, the boundary between secondary and primary metabolites becomes less distinct.

2.2.1.1 Green leaf volatiles

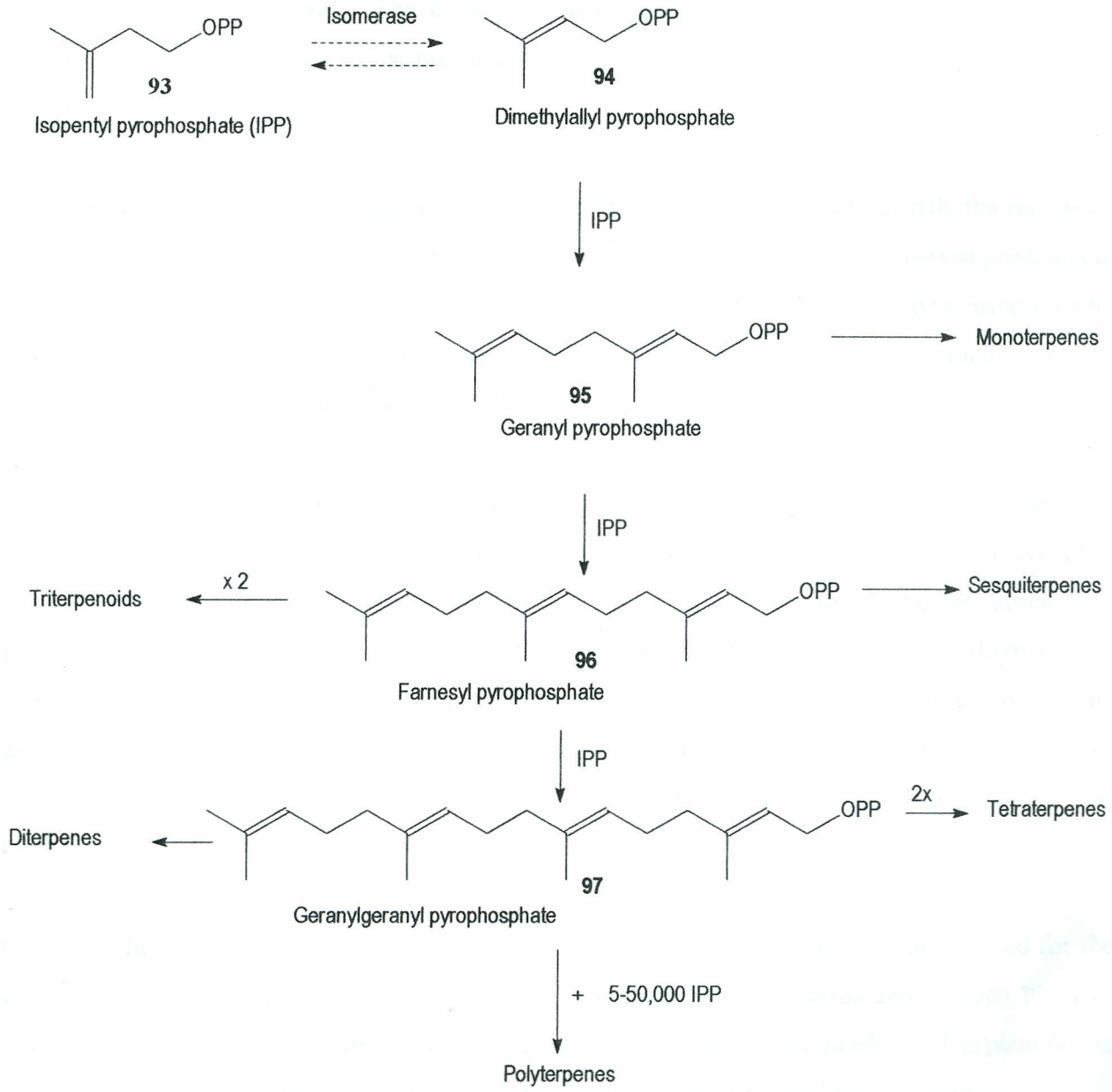
These are aliphatic six-carbon primary alcohols, aldehydes and acetates. Green leaf volatiles (GLVs) results from oxidative degradation of fatty acids and include; 2-hexenal, 1-hexanol, 2-hexen-1-ol and 3-hexen-1-ol and corresponding esters. The leaf aldehydes and alcohols have been reported as volatile components of numerous plant species belonging to a varierty of plant families (Visser *et al.*, 1979). The polyunsaturated fatty acids like linoleic (**83**) and linolenic (**84**) acids are oxidized by the action of lipoxygenase to hexanal (**85**) and *cis*-3-hexenal (**86**), respectively (Scheme 1) (Visser *et al.*; 1979; Galliard and Mathew, 1977; Wardale and Galliard, 1977; Kazeniac and Hall, 1970).

Alcohol dehydrogenase (ADH) in the presence of a coenzyme NADH converts hexanal to 1-hexanol (**87**), whereas *cis*-3-hexenal (**86**) is easily converted to *trans*-2-hexenal (**88**) with the help of isomerase and eventually to *cis*-3-hexen-1-ol (**89**). *Trans*-2-hexenal (**88**) leads to formation of *trans*-2-hexen1-ol (**90**). *Trans*-3-hexen-1-ol (**91**) and *cis*-2-hexen-1-ol (**92**) are believed to originate from isomerization during processing and storage of plant products. These biosynthetic routes are widely distributed in several plant species and are responsible for some volatiles in essential oils of many plants (Galliard and Mathew, 1977; Grosch *et al.*, 1976; Tappel, 1961).





Scheme 1: Biosynthesis of leaf aldehydes and alcohols constituting GLVs (Visser *et al.*, 1979).



Scheme 2: Biogenesis of terpenoids (Tedder, 1972).

The mono and sesquiterpenes are volatile compounds and their mixture is commonly referred to as essential oils. C_{20} and higher terpenes are non-volatile and are often referred to as higher terpenoids. Monoterpenes are C_{10} hydrocarbons or their oxygenated analogues. Biosynthesis of monoterpenoids involves the linking of isopentyl pyrophosphate (IPP) and dimethyl allylpyrophosphate (DMAP) to give geranyl pyrophosphate (GPP) and with elimination of a phosphate group via a cation (Hartmann, 1996; Pinder, 1960). Sesquiterpenes are C_{15} hydrocarbons or their oxygenated analogues. They arise

from farnesyl pyrophosphate. The cyclic sesquiterpenoids arise from the cyclisation of farnesyl pyrophosphate and subsequent rearrangements of the resulting carbonium ions (Nakanishi *et al.*, 1974).

Coincidentally the terpenoid constituents of the essential oils from plants are usually the repellent or insecticidal principles. Consequently they play a significant role in plant defense against predators and pests. Coincidentally, they may have the same effect on non-predator or non-pest insects such as mosquitoes. Essential oils and terpenoids from plants have shown repellency to adult mosquitoes (Curtis *et al.*, 1989, Leung and Foster, 1996).

The repellent properties of plants to mosquitoes and other insect pests were well known before the advent of synthetic chemicals (Curtis *et al.*, 1989). Smoke from open fire prevents insect attack, purportedly as a result of the repellent effect created by burning aromatic wood or other resinous plant parts (Curtis *et al.*, 1989; Snow *et al.*, 1987b). Neem oil has been reported as an alternative safe method of protection against mosquitoes (Sharma *et al.*, 1993b). A review on the use of botanical derivatives against mosquitoes has been given (Sukumar *et al.*, 1991). Over 1,000 plants have been reported to have anti-insect activity (Roark, 1947). Most natural repellents and folk remedy remains to be scientifically evaluated for their efficacy (Rozendal, 1997).

Owing to diversity in nature, it is believed that there are several plants not yet investigated for their insecticidal and repellent activity. The research project was geared towards investigating bio-active phytochemicals, from the flora of the coastal region of Kenya, against *An. gambiae*. The plant families considered included Annonaceae, Compositae, Euphorbiacae, Labiatae and Verbanaceae.

2.2.2 Annonaceae

A large family of about 120 genera and over 1000 species practically confined to tropical and subtropical regions, particularly in lowland vergreen forest (Verdcourt, 1971).

Members of this family have alternate leaves without stipules. They also have 2-3 sepals, 6 petals in one or two whorls and many stamens. The fruits are either syncarpous or consist of many free "monocarps" (Beentje, 1994).

Annona charimola, A. glabra, A. squamosa have been investigated and found to show larvicidal activity with *A. squamosa* also showing growth inhibition of mosquito larvae (Sukumar *et al.*, 1991).

2.2.2.1 *Mkilua* spp

This is a monotypic genus erected for a coastal E. African species which does not fit well into any genus previously known (Verdcourt, 1971).

2.2.2.1.2 *Mkilua fragrans*

This is a shrub or a tree (2.5-10 m). Its leaves are elliptic, base asymmetric and cuneate, apex obtuse to bluntly acuminate, glabrous except in young leaves (Plate 3). It has cream, yellow or orange flowers with a purple patch at the base, pendulous, in extra-axillary 1-3 flowered cymes; petals are 2-3.5 cm long. The flowers are usually sweetly scented. Fruit monocarps are red and oblong (Verdcourt, 1971; Beentje, 1994). Very little is known about this plant locally and it is believed to have found itself in Kenyan coast from Zanzibar where the flowers are widely used for perfumery by the Arab women (Verdcourt, 1971). The plant is distributed in Kenya (Kwale District-about half-way between Lungalunga and Msambweni, Mirima Hill), Tanganyika (Tanga district) and Zanzibar. It is found in evergreen forest and semi-deciduous coastal forest in the altitude 0-400 m. The plant was collected from Shimba Hills National Reserves at Mwele-Mkubwa.

Plate 3: ***Mkilua fragrans***

2.2.3 Euphorbiaceae

A very large family of some 300 genera and 5,000 species, the sixth largest among the flowering plants (after the *Orchidaceae*, *Compositae*, *Leguminosae*, *Gramineae* and *Rubiaceae*), sub-cosmopolitan, but with the greatest presentation in the humid tropics and subtropics of both hemispheres. In East Africa there are 77 genera and some 510 species, which represent a quarter of the total genera, and a tenth of the total species (Smith, 1987). The plants are monoecious or dioecious. Stipules, but may be quickly caduceus. Inflorescence is variable; flowers unisexual, usually small; calyx of 3-6 lobes or sepals; petals often absent; disk often present. Stamens are usually 3, ovary superior, usually 3-celled; styles usually three. Fruit often 3-lobed (Beentje, 1994).

2.2.3.1 *Croton* spp

A pantropical genus of about 800 species of which about 50 are African. Only 23 species occur in East Africa. *Croton tiglium* L., a native of tropical Asia, is sometimes cultivated for the seed oil, which has powerful purgative properties (Smith, 1987). Plants of this genus are mostly monoecious, often with stellate hairs or scales on the leaves. Leaves are alternate or sub-reticillate, simple, usually with two glands at the base of the blade; stipulates often present but caduceus. Infloresence mostly terminal, racemose, usually with flowers which has 4-6 petals. Fruit a dehiscent capsule which is 3-ported. Some species in this genus like *C. pseudopelchellus* have been used traditionally to repel insects (Kokwaro, 1993).

2.2.3.1.2 *Croton menyharthii*

This is a shrub (1.2-5 m) with several stems and virgate branches (Plate 4). The leaves are silvery beneath, turns orange when old, ovate, base rounded or sub-cordate, apex obtuse or emarginated, thinly stellate-pubescent above and densely scaly beneath. It has yellow flowers, which are monoecious in long racemes. It has trilobed yellow fruits with black spots (Beentje, 1994). The plant is found in *Aloe-Sasevieria* thickets and bushland ranging from *Terminalia-Combretum* type to dune bushland. It is locally known as Miama Wanyika (Giriama), Alkadhi (Pokomo) and Kobole (Borana and Somali). The plant was collected from Taru (on the way to the KBC transmitter station).

The Pokomo inhale the smoke of burnt leaves against pregnancy and menstrual pains (Beentje, 1994). Root decoction is drunk for the treatment of influeza and malaria (Kokwaro, 1993).

Plate 4: ***Croton menyharthii***

2.2.3.1.1 ***Croton pseudopulchellus***

It is a shrub or a small tree (1-6 m) with a rough bark, which is brown or pale grey. The leaves are sub-verticulate, silvery beneath, and doted brown, elliptic, base cuneate or less often rounded, apex obtuse, emerging or acute. It has white flowers which are monoecious and sometimes diocious in globular clusters. It is a common understory shrub of the drier lowland forests or woodland and is also common in parts of coastal evergreen bushland (Beentje, 1994). The plant was collected from Arabuko at Mida Creek (main entrance).

Locally known as Mwiani (Kilifi), Mukwamba (Digo), Mukunapaa (Swahili) and Muyama (Giriama). Leaves of the plant are boiled and applied to chest for colds. For treatment of asthma, a decoction of the roots are drunk. The juice from the boiled leaves and twigs are drunk for the treatment of gonorrhoea. An infusion of the leaves is given to cattle as a remedy for anthrax. The leaves are also burnt among crops as an insecticide (Kokwaro, 1993). The plant is traditionally used in South Africa to treat pulmonary diseases (Lall and Meyer, 1999).

2.2.4 Labiatae (Lamiaceae)

They are herbs or shrubs, often with square stems. Leaves are opposite and often aromatic. Flowers usually bisexual; corolla zygomorphic, 2-lipped, stamens 2-4; style emerging from the base of the ovary. Fruit consist of four small nutlets, mostly hidden in the persistent calyx (Beentje, 1994).

Some plants from this family have been used traditionally to ward off mosquitoes. Herbs of the mint family (Lamiaceae) have been used as mosquito repellents in East and West Africa (Curtis *et al.*, 1989). Shrubs of the genus *Ocimum* have been employed widely in Africa for their reputed mosquito repellent properties (White, 1973). Plants from the related Labiatae family like *Hyptis* spp. and *Leucas* spp. are also used. The plants are burnt to create mosquito-repellent smoke. Some communities use freshly harvested twigs or branches of the shrubs indoors at dusk. The foliage is banged sharply on the ground or the wall so as to enhance the plant odour realease (Kokwaro, 1993).

2.2.4.1 *Endostemon* spp

The genus Endostemon is widespread in tropical Africa with one species in India. It has 18 species (Paton *et al.*, 1994). This genus consist of shrubs or herbs with almost stalkless simple leaves and terminal racemes of few-red whorls or purple flowers; sepal tube bilaterally symmetrical with an expanded more or less circular upper lobe overlapping and covering linear to oblong lower teeth which curve upwards, all becoming stiff in fruit; petals hardly 2-lipped, lower side longer; stamens four, included in petal tube arching downwards; nutlets smooth or irregularly pitted, ovoid, rounded (Agnew, 1994).

2.2.4.1.1 *Endostemon tereticaulis*

It is an erect wiry annual or short-lived perennial herb, which grows to a height of 50 cm and is covered in sparse long hairs (Plate 5). Its leaves are toothed, lanceolate, with hairy veins and mildly aromatic. The flowers are small, purple and usually occur in pairs. The flowers have lateral sapal lobes, which are almost square at the apex with a central tooth and are long as the lower lobes (Agnew and Agnew, 1994). The plant is common in wooded grassland and *Acacia-Commiphora* bush land, especially on rocky shallow soils. The plant was collected from Taru (on the way to the KBC transmitter station).

Plate 5: ***Endostemon tereticaulis***

2.2.4.2 *Ocimum* spp

The genus *Ocimum*, collectively known as basil has long been recognized as diverse and rich source of essential oils (Simon *et al.*, 1990). The genus contains 50-150 species of herbs and shrubs from the tropical regions of Asia, Africa, Central and South America. They have square stems, fragrant opposite leaves and whorled flowers on spiked inflorescences.

The essential oils of *Ocimum basilicum* L. extracted via steam distillation from the leaves and flowering tops have been used to flavour foods, as dental and oral products, in fragrances, perfumes, traditional rituals and medicines (Guenther, 1949). Extracted essential oils have also been shown to contain biologically active constituents that are insecticidal (Deshpande and Tipnis, 1977; Chogo and Crank, 1981; Chavan and Nikam, 1982), nematicidal (Chatterjee *et al.*, 1982), fungistatic (Reuveni *et al.*, 1984) or anti-microbial (Ntezurubanza *et al.*, 1984). These properties have been frequently attributed to predominant essential oil constituents such as methylchavicol, eugenol, linalool, camphor and methyl cinnamate. Two minor components of the essential oil of *O. basilicum* L, juvocimene I and II, have been reported as potent juvenile hormone analogs (Nishida *et al.*, 1984) with insect growth regulating activity. The *Ocimum* spp. characteristically contains linalool, methylchavicol and to a lesser extent 1, 8-cineole, α-pinene, β-pinene, myrcene, ocimene, terpinolene, camphor,

terpenene-4-ol, α-terpineol, eugenol and sesquiterpenes (Guenther, 1949; Heath, 1981; Fleischer, 1981). These may vary quantitatively and qualitatively from one species to another.

2.2.4.2.1 *Ocimum fischeri*

Perennial, erect or straggling, aromatic shrubby herb to 1 m tall with woody rootstock. Has woody stem at the base with twiggy branches. Leaves are petiolate, usually with fascicles of young leaves in axils, green yellow; blade glandular-punctate, often folded along midrib on drying, margins entire, apex obtuse, base attenuate; pubescent, with short antrorse hairs. Has terminal inflorescence which are red or pinkish. Corolla bright mauve or purple and funnel shaped with the bilabiatae lips well separated. The plant is distributed in coastal parts of Kenya and Tanzania in the altitude range of 3-500 m. The plant is common in *Acacia-Commiphora* bushland, often on rocky ground (Lukhoba, 2001). The plant was collected from Tsavo West National Park along the road to Ngulia Lodge next to Mombasa-Nairobi oil pipeline through Man Eaters Gate.

The main components of the plant essential oil had been reported to be fenchone (41%) and linalool (37%) (Mwangi *et al.*, 1994)

2.2.4.2.2 *Ocimum forskolei*

The plant was formerly known as *Ocimum ladiensis*. It is an erect annual and often woody plant. It has lanceolate, elliptic hairless leaves. The flowers are large (12 mm long), purple or pink above and white below in simple lax racemes (Plate 6). The inflorescence hairs are often long and tinged purple. It is common in *Acacia-Comiphora* bushland (Agnew and Agnew, 1994). The plant is distributed in Ethopia, Kenya, Somalia and Yemen. The plant was collected from Taru (On the way to the KBC transmitter station).

Plate 6: ***Ocimum forskolei***

2.2.4.3. *Plectranthus* spp

This genus consists of about 300 species commonly found in Africa south of the Sahara, Arabia, India and Australia. 35 species are indigenous to Kenya (Lukhoba, 2001). It consists of annual or perenial mostly aromatic herbs or shrubs. The stems are herbaceous to slightly woody, succulent or not, glabrous to villous, hairs mostly retrorse, becoming more pubescent and glandular towards the inflorescecence. Leaves opposite and deccusate, shapes varying from ovate, deltoid to elliptic, oblong to oborate, margins serate to dentate, apex rounded, acute or acuminate. The inflorescence are usually terminal, sometimes axillary, spicate, racemes or panicles. Flowers in verticals, occasionally solitary (Lukhoba, 2001).

Many essential oil components of plants from labiatae family have been used as pesticides but the pesticidal activity of *Plectranthus* species are suspiciously scanty, both from ethno-botanical surveys and modern scientific investigations, notwithstanding the fact that there are many species that are particularly aromatic like *P. amboinicus*, *P. pseudomarrubiodes* and *P. cylindraceus* that could contain pesticidal substances (Lukhoba, 2001).

2.2.4.3.1 *Plectranthus longipes*

This is a succulent hairy herb with erect or semi-prostrate stems and circular, round-toothed leaves (Lukhoba, 2001) (Plate 7). The stems are succulent to woody. It has bright blue flowers with fruiting sepals about 4.55 mm long. The upper lip of the flowers are winged and curved back with the lower petal lip being about 7 mm long. It is found in evergreen woodland places, moist shaded thickets in dry rocky grassland, rock edge savanna bushland or disturbed cultivated arrears in the altitude of 60-2590 m (Agnew and Agnew, 1994; Lukhoba, 2001). The plant is distributed in Kenya (coastal), Ethopia, Eritrea, Uganda and Tanzania. The plant was collected from Taru (on the way to the KBC transmitter station).

Plate 7: ***Plectranthus longipes***

2.3 Hypothesis

The working assumption of the study was that there are repellent and insecticidal plants that have not been investigated which may contain phytochemicals useful for mosquito control.

2.4 Objectives

The general objective was to screen plants, from the families: Compositae, Labiatae, Euphorbiacae, Verbanaceae and Annonaceae from Coastal region of Kenya for their repellent and insecticidal activity against *An. gambiae* mosquitoes. To achieve this, specific objectives included:

- Screening and identification of candidate insecticidal/repellent plants.
- Extraction of potential insecticidal repellent plants.
- Detailed repellency and insecticidal assays on selected plants.
- Identification of the active component(s) in selected plants.
- Detailed repellency and insecticidal assay of the identified component(s).
- Evaluation of the protective efficacy of the identified component(s) against *An. gambiae* mosquitoes.

2.5 Justification

Although synthetic insecticides have been found to be of high efficacy against the target species they have adverse environmental effects. Physiological resistance and low susceptibility to many of these compounds have been reported.

Chemotherapy and prophylaxis have also been employed as malaria control strategy but with the increasing malarial resistance to drugs worldwide, these methods cannot be considered completely reliable.

DEET, which was once considered a golden standard of topical repellents, is proving to be less effective. Its application to the skin remain effective for only a few hours and even sophisticated formulations designed to extend the protection time, such as micro-encapsulation, do so only by a few hours (Mehr *et al.*, 1985). The main malaria vector in the tropics, *An. gambiae* has been found to be less susceptible to DEET (Curtis *et al.*, 1987).

Phytochemicals derived from various botanical sources have provided numerous beneficial compounds ranging from pharmaceuticals to insecticides. Thousands of plants have been tested as potential sources of insect repellents and insecticides. *p*-Menthane-3, 8-diol from *Eucalyptus citriodora* and pyrethrins from *Chrysanthemum* spp are living examples of effective plant-derived repellant (Schreck and Leonard 1991; Curtis, 1990).

There is therefore a need for investigation of phytochemical, repellents and insecticides against *An. gambiae*, which are more selective, potent, bio-degradable and user friendly.

CHAPTER 3

SCREENING FOR ANTI-INSECT PLANTS

3.1 Introduction

Search for bio-active principles normally entails bio-evaluation by making use of target organism. Bio-evaluation is the examination of biological resources (for example, plants, animals, micro-organisms) for features that may be of value for commercial development. It focuses on the discovery, and commercialization of valuable biological features. It concerns search for new chemicals: organic compounds in living things that will have some medical or commercial use. Bio-evaluation has the potential to uncover highly valuable bio-rational alternatives to synthetic organic products.

As is the case in the rest of the world, Kenya's biological diversity is being lost at an increasingly rapid rate, largely through activities like agriculture, urban development, deforestation, mining, dam construction, and over-exploitation. This calls for enhanced evaluation to discover the potential of bio-diversity to human life.

There are three main approaches in bio-evaluation of anti-arthropod botanicals that may contain new biological agents or provide crucial leads:

- Traditional knowledge: Ethnobotanical information is an important component of bio-diversity evaluation efforts, and has provided leads in a number of plant-based anti-arthropod products in the market today. Traditional information on the indigenous insecticidal use of a plant is important for plant collection. This is a suitable criterion for the selection and collection of botanical materials from communities that largely rely on botanical products for arthropod management.
- Taxonomic approach: Plants which belong to a given family or genus which had shown reliable anti-arthropod activity are sought for from probable localities of habitation.
- Chemotaxonomic approach: Plants which are known or suspected to contain particular class of compounds are targeted for bio-evaluation for anti-arthropod activity.

Several botanicals offer great promise as sources of phytochemicals for the control of mosquitoes. Plant families such as Labiatae/Lamiaceae, Compositae, Verbeneceae, Astaraceae, Cladophoraceae, Meliaceae, Oocystaceae and Rutaceae appear to have great potential as sources of anti-mosquito agents (Sukumar *et al.*, 1991; Omolo, 2002). Most members of these families have not been evaluated chemically or biologically. In this research project, 24 plants were investigated based on taxonomic information.

3.2 Extracts

The collected plant materials were extracted by both hydrodistillation and solvent (water, dichloromethane or chloroform). The yields for the individual plants extracted are presented in table 2.

Table 2: Yield of plant extracts

		Essential oil			Solvent extract		
PLant	Family	Material (g)	Amount (g)	% Yield	Material (g)	Amount (g)	% Yield
Mkilua fragrans	Annonaceae	5133.5	4.200	0.08	263.8	1.3	0.49
Orphrypeletum ordoratum	Annonaceae	897.5	0.127	0.01	456.1	2.6	0.57
Bidens schimperi	Compositae	898.8	0.198	0.02	373.0	1.3	0.35
Vernonia hildebrandtii	Compositae	2689.8	4.700	0.17	194.5	1.2	0.62
Vernonia sp A	Compositae	375.0	___	___	145.5	1.6	1.10
Croton megalocarpoides	Euphorbiaceae	1737.9	2.100	0.12	244.6	1.3	0.53
Croton menyharthii	Euphorbiaceae	2055.2	4.900	0.24	431.5	2.1	0.49
Croton pseudopulchellus	Euphorbiaceae	2442.0	4.300	0.18	1014.2	9.7	0.96
Croton sylvaticus	Euphorbiaceae	1066.2	0.041	0.00	617.7	1.0	0.16
Endostemon alba	Labiatae	912.3	0.100	0.01	372.4	1.3	0.35
Endostemon tereticaulis	Labiatae	1381.4	2.500	0.18	418.5	1.1	0.26
Erythroclamys spectabilis	Labiatae	644.4	0.140	0.02	351.0	2.7	0.77
Ocimum fischeri	Labiatae	2064.9	8.500	0.41	341.7	1.3	0.38
Ocimum forskolei	Labiatae	1057	21.560	2.04	415.1	1.5	0.36
Plectranthus cyneus	Labiatae	2019.0	0.200	0.01	745.7	1.3	0.17
Plectranthus flacifolius	Labiatae	547.2	0.872	0.16	218.9	1.3	0.59
Plecranthus longipes	Labiatae	4584.1	4.900	0.11	897.0	1.1	0.12
Plectranthus tellensis	Labiatae	2995.5	0.042	0.00	748.9	1.0	0.13
Clerodendrum incisum	Verbeneceae	658.4	___	___	139.1	3.9	2.80
Lippia carviodora	Verbeneceae	1428.0	1.000	0.07	432.0	1.4	0.32
Premna chrysoclada	Verbeneceae	534.0	0.053	0.01	304.9	2.7	0.89
Premna resinosa	Verbeneceae	1552.4	0.458	0.03	379.5	1.2	0.32
Vitex ferruginea	Verbeneceae	407.4	___	___	561.7	1.7	0.30
Vitex mombasae	Verbeneceae	2007.3	0.700	0.03	473.2	1.3	0.27

O. forskolei had the best yield of essential oil at 2.04% followed by, *C. menyharthii* (0.24%), *C. pseudopulchellus* (0.18%), *E. tereticaulis* (0.18%), *V. hildebrandtii* (0.17%), *P. flacifolius* (0.16%), *C. megalocarpoides* (0.12%), *P. longipes* (0.11%) and *M. fragrans* (0.08%). For the solvent extracts, *C. incisum* had the highest yield at 2.80% followed by *Vernonia sp A* (1.10%), *C. pseudopulchellus* (0.96%), *P. chrysoclada* (0.89%), *E. spectabilis* (0.77%), *V. hildebrandtii* (0.62%), *O. ordoratum* (0.57%) and *C. megalocarpoides* (0.53%).

3.3. Repellency assays

The three extracts; water, chloroform or dichloromethane and essential oils were tested as repellents against *An. gambiae*. The general repellent activity of the extracts was in the order essential oil> chloroform or dichloromethane> water extract.

3.3.1 Repellency of the water extracts

The water extracts showed repellent activity in the range of 18.81-79.38%. *E. spectabilis* exhibited the lowest repellence activity (18.81%). Statistically, its activity was not significantly different from that of the 4 other plants (*P. longipes*, *P. resinosa*, *O. fischeri* and *P. flacifolius*). The other 20 plants exhibited superior activity. *L. carviodora* had the highest activity (79.38%) over the other plants. However, its activity was not significantly different from that of *M. fragrans*, *B. schimperi* and *P. cyneus*. Its activity was outstanding over the other 20 plants. The results are summarized in the table 3.

Table 3: Repellency of the water extracts

Plant	% Protective efficacy ± SE
Mkilua fragrans	71.43 ± 6.023^{ijk}
Orphrypeletum ordoratum	50.56 ± 8.320^{pqr}
Bidens schimperi	67.28 ± 4.225^{klm}
Vernonia hildebrandtii	56.24 ± 5.953^{pqr}
Vernonia sp A	56.46 ± 10.145^{pqr}
Croton megalocarpoides	41.67 ± 10.853^{uvw}
Croton menyharthii	61.63 ± 10.513^{nop}
Croton pseudopulchellus	54.68 ± 2.280^{pqr}
Croton sylvaticus	62.35 ± 3.377^{nop}
Endostemon alba	37.78 ± 17.301^{uvw}
Endostemon tereticaulis	44.21 ± 21.613^{u}
Erythroclamys spectabilis	18.81 ± 17.902^{z}
Ocimum fischeri	27.13 ± 10.111^{y}
Ocimum forskolei	47.58 ± 19.203^{rst}
Plectranthus cyneus	72.63 ± 6.904^{hij}
Plectranthus flacifolius	20.00 ± 7.005^{z}
Plecranthus longipes	34.26 ± 11.423^{vwx}
Plectranthus tellensis	38.75 ± 12.671^{uvw}
Clerodendrum incisum	54.70 ± 6.593^{pqr}
Lippia carviodora	79.38 ± 8.004^{fgh}
Premna chrysoclada	55.05 ± 4.542^{pqr}
Premna resinosa	31.75 ± 15.825^{wxy}
Vitex ferruginea	52.83 ± 3.157^{pqr}
Vitex mombasae	43.56 ± 7.262^{uv}

Means followed by same letter are not significantly different at $p<0.05$

3.3.2 Repellency of the organic solvent extracts

The percentage repellency of the DCM or chloroform extracts at 0.05 g/ml (5% solution) ranged from 57.17-97.44 %. Statistical analysis of the repellency data showed the superiority of *Ocimum forskolei*

($CHCl_3$) (97.44%), *Plectranthus cyneus* (DCM) (94.43%), *Croton pseudopulcheles* (DCM) (94.10%), *Vernonia hildebrandtii* ($CHCl_3$) (93.45%), and *Croton meryharthii* (DCM) (91.98%), even though their activity was not significantly different from that of *Bidens schemperi* ($CHCl_3$), *Clerodendrum incisum* (DCM), *Plectranthus chrysoclada* (DCM), *Plectranthus longipes* ($CHCl_3$), *Premna resinosa* (DCM) and *Erythroclamys spectabilis* (DCM). However, they were significantly more active than the 13 other extracts. The least active extracts were from *Plectranthus flacifolius* (DCM) and *Endostemon alba* (DCM) (Table 4). Subsequenly, the five leading extracts were subjected to detailed bio-assays.

Table 4: Preliminary *An. gambiae* repellency assay data for DCM or $CHCl_3$ plant extracts

	% Protective efficacy ± SE		
Concentration (g/ml)	5.0×10^{-6}	5.0×10^{-4}	5.0×10^{-2}
Mkilua fragrans (D)	58.89 ± 14.028^{pq}	48.40 ± 7.614^{rst}	71.47 ± 5.073^{ijk}
Orphrypeletum ordoratum (C)	-7.50 ± 23.585^{yz}	44.00 ± 5.776^{stu}	75.30 ± 8.602^{hi}
Bidens schimperi (C)	47.81 ± 9.876^{rst}	39.06 ± 7.593^{uvw}	73.19 ± 4.342^{hij}
Vernonia hildebrandtii (C)	29.81 ± 7.986^{y}	67.43 ± 7.167^{klm}	93.45 ± 4.366^{bc}
Vernonia sp A (C)	34.523 ± 5.386^{vwx}	46.43 ± 7.816^{stu}	62.74 ± 11.121^{nop}
Croton megalocarpoides (D)	42.42 ± 7.987^{uv}	53.43 ± 7.490^{pqr}	67.22 ± 8.902^{klm}
Croton menyharthii (D)	49.92 ± 10.151^{rst}	57.71 ± 4.587^{pq}	91.98 ± 2.635^{cd}
Croton pseudopulchellus (D)	62.62 ± 7.736^{nop}	67.51 ± 6.976^{klm}	94.10 ± 2.700^{bc}
Croton sylvaticus (C)	51.03 ± 11.668^{qrs}	52.117 ± 4.406^{qrs}	73.57 ± 3.717^{hij}
Endostemon alba (D)	33.14 ± 5.167^{wxy}	42.78 ± 4.125^{uv}	57.17 ± 5.672^{pq}
Endostemon tereticaulis (D)	32.83 ± 5.873^{wxy}	36.09 ± 8.093^{uvw}	58.78 ± 9.717^{pq}
Erythroclamys spectabilis (D)	66.56 ± 9.783^{klm}	69.46 ± 7.463^{jkl}	82.36 ± 4.606^{efg}
Ocimum fischeri (C)	43.95 ± 10.000^{uv}	46.10 ± 8.261^{stu}	71.83 ± 5.833^{ijk}
Ocimum forskolei (C)	43.98 ± 7.410^{uv}	37.64 ± 8.724^{uvw}	97.44 ± 1.658^{ab}
Plectranthus cyneus (D)	52.95 ± 7.939^{qrs}	62.50 ± 3.227^{nop}	94.43 ± 2.788^{bc}
Plectranthus flacifolius (D)	49.81 ± 6.063^{rst}	49.36 ± 11.946^{rst}	65.92 ± 5.469^{lmn}
Plecranthus longipes (C)	34.93 ± 11.507^{vwx}	47.02 ± 8.66^{rst}	85.43 ± 4.87^{de}
Plectranthus tellensis (D)	56.62 ± 5.575^{pqr}	52.80 ± 4.930^{qrs}	71.39 ± 11.631^{ijk}
Clerodendrum incisum (D)	39.32 ± 5.928^{uvw}	72.71 ± 6.705^{hij}	78.07 ± 5.895^{ghi}
Lippia carviodora (D)	31.65 ± 10.575^{wxy}	32.30 ± 8.756^{wxy}	71.540 ± 7.993^{ijk}
Premna chrysoclada (D)	49.37 ± 14.724^{rst}	48.413 ± 13.309^{rst}	88.69 ± 5.410^{cd}
Premna resinosa (D)	46.98 ± 16.487^{stu}	55.39 ± 8.286^{pqr}	84.00 ± 3.530^{def}
Vitex ferruginea (D)	40.97 ± 4.359^{uv}	50.88 ± 9.171^{rst}	71.32 ± 15.316^{ijk}
Vitex mombasae (D)	33.41 ± 9.009^{wxy}	58.94 ± 5.225^{pq}	73.19 ± 4.342^{hij}

Means followed by same letter are not significantly different at $p<0.05$, D = DCM, C = $CHCl_3$

3.3.2.1 Detailed mosquito repellency assay of active solvent extracts

Detailed evaluation of the active solvent extracts revealed the order of repellency to be *O. forskolei* > *C. pseudopulcheles* > *P. cyneus* > *C. menyharthii* > *V. hildebrandtii*. However, there was no significant difference in their repellency at 5% concentration (Table 5).

Table 5: Detailed *An. gambiae* repellency assay data for solvent extracts

	% Protective efficacy ± SE				
Conc. x 8.54 (mg/cm^2)	VH	CM	CP	OFO	PC
10^{-6}	24.99 ± 4.728^{ij}	21.26 ± 6.540^{j}	29.51 ± 9.926^{hij}	25.08 ± 8.018^{ij}	38.21 ± 9.376^{gh}
10^{-5}	37.50 ± 5.990^{ghi}	44.88 ± 9.165^{fgh}	38.26 ± 11.605^{gh}	32.07 ± 11.260^{hij}	43.21 ± 8.056^{fgh}
10^{-4}	50.88 ± 9.171^{efg}	45.17 ± 7.897^{fgh}	44.43 ± 10.579^{fgh}	40.60 ± 9.045^{gh}	49.92 ± 10.151^{fgh}
10^{-3}	57.36 ± 5.191^{def}	67.17 ± 3.269^{cd}	55.09 ± 12.011^{def}	63.05 ± 3.885^{de}	58.55 ± 6.11^{def}
10^{-2}	71.21 ± 4.106^{bcd}	73.42 ± 5.815^{bc}	75.99 ± 11.992^{ab}	82.64 ± 2.269^{a}	75.50 ± 4.586^{b}

Means followed by the same letter are not significantly different at $p < 0.05$; VH- *V. hildebrandtii*, CM- *C. menyharthii*, CP- *C. pseudopulchellus*, OFO- *O. forskolei*, PC- *P. cyneus*

Probit analysis was carried out on the repellency data of solvent extracts (Table 6-10) and regression equation determined for each (Finney, 1971).

Table 6: Probit analysis of *An. gambiae* repellency data for *Vernonia hildebrandtii* solvent extract

Dose x 8.54 (mg/cm^2)	% Repellency	Log Dose + 6	Emperical Probit	Expected Probit	Working Probit
10^{-6}	24.99	0.931	4.33	4.4	4.33
10^{-5}	37.50	1.931	4.69	4.7	4.68
10^{-4}	50.88	2.931	5.03	5.0	5.03
10^{-3}	57.36	3.931	5.18	5.2	5.19
10^{-2}	71.21	4.931	5.55	5.5	5.56

Regression equation, $Y = 0.293X + 4.0972$

Table 7: Probit analysis of *An. gambiae* repellency data for *Croton menyharthii* solvent extract

Dose x 8.54 (mg/cm^2)	% Repellency	Log Dose + 6	Emperical Probit	Expected Probit	Working Probit
10^{-6}	21.26	0.931	4.19	4.3	4.21
10^{-5}	44.88	1.931	4.87	4.7	4.88
10^{-4}	45.17	2.931	4.87	5.0	4.88
10^{-3}	67.17	3.931	5.44	5.3	5.44
10^{-2}	73.42	4.931	5.61	5.7	5.62

Regression equation, $Y = 0.341X + 3.9965$

Table 8: Probit analysis of *An. gambiae* repellency data for *Croton pseudopulchellus* solvent extract

Dose x 8.54 (mg/cm^2)	% Repellency	Log Dose + 6	Emperical Probit	Expected Probit	Working Probit
10^{-6}	29.51	0.931	4.48	4.4	4.46
10^{-5}	38.26	1.931	4.69	4.7	4.70
10^{-4}	44.43	2.931	4.85	5.0	4.87
10^{-3}	55.09	3.931	5.13	5.3	5.12
10^{-2}	75.99	4.931	5.71	5.6	5.70

Regression equation, Y= 0.290X + 4.1220

Table 9: Probit analysis of *An. gambiae* repellency data for *Ocimum forskolei* solvent extract

Dose x 8.54 (mg/cm^2)	% Repellency	Log Dose + 6	Emperical Probit	Expected Probit	Working Probit
10^{-6}	25.08	0.931	4.33	4.2	4.34
10^{-5}	32.07	1.931	4.53	4.6	4.53
10^{-4}	40.60	2.931	4.77	5.0	4.77
10^{-3}	63.05	3.931	5.33	5.4	5.33
10^{-2}	82.64	4.931	5.95	5.8	5.93

Regression equation, Y= 0.404X + 3.7979

Table 10: Probit analysis of *An. gambiae* repellency data for *Plectranthus cyneus* solvent extract

Dose x 8.54 (mg/cm^2)	% Repellency	Log Dose + 6	Emperical Probit	Expected Probit	Working Probit
10^{-6}	38.21	0.931	4.69	4.6	4.70
10^{-5}	43.21	1.931	4.82	4.8	4.83
10^{-4}	49.92	2.931	5.00	5.1	5.00
10^{-3}	58.55	3.931	5.23	5.3	5.21
10^{-2}	75.50	4.931	5.71	5.6	5.69

Regression equation, Y= 0.245X + 4.3719

From the regression equations the RC values for each extract were calculated (Table 11).

Table 11: *An. gambiae* RC levels for the solvent extracts

	RC values (mg/cm^2)		
Plant extract	$RC_{25} \times 10^{-6}$	$RC_{50} \times 10^{-4}$	$RC_{75} \times 10^{-2}$
V. hildebranditi	6.23	12.06	23.33
C. menyharthii	9.51	8.77	8.084
C. pseudopulcheles	5.22	10.66	21.77
O. forskolei	20.75	9.45	4.304
P. cyneus	0.67	3.66	19.88

The RC_{50} values shows that the extracts are of relatively low repellent activity as compared to DEET (RC_{50} = 2.6 x 10^{-4}) (Jerome and Mustapha, 2000) and may not provide adequate protection of the subjects.

3.3.3 Repellency of the essential oils

The highest concentration tested for the plants that had sufficient yields of essential oil was 0.1 g/ml (10%) and those with moderate yields 0.01 g/ml (1%). Out of the 24 plants, the essential oils from 10 were tested up to 10% and 5 oils to 1% concentration. The protective efficacy (PE) for the 10 oils tested at 10% concentration ranged from 62.5-100% while that of the other 5 ranged from 73.9-92.66% at 1%. Statistical analysis (SAS, 1999-2000) of the repellency data at 10% revealed three categories of the oils; highly, moderately and least active oil(s). The highly active oils were from *Ocimum fischeri*, *Mkilua fragrans*, *Plecranthus longipes*, *Vernonia hildebrandtii*, *Croton megalocarpoides*, *Croton menyharthii* and *Ocimum forskolei*. The moderately active ones were from *Endostemon tereticaulis* and *Croton pseudopulchellus* while the least active was from *Plectrunthus flacifolius*. The oils that were tested up to 1% concentration revealed the superiority of *Bidens schimperi* (92.66%) even though the activity was not significantly different from that of *Vitex mombasae* (86.64%). *Premna resinosa* was the least active (73.90%) at this concentration (Table 12).

Table 12: Preliminary *An. gambiae* repellency assay data for plant essential oils

	% Protective efficacy ± SE			
Concentration (g/ml)	10^{-5}	10^{-3}	10^{-2}	10^{-1}
M. fragrans	45.05 ± 12.487^{stu}	66.39 ± 4.355^{klm}		100.00^{a}
O. ordoratum	49.54 ± 8.796^{rst}	55.56 ± 7.286^{pqr}	80.12 ± 4.988^{fgh}	
B. schimperi	54.26 ± 3.384^{pqr}	65.82 ± 9.636^{lmn}	92.66 ± 3.837^{c}	
V. hildebrandtii	46.16 ± 8.589^{rst}	53.60 ± 11.516^{pqr}		100.00^{a}
C. megalocarpoides	45.05 ± 12.487^{stu}	66.39 ± 4.355^{klm}		100.00^{a}
C. menyharthii	20.76 ± 8.444^{z}	41.11 ± 9.375^{stu}		100.00^{a}
C. pseudopulchelles	56.96 ± 10.451^{pqr}	70.42 ± 7.438^{jkl}		91.83 ±2.088^{cd}
E. tereticaulis	40.75 ± 8.651^{uv}	65.44 ± 5.957^{lmn}		92.63 ± 1.848^{c}
O. fischeri	35.97 ± 8.565^{uvw}	45.06 ± 8.321^{stu}		100.00^{a}
O. forskolei	22.650 ± 8.776^{y}	44.18 ± 5.264^{stu}		98.718 ± 1.282^{a}
P. flacifolius	45.93 ± 4.522^{stu}	48.71 ± 7.822^{rst}		62.50 ± 7.217^{nop}
P. longipes	52.20 ± 13.307^{qrs}	48.33 ± 11.949^{rst}		100^{a}
L. carviodora	45.52 ± 7.476^{stu}	63.22 ± 5.623^{lmn}	84.27 ± 3.688^{def}	
P. resinosa	52.09 ± 7.182^{qrs}	65.69 ± 9.876^{lmn}	73.90 ± 3.337^{hij}	
V. mombasae	67.77 ± 5.114^{klm}	73.49 ± 10.668^{hij}	86.64 ± 7.401^{de}	

Means followed by same letter are not significantly different at $p<0.05$

The essential oils from 9 plants that showed good activity at 10% were subjected to detailed bio-assay to verify their activity.

3.3.3.1 Detailed mosquito repellency assay of active plant essential oils

The detailed repellency assay confirmed the superiority of *M. fragrans* (100%), *O. fischeri* (100%), *P. longipes* (99.52%), *C. menyharthii* (96.50%), *E. tereticaulis* (95.78%), *O. forskolei* (93.74%) and *C. pseudopulchellus* (90.17%) at 10^{-1} g/ml (10% solution) concentration. There was no significant difference in the repellent activity of *M. fragrans*, *O. fischeri* and *P. longipes*. Similarly, there was no significant difference in the repellency of *C. menyharthii*, *E. tereticaulis* and *O. forskolei* essential oil. The repellent activity of *C. pseudopulchellus* was significantlly different from those of the most active oils. *C. megalocarpoides* essential oil had the lowest activity (85.35%) at this concentration and the activity was significantly different from all the others (Table 13).

Table 13: Detailed *An. gambiae* repellency assay data for essential oils

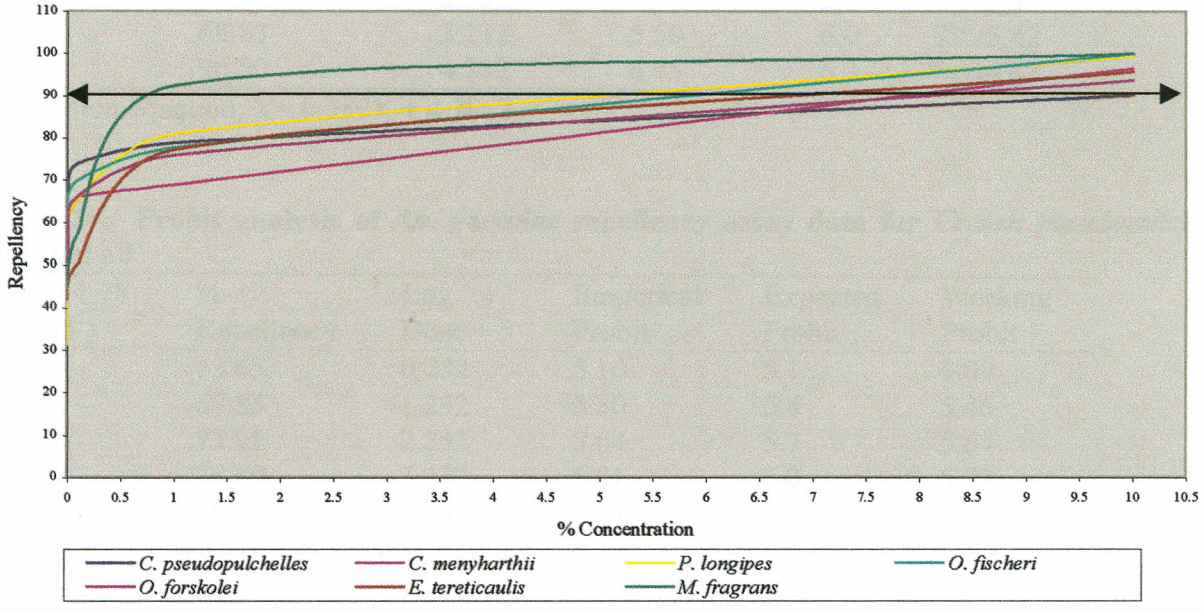
	% Protective efficacy ± SE				
Conc. (g/ml)	10^{-5}	10^{-4}	10^{-3}	10^{-2}	10^{-1}
M. fragrans	44.67 ± 12.135^{tu}	50.09 ± 7.879^{rst}	57.51 ± 7.758^{pqr}	92.31 ± 2.490^{bc}	100.00^{a}
V. hildebraniiti	26.17 ± 9.365^{y}	36.34 ± 10.296^{uvw}	55.37 ± 4.395^{pqr}	55.09 ± 5.244^{pqr}	87.602 ± 2.645^{d}
C. megalocarpoides	64.96 ± 9.534^{lmn}	72.305 ± 8.185^{hij}	60.74 ± 2.759^{op}	76.15 ± 3.489^{ghi}	85.35 ± 2.563^{de}
C. menyharthii	54.95 ± 8.093^{pqr}	56.37 ± 6.321^{pqr}	65.97 ± 4.004^{lmn}	68.83 ± 10.663^{klm}	96.50 ± 1.714^{b}
C. pseudopulchellus	53.63 ± 10.290^{pqr}	67.83 ± 7.340^{klm}	73.91 ± 5.190^{hij}	78.89 ± 4.476^{ghi}	90.17 ± 2.546^{cd}
E. tereticaulis	42.56 ± 6.732^{uv}	47.06 ± 10.817^{rst}	50.38 ± 16.905^{rst}	77.18 ± 4.073^{ghi}	95.78 ± 3.090^{b}
O. fischeri	54.89 ± 8.136^{pqr}	60.63 ± 6.458^{op}	69.85 ± 2.638^{jkl}	77.57 ± 4.613^{ghi}	100.00^{a}
O. forskolei	41.80 ± 9.973^{uv}	54.22 ± 3.467^{pqr}	66.18 ± 4.889^{klm}	75.80 ± 5.335^{hi}	93.74 ± 3.649^{bc}
P. longipes	31.46 ± 10.167^{wxy}	52.06 ± 9.099^{qrs}	65.27 ± 4.105^{lmn}	80.864 ± 8.679^{efg}	99.52 ± 0.476^{a}

Means followed by the same letter are not significantly different at $p<0.05$

The repellency data was subjected to regression analysis (Hassanali *et al.*, 1990) and the best fitting curves determined (Figure 2). The regression analysis is based on the assumption that repellency is exponentially related to concentration of test repellent as expressed by the mathematical relationship: % Repellency = $e^{(b\log x + \log a)}$ (Hassanali *et al.*, 1990), where x is concentration, a is intercept and b is slope obtained when repellency is plotted against logarithm of concentration. The values obtained were: a = 81.300, b = 8.414 for *C. pseudopulchellus*, a =78.080, b = 9.556 for *C. menyharthii*, a = 82.326, b = 16.492 for *P. longipes*, a = 83.304, b = 10.716 for *O. fischeri*, a = 78.894, b = 12.546 for

O. forskolei, a = 76.248, b = 13.656 for *E. tereticaulis* and a = 84.204, b = 15.288 for *M. fragrans*. The regression plots shows that most of the oils provided better protection at concentrations >8%.

Figure 2: Regression plot of % protective efficacy against concentration of the oils.



The bold longitudinal line indicates the 90% protection level.

Probit analysis (Finney, 1971) was carried out on the repellency data for the repellent essential oils (Table 8-14) and the regression equation for each determined.

Table 14: Probit analysis of *An. gambiae* repellency assay data for *Mkilua fragrans* essential oil

Dose x 1.78 (mg/cm^2)	% Repellency	Log Dose + 5	Emperical Probit	Expected Probit	Working Probit
10^{-5}	44.67	0.232	4.87	4.7	4.88
10^{-4}	50.09	1.232	5.00	5.1	5.00
10^{-3}	57.51	2.232	5.20	5.6	5.15
10^{-2}	92.31	3.232	6.41	6.1	6.37
10^{-1}	100	4.232	----	----	----

Regression equation, Y= 0.482X + 4.5352

Table15: Probit analysis of *An. gambiae* repellency assay data for *Croton menyharthii* essential oil

Dose x 1.78 (mg/cm^2)	% Repellency	Log Dose + 5	Emperical Probit	Expected Probit	Working Probit
10^{-5}	54.95	0.232	5.13	4.9	5.12
10^{-4}	56.37	1.232	5.15	5.2	5.16
10^{-3}	65.97	2.232	5.41	5.6	5.40
10^{-2}	68.83	3.232	5.50	6.0	5.42
10^{-1}	96.50	4.232	6.75	6.3	6.67

Regression equation, Y= 0.359X + 4.7867

Table 16: Probit analysis of *An. gambiae* repellency assay data for *Croton pseudopulchellus* essential oil

Dose x 1.78 (mg/cm^2)	% Repellency	Log Dose + 5	Emperical Probit	Expected Probit	Working Probit
10^{-5}	53.63	0.232	5.10	5.1	5.09
10^{-4}	67.83	1.232	5.50	5.4	5.46
10^{-3}	73.91	2.232	5.64	5.7	5.64
10^{-2}	78.89	3.232	5.81	6.0	5.78
10^{-1}	90.70	4.232	6.34	6.2	6.31

Regression equation, Y= 0.279X + 4.8410

Table 17: Probit analysis of *An. gambiae* repellency assay data for *Endostemon tereticaulis* essential oil

Dose x 1.78 (mg/cm^2)	% Repellency	Log Dose + 5	Emperical Probit	Expected Probit	Working Probit
10^{-5}	42.56	0.232	4.82	4.5	4.83
10^{-4}	47.06	1.232	4.92	5.0	4.93
10^{-3}	50.38	2.232	5.00	5.4	4.99
10^{-2}	77.18	3.232	5.74.	5.9	5.73
10^{-1}	95.78	4.232	6.75	6.4	6.66

Regression equation, Y= 0.468X + 4.4014

Table 18: Probit analysis of *An. gambiae* repellency assay data for *Ocimum fischeri* essential oil

Dose x 1.78 (mg/cm^2)	% Repellency	Log Dose + 5	Emperical Probit	Expected Probit	Working Probit
10^{-5}	54.89	0.232	5.13	5.1	5.12
10^{-4}	60.63	1.232	5.28	5.3	5.27
10^{-3}	69.85	2.232	5.52	5.5	5.52
10^{-2}	77.57	3.232	5.77	5.8	5.75
10^{-1}	100	4.232	-----	------	------

Regression equation, Y= 0.216X + 5.0509

Table 19: Probit analysis of *An. gambiae* repellency assay data for *Ocimum forskolei* essential oil

Dose x 1.78 (mg/cm^2)	% Repellency	Log Dose + 5	Emperical Probit	Expected Probit	Working Probit
10^{-5}	41.80	0.232	4.80	4.7	4.80
10^{-4}	54.22	1.232	5.10	5.1	5.11
10^{-3}	66.18	2.232	5.41	5.5	5.42
10^{-2}	75.80	3.232	5.71	5.9	5.68
10^{-1}	93.74	4.232	6.55	6.3	6.50

Regression equation, Y= 0.411X + 4.5966

Table 20: Probit analysis of *An. gambiae* repellency assay data for *Plectranthus longipes* essential oil

Dose x 1.78 (mg/cm^2)	% Repellency	Log Dose + 5	Emperical Probit	Expected Probit	Working Probit
10^{-5}	31.46	0.232	4.50	4.5	4.51
10^{-4}	52.06	1.232	5.05	5.0	5.06
10^{-3}	65.27	2.232	5.39	5.4	5.40
10^{-2}	80.86	3.232	5.88	5.9	5.86
10^{-1}	99.52	4.232	-----	-----	-----

Regression equation, Y= 0.448X + 4.429

From probit analysis and regression equations the repellent concentration levels were calculated (Table 21) (Finney, 1971).

Table 21: *An. gambiae* RC levels for essential oils from repellent plants

	RC values (mg/cm^2)		
Plant oil	$RC_{25} \times 10^{-6}$	$RC_{50} \times 10^{-5}$	$RC_{75} \times 10^{-3}$
M. fragrans	3.752	9.211	2.261
C. menyharthii	3.494	3.928	2.887
C. pseudopulchellus	0.1474	3.714	9.361
E. tereticaulis	7.038	1.515	4.828
O. fischeri	4.597	0.581	7.349
O. forskolei	2.246	9.583	4.021
P. longipes	6.012	1.882	5.890

The results show that the order of repellency of the plant essential oils at RC_{50} level is *O. fischeri* > *E. tereticaulis* > *P. longipes* > *C. pseudopulchellus* > *C. menyharthii* > *M. fragrans* >*O. forskolei*.

3.4 Mosquitocidal assays

3.4.1 Tarsal contact mosquitocidal assays

The solvent extracts were also evaluated for their mosquitocidal activity using tarsal contact assay. However, none of the extracts from the evaluated plants showed mosquitocidal activity by this method.

3.4.2 Fumigant mosquitocidal assays

The essential oils were subjected to mosquitocidal assay by fumigation. Out of the 10 essential oils tested at 10% concentration, only 3 exhibited mosquitocidal activity by fumigation. *O. forskolei* had the highest insecticidal action with T_{i100} and T_{KD100} values of 3.97 and 0.47 hours, respectively. *P. longipes* and *M. fragrans* did not exhibit 100% knockdown or insecticidal action during the 24 hours test period. The percentage mortality at 24 hours for the two least active oils was not significantly different from that after 6 hours of the test period (Table 22). The data was subjected to probit analysis (Table 23-25). The order of insecticidal activity was determined to be *O. forskolei* > *P. longipes* > *M. fragrans* with LC_{50} values of 3.044×10^{-3}, 3.392×10^{-3} and 5.106×10^{-3} mg/cm^3, respectively.

Table 22: Mosquitocidal activity data for plant essential oils on *An. gambiae* at 10% concentration

	% Mortality		
Time (hours)	*M. fragrans*	*O. forskolei*	*P. longipes*
1	22.00 ± 5.033^{h}	10.67 ± 2.404^{l}	0.00^{p}
2	30.67 ± 4.372^{gh}	68.00 ± 6.110^{d}	7.33 ± 1.764^{m}
3	35.33 ± 3.412^{f}	99.33 ± 0.667^{a}	16.67 ± 1.764^{j}
4	46.00 ± 4.163^{e}	100.00^{a}	28.00 ± 3.055^{gh}
5	56.67 ± 4.410^{e}		53.33 ± 4.055^{e}
6	73.33 ± 2.848^{c}		84.67 ± 3.528^{b}
24	76.00 ± 3.055^{c}		88.00 ± 2.000^{b}

Means followed by the same letter are insignificantly different at $p<0.05$

The mosquitocidal assay data was subjected to probit analysis.

Table 23: Probit analysis of fumigant activity of *Mkilua fragrans* essential oil on *An. gambiae* after 6 hours exposure

Dose x 10^{-3} (mg/cm^3)	% Mortality (Corrected)	Log Dose + 5	Emperical Probit	Expected Probit	Working Probit
1.408	7.34	2.149	3.52	3.6	3.55
2.817	33.00	2.450	4.56	4.3	4.59
4.225	37.34	2.626	4.67	4.8	4.68
5.634	50.00	2.751	5.00.	5.1	5.00
7.042	67.34	2.848	5.44	5.4	5.45

Regression equation, Y= 2.528X – 1.846

Table 24: Probit analysis of fumigant activity of *Ocimum forskolei* essential oil on *An. gambiae* after 4 hours exposure

Dose x 10^{-3} (mg/cm^3)	% Mortality (Corrected)	Log Dose + 5	Emperical Probit	Expected Probit	Working Probit
1.408	5.34	2.149	3.36	3.2	3.42
2.817	29.66	2.450	4.48	4.8	4.48
4.225	83.00	2.626	5.95	5.8	5.94
5.634	90.66	2.751	6.34.	6.4	6.32
7.042	98.00	2.848	7.05	7.0	7.05

Regression equation, Y= 5.349X – 8.284

Table 25: Probit analysis of fumigant activity of *Plectranthus longipes* essential oil on *An. gambiae* after 6 hours exposure

Dose x 10^{-3} (mg/cm^3)	% Mortality (Corrected)	Log Dose + 5	Emperical Probit	Expected Probit	Working Probit
1.408	13.66	2.149	3.92	3.9	3.91
2.817	44.66	2.450	4.87	4.8	4.86
4.225	56.66	2.626	5.18	5.3	5.16
5.634	72.00	2.751	5.58.	5.6	5.58
7.042	82.34	2.848	5.92	5.9	5.93

Regression equation, Y= 2.769X – 2.001

From the regression equations the LC values were calculated (Table 26).

Table 26: *An. gambiae* LC levels for the fumigant essential oils

		LC values (x 10^{-3} mg/cm^3)		
Plant oil	Time (hours)	LC_{25}	LC_{50}	LC_{75}
O. fischeri	4	2.281	3.044	4.062
P. longipes	6	1.943	3.392	5.922
M. fragrans	6	2.774	5.106	9.399

The quantitative difference in activity of the oils suggested a likelihood of their qualitative differences. This called for the analysis of the chemical composition of the essential oils so as to evaluate the bio-active principles therein.

CHAPTER 4

CHEMICAL COMPOSITION OF ESSENTIAL OIL FROM ANTI-INSECT PLANTS

4.1 Chemical constituents of the selected plant essential oils

GC and GC-MS was used to analyze the constituents of the bio-active essential oils. The comparison of the MS data of the components with the library spectra provided clues of the identities of the components (Waller, 1972; Adams, 1989). Some of the components of the oils were confirmed through peak enhancement by GC-coinjection with standards. Standards were acquired from commercial sources or synthesized by one step conversions. For instance 4-isopropylbenzenemethanol was made by reduction of 4-isopropylbenzaldehyde with sodium borohydride (Solomons, 1990). Spectroscopic analysis confirmed the product as 4-isopropylbenzenemethanol (Appendices). The relative percentage compositions of the oils were analyzed based on the GC-MS detectable peaks with the least component being 0.0003 (0.03%) of the most abundant one in the individual essential oils.

4.1.1 *Mkilua fragrans*

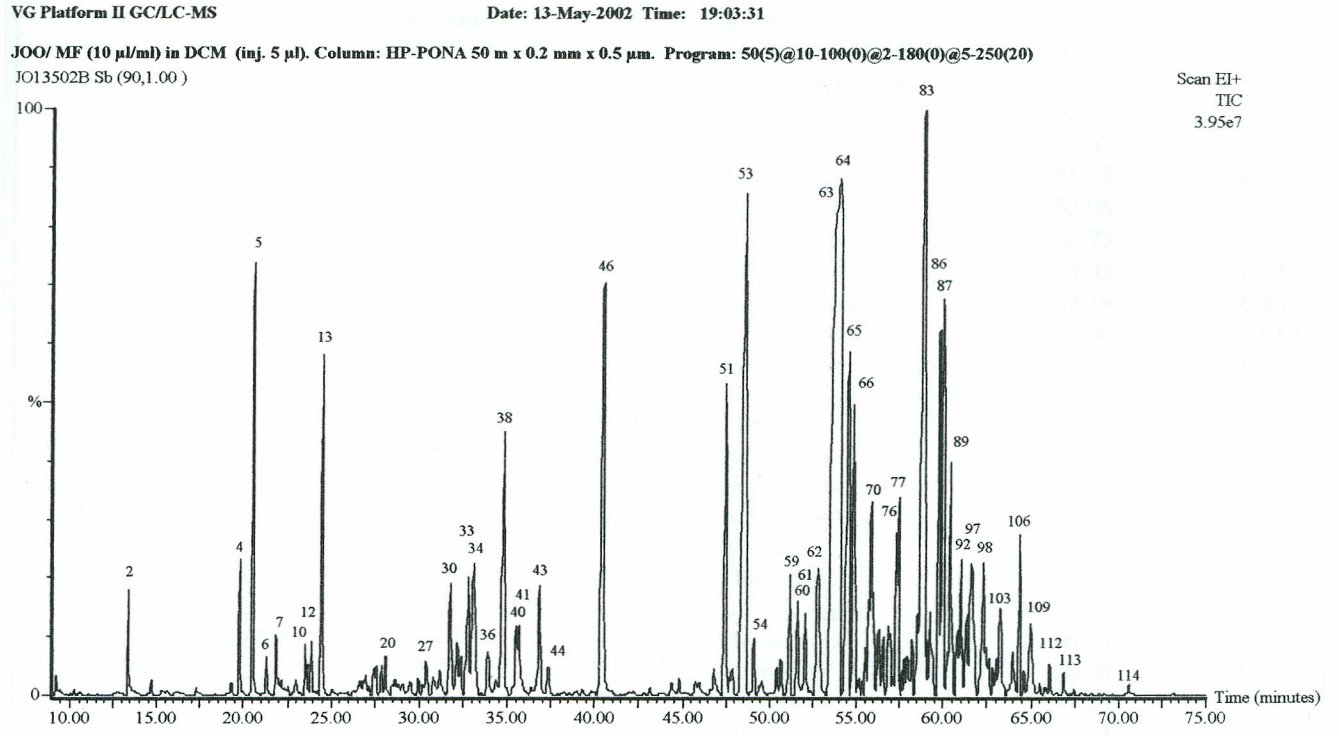
The oil was subjected to GC-MS analysis (Figure 3) and 114 components that had relative percentage of ≥0.05 constituting 97.12% of the oil proposed by MS matching (Table 27). The suggested major components were sesquiterpenes (51.66%), oxygenated terpenoids (19.2%) and 35 unidentified compounds constituting 18.46% of the oil. The proposed major components included caryophyllene oxide (8.63%), (-)-dehydroaromadendrene (7.50%), β-elemene (7.10%), *endo*-bornyl acetate (4.48%), β-selinene (4.18%), torreyol (3.09%), valencene (3.02%), camphene (3.01%), chrysanthenone (2.72%), α-ylangene (2.62%), humuladione (2.32%), limonene (2.23%), α-muurolene (1.93%), ledonoxide I (1.73%), α-guaiene (1.55%), juniper camphor (1.35%), 4-isopropylbenzenemethanol (1.29%) and (+)-oxo-α-ylangene (1.21%). Out of the 79 components suggested by spectral matching, 22 were confirmed by GC-coinjection with standards. However, 9 major (≥ 1%) compounds (chrysanthenone, α-muurolene, α-guaiene, β-elemene, humuladione, torreyol, ledonoxide I, juniper camphor, (+)-oxo-α-ylangene and 2 other unknowns) were not confirmed by GC-coinjection due to the absence of standards.

Table 27: Chemical composition of *Mkilua fragrans* essential oil

Components	Rt (min)	RE%	Components	Rt (min)	RE%
1. 2-Methylbutanal*	9.23	0.09	58. β-Caryophyllene*	50.68	0.25
2. Hexanal*	13.38	0.59	59. Unknown	51.18	0.82
3. Tricylene	19.28	0.07	60. Unknown	51.63	0.82
4. α-Pinene*	19.78	0.75	61. δ-Guaiene	52.08	0.72
5. Camphene*	20.55	3.01	62. α-Muurolene	52.78	1.93
6. *m*-Cymene*	21.30	0.24	63. 2 Unknowns	53.83	9.06
7. 2-Pentylfuran	21.85	0.36	64. (-)-Dehydroaroma-dendrene*	54.05	7.50
8. 4-Methyl-1-sopropylcy-clohexane	22.15	0.11	65. β-Selinene	54.55	4.18
9. 2-Carene*	22.98	0.14	66. Valencene	54.80	3.02
10. Unknown	23.53	0.17	67. Unknown	55.18	0.05
11. *o*-Cymene*	23.65	0.07	68. α-Gurjunene	55.53	0.20
12. *p*-Cymene*	23.88	0.27	69. *Cis*-Calamenene	55.70	0.38
13. Limonene*	24.48	2.23	70. α-Guaiene	55.85	1.55
14. γ-Terpinene*	25.95	0.02	71. Unknown	55.95	0.57
15. Unknown	26.68	0.05	72. Unknown	56.30	0.55
16. *o*-Allyltoluene	26.98	0.09	73. α-Calocorene	56.58	0.39
17. Unknown	27.43	0.22	74. Hedycaryol	56.85	0.55
18. Rosefuran	27.58	0.19	75. Nor-copaene	56.98	0.28
19. Linalool*	27.88	0.19	76. Unknown	57.30	0.51
20. Perillene	28.10	0.24	77. 1-Ethyl-3, 5-diisopro-pylbenzene	57.45	0.70
21. Hexahydro-3-methylen-6-methylbenzofurane	28.63	0.05	78. Unknown	57.63	0.10
22. 4-Methylene-1-isopropyl bicyclo[3.1.0]hexan-3-ol	29.08	0.07	79. Unknown	57.80	0.15
23. Unknown	29.43	0.05	80. Unknown	57.95	0.18
24. α-Campholene aldehyde	29.50	0.11	81. 4-Oxo-β-ionone	58.23	0.29
25. 3, 4-Dimethyl-2, 4, 6-octatriene	29.95	0.11	82. Unknown	58.53	0.66
26. *Trans-p*-mentha-2,8-dien-1-ol	30.23	0.06	83. Caryophylene oxide*	58.88	8.63
27. *Cis*-limonene oxide*	30.40	0.27	84. Unknown	59.03	0.19
28. Camphor*	30.80	0.15	85. Unknown	59.25	0.49
29. Unknown	31.18	0.23	86. Humuladione	59.75	2.32
30. *p*-Mentha-1,5-dien-8-ol	31.78	0.94	87. Torreyol	60.00	3.09
31. *Trans-p*-mentha-2-en-1,8-diol	32.18	0.29	88. Unknown	60.23	0.18
32. Unknown	32.38	0.16	89. Ledonoxide-(I)	60.40	1.73
33. *p*-Cymen-8-ol	32.78	0.92	90. Unknown	60.48	0.34
34. 4-Isopropylbenzene-methanol*	33.13	1.29	91. 2-Methyl-3-isopro-pylcyclohexyl acetate	60.85	0.32
35. Unknown	33.23	0.12	92. Caryophylla-3, 8-dien-5-β-ol	61.03	0.75
36. Cyclododecyne	33.93	0.43	93. Unknown	61.28	0.11
37. Unknown	34.35	0.08	94. Unknown	61.352	0.38
38. Chrysanthenone	34.83	2.72	95. Juniper camphor	61.60	1.35
39. Unknown	35.33	0.06	96. Cadalin	61.75	0.27
40. *Cis*-dihydrocarvone	35.53	0.66	97. Unknown	62.20	0.48
41. Eucarvone	35.68	0.78	98 (+)-Oxo-α-ylangene	62.30	1.21
42. Unknown	36.35	0.05	99. Unknown	62.43	0.37
43. Carvone*	36.83	0.93	100. Unknown	62.60	0.25
44. Tripal	37.35	0.26	101. Valerenal	62.85	0.11
45. 2,6,6-Trimethyl-1-cyclo-hexene-1-acetaldehyde	38.25	0.06	102. Unknown	63.05	0.17
46. Bornyl acetate*	40.48	4.48	103. Unknown	63.30	0.74
47. Carvyl acetate*	43.13	0.07	104. Unknown	63.98	0.29
48. Farnesyl acetate	44.80	0.12	105. Unknown	64.03	0.12
49. β-Terpenyl acetate	45.75	0.07	106. Alloaromadendrene	64.35	0.92
50. Methyleugenol	46.80	0.34	107. Dispiro[2.6.2.5]un-decane	64.63	0.12
51. α-Ylangene*	47.45	2.65	108. Unknown	64.88	0.11
52. Unknown	47.85	0.12	109. 3, 12-diethyl-2, 5, 9-tetradecatriene	64.98	0.63
53. β-Elemene	48.60	7.10	110. Unknown	65.50	0.07
54. β-Copaen-4-α-ol	49.10	0.41	111. 8-Oxo-neoisolo-ngifolene	65.80	0.07
55. Unknown	49.40	0.06	112. Nootkatone	66.05	0.18
56. α-Ionone	49.55	0.12	113. 17-Acetoxy-19-kauranal	66.88	0.18
57. Unknown	50.43	0.19	114. Phytol*	70.60	0.08

*-Component confirmed through co-injection with standards

Figure 3: GC profile of *Mkilua fragrans* essential oil



4.1.2 *Croton menyharthii*

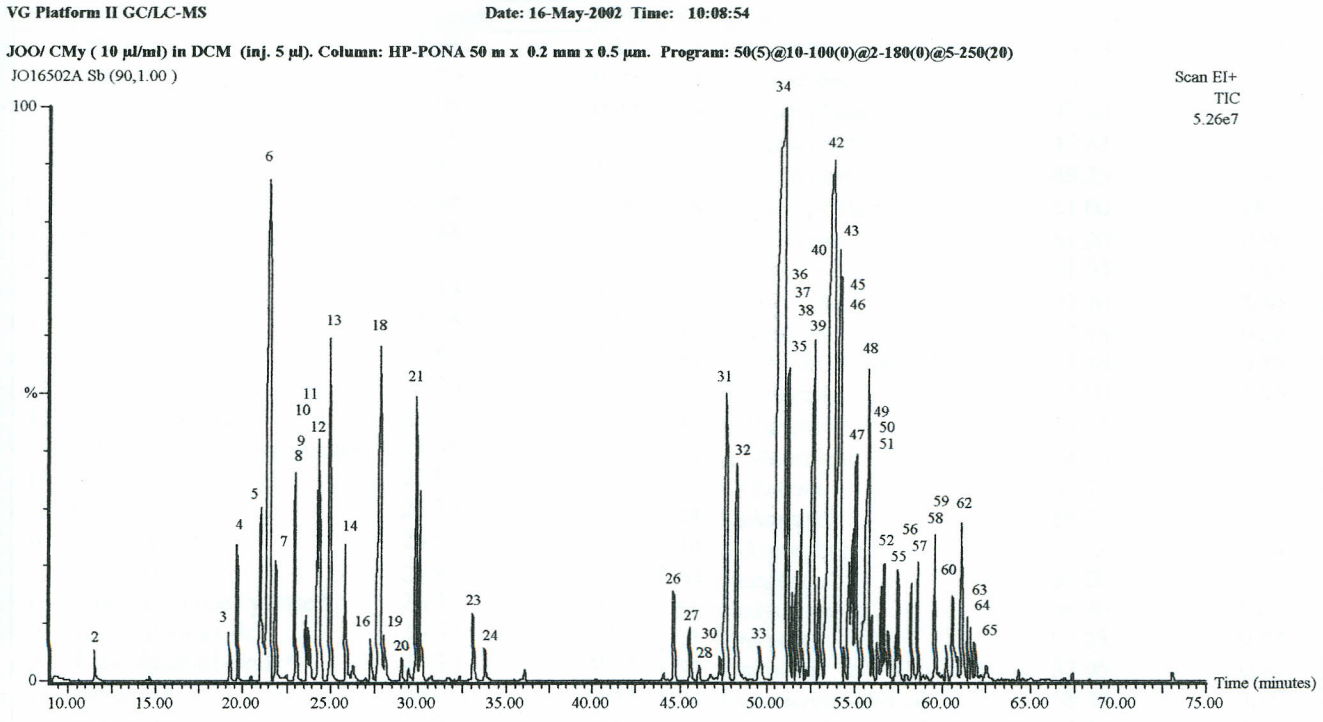
The essential oil was subjected to GC-MS analysis (Figure 4). The oil had 65 components with relative percentage composition of ≥0.05% constituting 97.92%. The monoterpenes constituted 20.32% and the sesquiterpene hydrocarbons 69.97% of the oil (Table 28). The major components of the oil were suggested to be non-oxygenated terpenes by mass spectral matching. They included *cis*-caryophyllene (18.28%), acoradiene (13.18%), sabinene (6.85%), β-sesquiphellandrene (6.62%), α-caryophyllene (4.69%), δ-amorphene (4.29%), linalool (3.11%), α-ylangene (3.06%), β-ocimene (2.75%), germacrene D (2.73%), (+)-aromadendrene (1.44%) 3, 4-dimethyl-2, 4, 6-octatriene (1.33%), α-phellandrene (1.32%), 2-dodecen-4-yne (1.23%), α-amorphene (1.21%), α-cadinol (1.17%) and limonene (1.09%) (Table 28). Out of the 65 compounds suggested by mass spectral matching with library spectra, 26 were confirmed by GC coinjection (Table 28). However 9 major (≥ 1%) compounds (*cis*-caryophyllene, acoradiene, β-sesquiphellandrene, δ-amorphene, α-caryophyllene, germacrene D, α-amorphene, 2-dodecen-4-yne and 3, 4-dimethyl-2, 4, 6-octatriene) were not confirmed by coinjection due to unavailability of authentic standards.

Table 28: Chemical composition of *Croton menyharthii* essential oil

Components	Rt (min)	RE%	Components	Rt (min)	RE%
1. 2-Methylbutanol*	9.23	0.25	33. Isocaryophyllene*	49.63	0.44
2. 1-Methylpyrole*	11.53	0.15	34. *Cis*-caryophyllene	50.60	18.28
3. α-Thujene	19.25	0.19	35. Germacrene D	51.33	2.73
4. α-Pinene*	19.73	0.70	36. α-Guaiene	51.53	0.54
5. 1-Octen-3-ol*	21.08	1.33	37. E-β-Farnesene*	51.78	0.71
6. Sabinene*	21.60	6.85	38. α-Armorphene	52.05	1.21
7. β-Myrcene*	21.95	0.66	39. α-humulene	52.75	4.69
8. α-Phellandrene*	23.05	1.32	40. β-Himachalene	53.03	0.91
9. 2-Carene*	23.68	0.28	41. α-Curcumene	53.48	0.26
10. *p*-Cymene*	23.83	0.23	42. Acoradiene	53.88	13.18
11. 3, 6, 6-Trimethylbicyclo [3.1.1]hept-2-ene	24.30	0.50	43. β-Sesquiphellandrene	54.18	6.62
12. Limonene*	24.43	1.09	44. (+) -Cyclosativene	54.43	0.17
13. β-Ocimene*	25.03	2.75	45. Torreyol	54.73	0.57
14. γ-Terpinene*	25.93	0.90	46. β-Bisabolene	54.95	0.76
15. *Trans*-sabinene hydrate*	26.30	0.13	47. (+)-Aromadendrene*	55.15	1.44
16. Fenchone*	27.33	0.28	48. δ-Amorphene	55.83	4.29
17. Terpinolene*	27.78	0.49	49. Sinensal	56.03	0.39
18. Linalool*	27.90	3.11	50. Unknown	56.33	0.25
19. 1-Octen-3-yl acetate	28.13	0.23	51. α-Calocorene[a]	56.55	0.43
20. Unknown	29.05	0.17	52. Hedycaryol	56.75	0.71
21. 3, 4-Dimethyl-2, 4, 6-octatriene	29.95	2.27	53. Nerolidol*	56.98	0.32
22. 2-Dodecen-4-yne	30.18	1.23	54. Calocorene[a]	57.38	0.14
23. Terpene-4-ol*	33.15	0.50	55. Unknown	57.53	0.57
24. α-Terpeneol*	33.78	0.29	56. Germacrene-D-4-ol	58.25	0.66
25. γ-Elemene	44.10	0.05	57. Caryophyllene oxide*	58.63	0.70
26. Bicycloelemene	44.70	0.67	58. Mansonone	59.63	0.93
27. α-Cubebene	45.63	0.41	59. Unknown	60.23	0.21
28. Unknown	46.10	0.12	60. Unknown	60.63	0.97
29. Methyleugenol	46.78	0.06	61. Unknown	60.83	0.12
30. (+)-Cycloisosativene*	47.30	0.20	62. α-Cadinol	61.13	1.17
31. α-Ylangene*	47.73	3.06	63. Juniper camphor	61.48	0.37
32. γ-Gurjunene*	48.35	2.27	64. Cadalin	61.68	0.29
			65. α-Bisabolol	61.88	0.31

*-Component confirmed through co-injection with standard; [a]-isomer not established

Figure 4: GC profile of *Croton menyharthii* essential oil



4.1.3 *Croton pseudopulchellus*

GC-MS analysis (Figure 5) revealed 143 components of which 65 had relative percentage constitution of ≥0.05% and constituted 97.5% of the oil. A total of 15 compounds were not identified at all. The oil mainly consisted of non-oxygenated terpenes (70.7%) (Table 29).

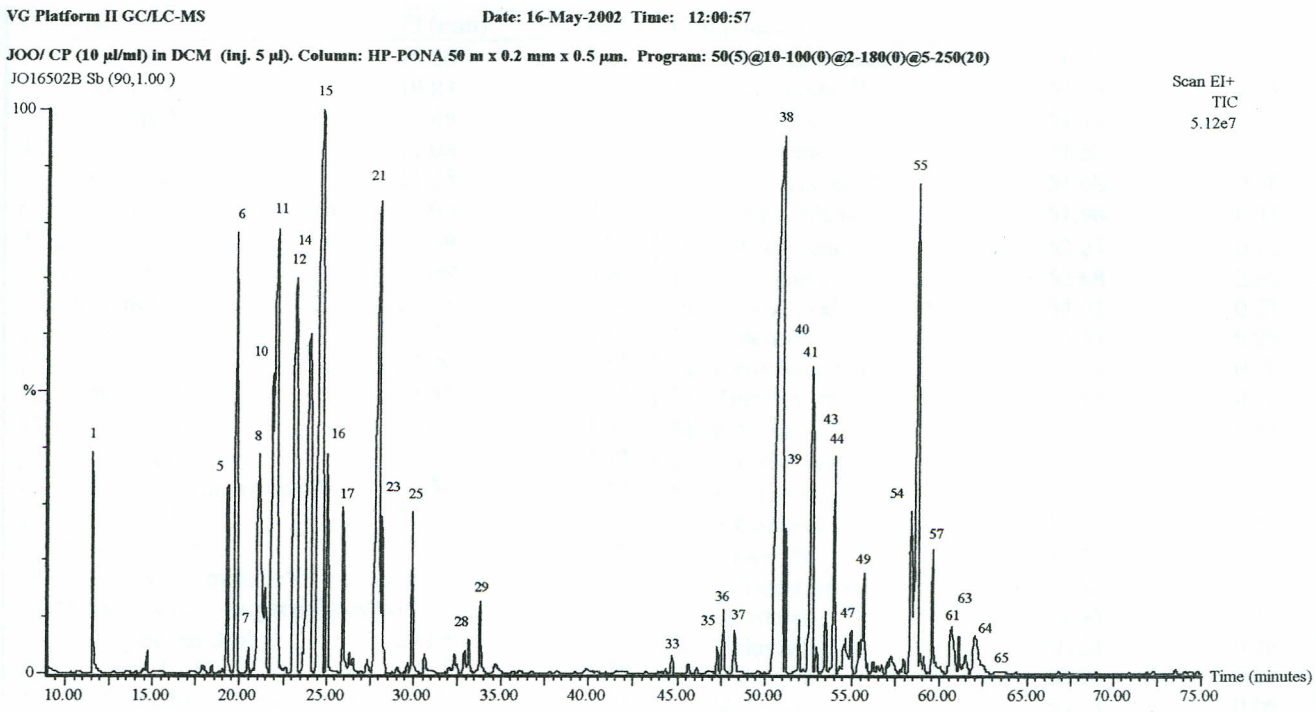
The major components of the oil included β-caryophyllene (14.95%), limonene (12.48%), β-myrcene (8.22%), *p*-cymene (6.79%), α-phellandrene (6.70%), linalool (6.33%), caryophyllene oxide (5.47%), α-pinene (3.98%), α-caryophyllene (3.45%), 2-ethyl-3-propyl-*trans*-3-oxirane (3.29%), β-pinene (2.70%), germacrene D (1.95%), β-ocimene (1.44%), α-thujene (1.35%), 3, 4-dimethyl-2, 4, 6-octatriene (1.30%), γ-terpinene (1.13%), 1-methylpyrole (1.11%), unknown (1.07%), germacrene-D-4-ol (1.07%). Out of the 50 compounds proposed by MS matching with library data, 25 were confirmed by coinjection with standards. However, 9 major (≥ 1%) compounds (α-thujene, 2-ethyl-3-propyl-*trans*-3-oxirane, 3, 4-dimethyl-2, 4, 6-octatriene, α-caryophyllene, germacrene D, germacrene-D-4-ol, humuladienone, β-cubebene and one unknown) were not confirmed by coinjection due to unavailability of standards.

Table 29: Chemical composition of *Croton pseudopulchellus* essential oil

Components	Rt (min)	RE%	Components	Rt (min)	RE%
1. 1-Methylpyrole*	11.55	1.11	33. Unknown	44.68	0.13
2. Toluene*	12.75	0.06	34. α-Cubebene	45.63	0.08
3. Octane*	14.05	0.09	35. α-Amorphene	47.28	0.23
4. 2-Octene	14.50	0.11	36. α-Ylangene*	47.63	0.55
5. α-Thujene	19.30	1.35	37. β-Bourbonene	48.25	0.42
6. α-Pinene*	19.80	3.98	38. β-Caryophyllene*	51.00	14.95
7. Camphene*	20.48	0.15	39. β-Cubebene	51.20	0.96
8. 2-Ethyl-3-propyl-*trans*-3-oxirane	21.13	3.29	40. Unknown	51.95	0.43
9. Sabinene*	21.48	0.45	41. α-Humulene	52.70	3.45
10. β-Pinene*	21.88	2.70	42. γ-Amorphene	52.95	0.22
11. β-Myrcene*	22.20	8.22	43. (+)-Aromadendrene*	53.48	0.55
12. α-Phellandrene*	23.25	6.70	44. Germacrene-D	54.00	1.95
13. 2-Methyl-2-heptanyl acetate	23.65	0.12	45. β-Cubebene	54.53	0.13
14. *p*-Cymene*	24.05	6.79	46. α-Muurolene	54.63	0.19
15. Limonene*	24.70	12.48	47. Unknown	54.95	0.24
16. β-Ocimene*	25.03	1.44	48. Unknown	55.55	0.12
17. γ-Terpinene*	25.95	1.13	49. δ-Amorphene	55.68	0.64
18. *Trans*-sabinene hydrate*	26.33	0.10	50. Junipene	56.20	0.10
19. *Cis*-linalool oxide*	26.50	0.05	51. Hedycaryol	56.70	0.05
20. *Trans*-linalool oxide*	27.33	0.13	52. Unknown	57.25	0.23
21. Linalool*	28.03	6.33	53. Unknown	57.95	0.10
22. Terpinolene*	27.80	0.89	54. Germacrene-D-4-ol	58.38	1.07
23. Octenyl acetate	28.18	0.64	55. Caryophyllene oxide*	58.73	5.47
24. Terpene-1-ol	29.65	0.09	56. Salivial-4-en-1-one	58.90	0.08
25. 3,4-Dimethyl 2,4,6-octatriene-	29.93	1.30	57. Humuladienone	59.60	0.99
26. 1-Methyl-4-isopropyl-2-cyclohexen-1-ol	30.63	0.17	58. Unknown	59.75	0.21
27. Unknown	32.90	0.15	59. Unknown	60.05	0.09
28. Terpene-4-ol*	33.15	0.24	60. ζ-Cadinol	60.57	0.32
29. α-Terpeneol*	33.81	0.65	61. Unknown	60.68	0.60
30. *Cis*-sabinol	34.70	0.24	62. α-Cadinol	61.08	0.22
31. 4-Isopropylbenzalde-hyde*	36.75	0.06	63. Unknown	61.45	0.24
32. Unknown	39.85	0.06	64. Unknown	62.00	1.01
			65. Unknown	64.65	0.06

*-Component confirmed through co-injection with standard

Figure 5: GC profile of *Croton pseudopulchellus* essential oil



4.1.4 *Endostemon tereticaulis*

Out of the 189 GC-MS (Figure 6) peaks, 83 components had relative percentage composition of ≥0.05% constituting 96.54% of the essential oil. A total of 18 compounds could not be identified by GC-MS library spectral comparison. The main components of the oil were non-oxygenated terpenes (82.18%) (Table 30).

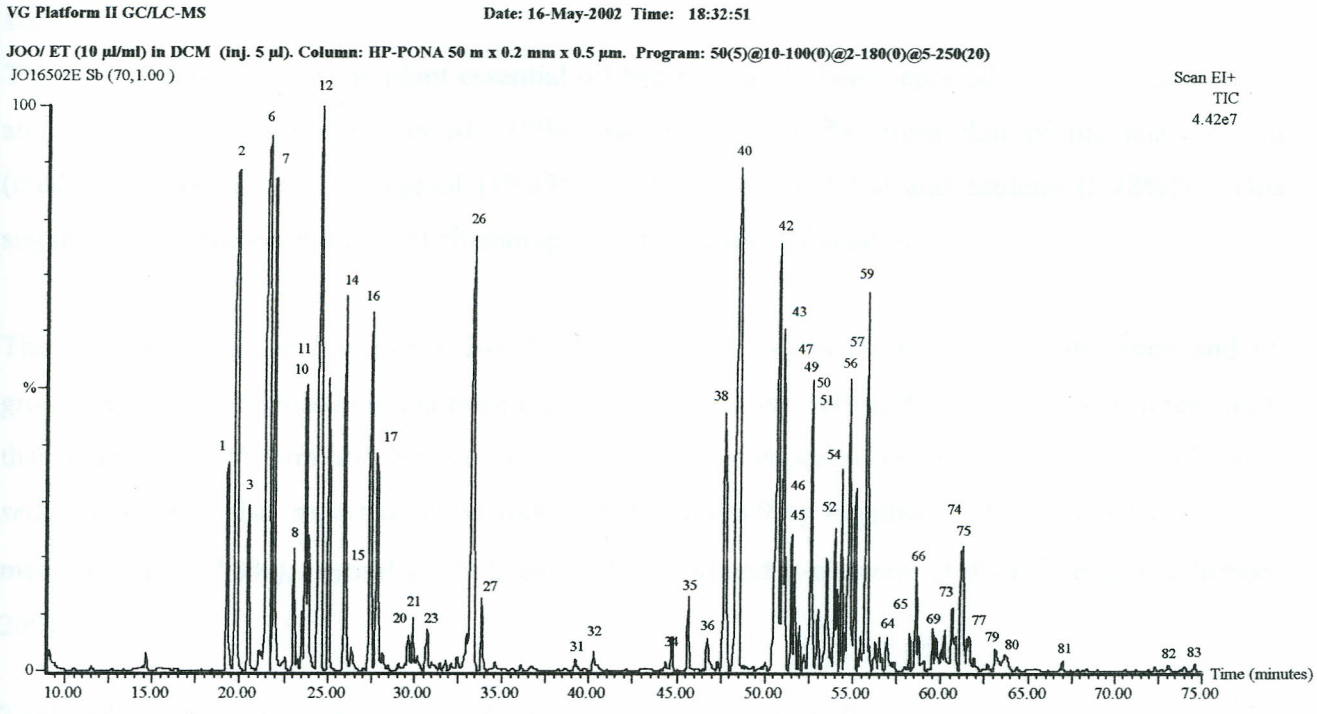
The main components of the oil were 3-methylene-1, 6-heptadiene (9.30%), β-phellandrene (7.54%), β-elemene (6.32%), terpene-4-ol (6.23%), α-pinene (5.51%), β-pinene (5.07%), *E*-β-caryophyllene (4.69%), fenchone (3.96%), δ-amorphene (3.41%), γ-terpinene (3.22%), α-ylangene (3.00%), α-selinene (2.79%), α-caryophylene (2.69%), germacrene B (2.54%), linanyl acetate (1.99%), β-bourbonene (1.98%), *E*-β-ocimene (1.89%), α-thujene (1.67%), 2-carene (1.61%), β-selinene (1.55%), camphene (1.22%) and δ-guaiene (1.15%) (Table 30). Out of the 65 compounds tentatively identified by GC-MS library data spectral matching, 28 were confirmed by coinjection with standards. However, 11 major (≥ 1%) compounds (α-thujene, 3-methylene-1, 6-heptadiene, β-bourbonene, β-elemene, germacrene B, α-caryophyllene, δ-amorphene, β-selinene, α-selinene and δ-guaiene) were not confirmed by coinjection due to the absence of authentic standards.

Table 30: Chemical composition of the *Endostemon tereticaulis* oil

Components	Rt (min)	RE%	Components	Rt (min)	RE%
1. α-Thujene	19.30	1.67	42. β-Caryophyllene*	50.78	4.69
2. α-Pinene*	19.83	5.51	43. Germacrene B	51.03	2.54
3. Camphene*	20.48	1.22	44. Unknown	51.15	0.58
4. 1-Octen-3-ol*	21.08	0.15	45. α-Guaiene	51.50	0.81
5. 3-Octanone	21.25	0.06	46. *E*-β-Farnesene*	51.68	0.58
6. 3-Methylene-1,6-heptadiene	21.65	9.30	47. α-Amorphene	51.98	0.33
7. β-Pinene*	21.98	5.07	48. α-Cubebene	52.23	0.11
8. α-Phellandrene*	23.08	0.80	49. α-Humulene	52.68	2.69
9. 3-Carene*	23.53	0.30	50. Alloaromadendrene*	53.03	0.57
10. 2-Carene*	23.75	1.61	51. Valencene	53.53	0.99
11. *p*-Cymene*	23.90	0.52	52. Germacrene D	53.98	0.70
12. Limonene*	24.58	7.54	53. Zingiberene	54.13	0.24
13. β- Ocimene*	25.05	1.89	54. β-Selinene	54.38	1.55
14. γ-Terpinene*	26.00	3.22	55. β-Cubebene	54.55	0.14
15. *Trans*-sabinene hydrate*	26.38	0.17	56. Unknown	54.80	2.79
16. Fenchone*	27.50	3.96	57. δ-Guaiene	55.13	1.15
17. Terpinolene*	27.83	1.99	58. Unknown	55.43	0.15
18. *Cis*-sabinene hydrate*	28.18	0.10	59. δ-Amorphene	55.80	3.41
19. 4-Methylene-1-isopropylbicyclo [3.1.0] hexan-3-ol	29.08	0.05	60. Unknown	55.95	0.10
20. Terpene-1-ol	29.65	0.26	61. Unknown	56.28	0.26
21. 3,4-Dimethyl-2,4,6-octatriene	29.90	0.30	62. Unknown	56.50	0.19
22. Epoxyocimene	30.18	0.07	63. Valencene	56.63	0.06
23. Camphor*	30.75	0.34	64. Nerolidol*	56.95	0.26
24. Pinacarvone	31.80	0.09	65. Unknown	58.23	0.25
25. Unknown	33.00	0.42	66. Caryophyllene oxide*	58.58	0.52
26. Terpene-4-ol*	33.38	6.23	67. Unknown	58.75	0.12
27. α-Terpeneol*	33.85	0.60	68. Unknown	59.03	0.08
28. Unknown	34.63	0.12	69. Humuladienone	59.55	0.23
29. Unknown	36.10	0.06	70. Unknown	59.73	0.20
30. Cuminal	36.68	0.06	71. Unknown	60.05	0.08
31. Phellandral	39.20	0.13	72. Unknown	60.23	0.18
32. Bornyl acetate*	40.25	0.16	73. α-Cadinol	60.65	0.68
33. Thymol*	40.40	0.08	74. Unknown	61.13	0.74
34. δ-Elemene	44.70	0.25	75. Thunbergol	61.23	0.89
35. α-Cubebene*	45.65	0.57	76. Unknown	61.48	0.07
36. Methyleugenol	46.73	0.34	77. Spathulenol	61.60	0.11
37. Unknown	47.33	0.07	78. α-Bisabolol	61.88	0.07
38. α- Ylangene*	47.73	3.00	79. Unknown	63.73	0.25
39. β-Bourbenene	48.35	1.98	80. Neophytadiene	66.98	0.05
40. β-Elemene	48.53	6.32	81. Unknown	73.10	0.06
41. (-)-Isoledene*	50.00	0.06	82. Unknown	74.60	0.06
			83. Phytol*	74.98	0.08

*-Component confirmed through co-injection with standard

Figure 6: GC profile of *Endostemon tereticaulis* essential oil



4.1.5 *Ocimum fischeri*

GC-MS analysis revealed 127 peaks (Figure 7) with 64 components having relative percentage composition of ≥0.05%. The 64 components (Table 31) constituted 98.62% of the plant essential oil. Oxygenated and non-oxygenated monoterpenes constituted 45.73 and 18.67% of the oil, respectively. A total of 18 components could not be identified by MS matching with library spectra. The non-oxygenated sesquiterpene hydrocarbons constituted 30.28% of the oil (Table 31). Non-terpenoid components constituted 0.52 % of the oil while 3.48% of the oil was composed by unknowns. The main constituents of the oil were methylchavicol (20.97%), eugenol (19.35%), *E*-β-ocimene (11.97%), azulene (5.78%), α-caryophyllene (3.08%), β-elemene (2.84%), α-guaiene (1.87%), δ-guaiene (1.82%), germacrene D (1.69%), limonene (1.69%), α-ylangene (1.65%), hedycaryol (1.47%), β-bourbonene (1.42%), valencene (1.37%), terpinolene (1.26%), naphthalenol (1.17%), (Z)-β-caryophyllene (1.13%), β-myrcene (1.08%) and δ-amorphine (1.08%) (Table 29). Out of the 44 compounds suggested by GC-MS library spectral matching, 24 were confirmed by GC-coinjection with standards. However, 12 major (≥ 1%) compounds (methylchavicol, β-bourbonene, β-elemene, valencene, α-guaiene, α-caryophyllene, germacrene D, δ-guaiene, azulene, δ-amorphine, hedycaryol

and naphthalenol) were not confirmed by coinjection due to the unavailability of the authentic standards.

The main components of the plant essential oil had previously been reported to be fenchone (41%) and linalool (37%) (Mwangi *et al.*, 1994), which totally differ from that of the analysed oil (methylchavicol (20.97%), eugenol (19.35%), *E*-β-ocimene (11.97%) and azulene (5.78%)). This suggests the existence of different chemotypes for this particular species.

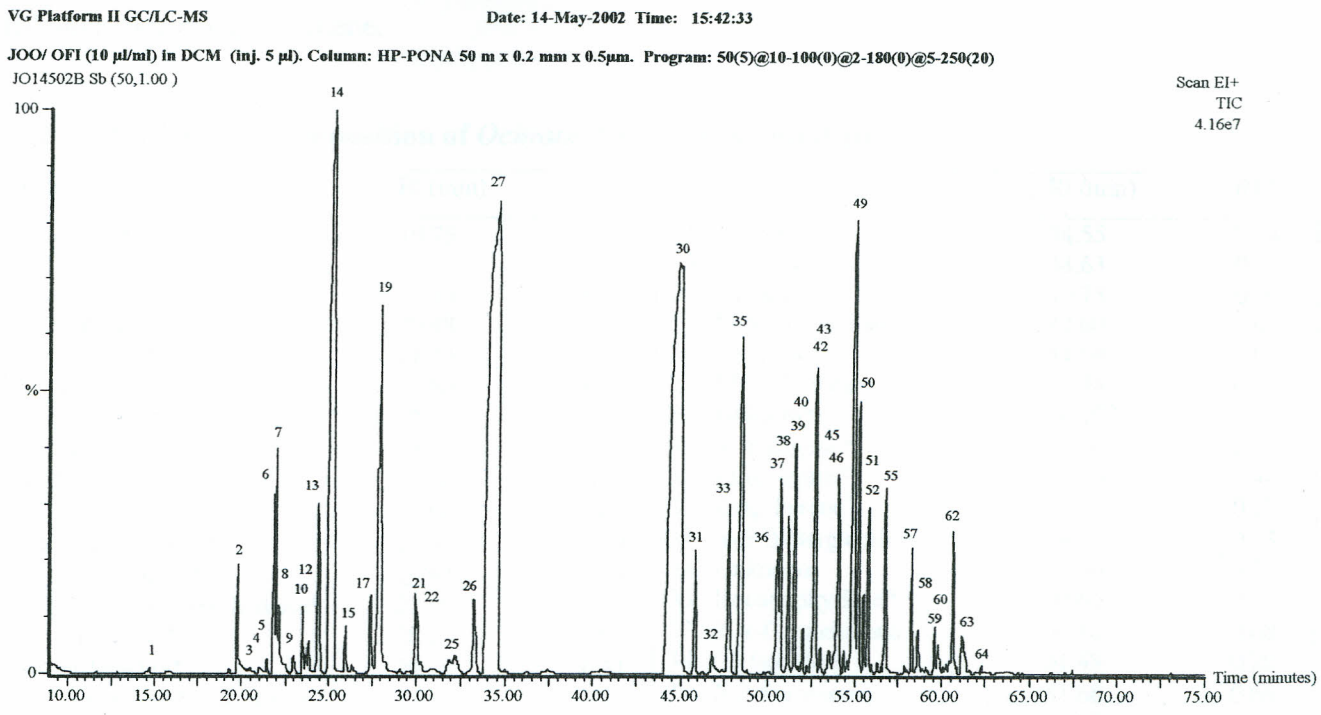
The other members in this genus like *O. basilicum*, *O. campechianum*, *O. americanum* and *O. gratissimum* have essential oil compositions which are qualitatively and quantitatively different from that of this plant (Vieira and Simon, 2000). The main component of the essential oil of *Ocimum selloi*, has been also reported to be methylchavicol (38.9%) together with β-bisabolene (10%), methyleugenol (7.3%), thymol (4.3%), eugenol (2.1%) and β-elemene (1.4%) (Vieira and Simon, 2000).

Table 31: Chemical composition of *Ocimum fischeri* essential oil

Components	Rt (min)	RE%	Components	Rt (min)	RE%
1. *Cis*-3-Hexenol*	15.80	0.08	33. α-Ylangene*	47.78	1.65
2. α-Pinene*	19.75	0.56	34. β-Bourbonene	48.43	1.42
3. Benzaldehyde*	19.85	0.11	35. β-Elemene	48.53	2.84
4. 1-Octen-3-ol*	21.00	0.07	36. Unknown	50.55	0.61
5. Unknown	21.45	0.09	37. β-Caryophyllene*	50.75	1.13
6. β-Pinene*	21.85	0.85	38. Valencene	51.18	1.37
7. β-Myrcene*	22.00	1.08	39. α-Guaiene	51.58	1.87
8. *Cis*-3-hexenylacetate*	22.13	0.20	40. Unknown	52.03	0.36
9. *p*-Mentha-1, 5, 8-triene	22.95	0.17	41. α-Cubebene	52.28	0.06
10. 3-Carene*	23.50	0.38	42. α-Caryophyllene	52.73	3.08
11. 2-Carene*	23.70	0.06	43. Unknown	53.00	0.19
12. *p*-Cymene*	23.85	0.17	44. Unknown	53.53	0.25
13. Limonene*	24.43	1.69	45. Unknown	53.90	0.30
14. β-Ocimene*	25.30	11.97	46. Germacrene D	54.05	1.69
15. γ-Terpinene*	26.00	0.29	47. Unknown	54.35	0.17
16. *Trans*-sabinene hydrate*	26.38	0.07	48. Unknown	54.58	0.06
17. Fenchone*	27.40	0.52	49. δ-Guaiene	54.98	5.78
18. Terpinolene*	27.85	1.26	50. Azulene	55.23	1.82
19. Linalool*	27.98	3.50	51. α-Amorphene	55.50	0.46
20. *Cis*-sabinene hydrate*	28.23	0.10	52. δ- Amorphene	55.75	1.08
21. 3, 4-Dimethyl-2, 4, 6-octatriene	29.95	0.57	53. Unknown	55.95	0.05
22. Unknown	30.05	0.41	54. Unknown	56.28	0.08
23. Unknown	30.23	0.12	55. Hedycaryol/Elemol	56.75	1.47
24. 2-Pinen-3-ol	30.93	0.06	56. Unknown	57.85	0.05
25. *p*-Mentha-1, 5-dien-8-ol	2.18	0.17	57. Spathulenol	58.28	0.92
26. 4-Terpeneol*	33.25	0.91	58. Caryophyllene oxide*	58.60	0.38
27. Methylchavicol	34.58	20.97	59. Humuladienone	59.58	0.27
28. Phenyl acetate	37.38	0.08	60. Unknown	59.78	0.20
29. Unknown	40.45	0.05	61. Unknown	60.40	0.07
30. Eugenol*	44.88	19.35	62. Naphthalenol	60.63	1.17
31. α-Cubebene*	45.85	0.99	63. Unknown	61.13	0.41
32. Methyleugenol	46.83	0.26	64. Unknown	61.30	0.20

*-Component confirmed through co-injection with standard

Figure 7: GC profile of *Ocimum fischeri* essential oil



4.1.6 *Ocimum forskolei*

GC-MS analysis (Figure 8) revealed 73 compounds out of which 49 (Table 32) had relative percentage constitution of ≥0.05. The 49 components constituted 98.59% of the oil, whereas the remaining 23 were responsible for only 1.41%. A total of 9 compounds could not be identified by mass spectral matching. The proposed components were mainly monoterpenes (81.04%) (Table 32). Oxygenated monoterpenes constituted 58.19% of the oil while the non-oxygenated sesquiterpene were responsible for 15.92% and 1.61% due to unknown components. The most abundant components were fenchone (49.86%), limonene (14.08%), zingeberene (6.62%), camphor (5.93%), α-caryophyllene (4.46%), β-myrcene (2.91%), E-β-ocimene (2.42%) and α-pinene (1.47%). Out of the 40 proposed compounds, 21 were confirmed by GC coinjection with standards. However, 2 major (≥ 1%) compounds (α-caryophyllene and zingeberene) were not confirmed by coinjection due to the unavailability of the standards.

The main components of essential oil of the same plant from Ethiopia has been reported as (E)-methyl cinnamate (33.0%), eugenol (25.0%), myrcene (24.2%), methylchavicol (19.3%), linalool (17.3%) (Demissew, 1993). The composition of the oil reveals that the plant consists of different chemotypes and this can be attributed to variation in climatic and geographical conditions. It is

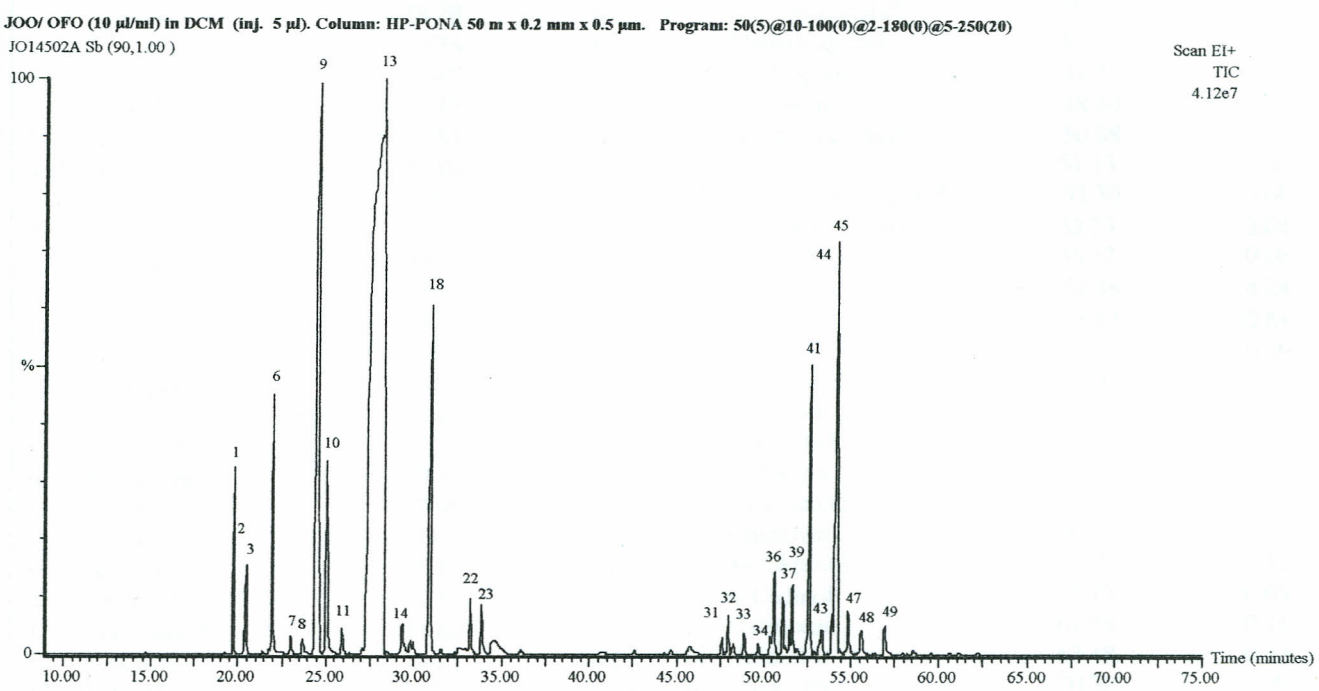
interesting to note that the major constituents in the Kenyan chemotype are not the same as in the Ethopian one except myrcene.

Table 32: Chemical composition of *Ocimum forskolei* essential oil

Components	Rt (min)	RE%	Components	Rt (min)	RE%
1. α-Pinene*	19.75	1.47	24. Myrtenol*	34.55	0.34
2. 7,7-Dimethyl-2-methylene-bicyclo[2.2.1]heptane	20.35	0.20	25. Unknown	34.63	0.77
3. Camphene*	20.48	0.75	26. Unknown	40.75	0.06
4. Sabinene*	21.43	0.05	27. Myrtenyl acetate	42.60	0.05
5. β-Pinene*	21.80	0.05	28. Unknown	44.68	0.06
6. β-Myrcene*	22.00	2.91	29. Methyl cinnamate	45.78	0.27
7. α-Phellandrene*	23.03	0.25	30. Unknown	46.102	0.06
8. 2-Carene*	23.70	0.20	31. α-Ylangene*	47.60	0.21
9. Limonene*	24.60	14.08	32. Zingiberene[a]	47.95	0.48
10. E-β-Ocimene*	25.03	2.42	33. Zingeberene[a]	48.88	0.27
11. γ-Terpinene*	25.93	0.37	34. α-*E*-β-Bergamatone	49.70	0.13
12. *Trans*-sabinene hydrate*	27.10	0.06	35. Unknown	50.40	0.12
13. Fenchone*	28.25	49.86	36. Isocayophyllene*	50.60	0.90
14. α-Fenchol*	29.35	0.31	37. *Z*-α-Bergamatone	51.10	0.68
15. 3-Isopropylcyclohexane	29.43	0.10	38. (-)-Isoledene	51.48	0.20
16. *Trans/cis*-2-pipanal	29.83	0.10	39. *E*-β-Farnesene	51.68	0.66
17. (2-methyl-1-propenylidene)-trimethylcyclopropane	30.00	0.10	40. Unknown	51.90	0.06
18. Camphor*	30.98	5.93	41. α-Caryophyllene	52.68	4.46
19. 2,3,3-Trimethylbicyclo [2.2.1]heptan-2-ol	31.58	0.05	42. α-Curcumene	53.20	0.15
20. Borneol*	32.58	0.15	43. Unknown	53.33	0.33
21. Geranial*	32.93	0.06	44. Germacrene-D	53.93	0.23
22. Terpene-4-ol*	33.23	0.68	45. Zingiberene[a]	54.20	6.62
23. α-Terpeneol*	33.85	0.70	46. Unknown	54.38	0.05
			47. β-Bisabolene	54.85	0.53
			48. β-Sesquiphellandrene	55.58	0.40
			49. Unknown	56.93	0.40

*-Component confirmed through co-injection with standard

Figure 8: GC profile of *Ocimum forskolei* essential oil.



4.1.7 *Plectranthus longipes*

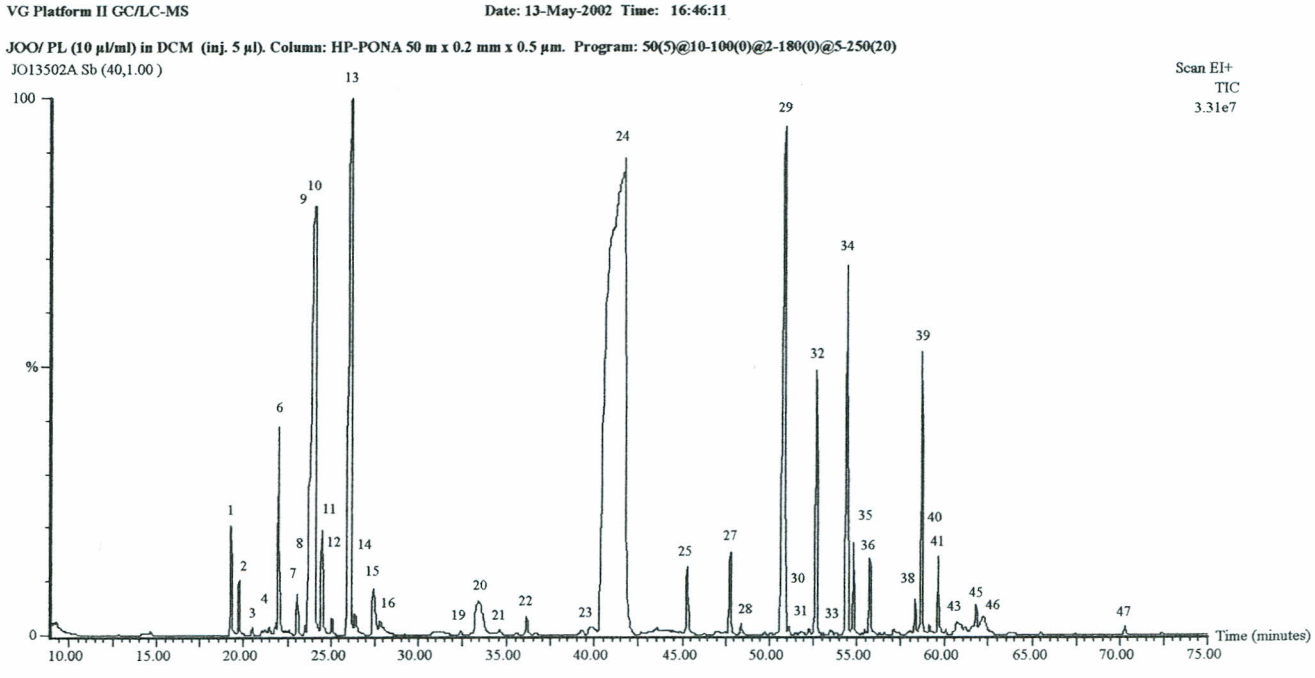
The relative percentage composition of the oil was analyzed based on the 87 peaks detected by GC-MS (Figure 9). Out of these, 47 of the components representing 99.23% of the oil had relative percentage composition of ≥0.05% (Table 33). Out of the 87 GC-MS detectable components, 40 representing 0.77% of the oil had relative abundance of <0.05%. Monoterpenes constituted 75.08% of the oil while 21.81% was due to sesquiterpenes and 1.39% was attributed to unknown components. The oxygenated and non-oxygenated monoterpenes constituted 49.73 and 25.35% of the oil, respectively, while the oxygenated and non-oxygenated sesquiterpenes constituted 18.43 and 3.38% of the oil, respectively. The most abundant components were carvacrol (47.17%), *p*-cymene (9.83%), γ-terpinene (9.16%), isocaryophyllene (8.33%), β-selinene (4.24%), α-caryophyllene (3.04%), caryophyllene oxide (2.73%), β-myrcene (1.71%), α-terpinene (1.57%), terpene-4-ol (1.22%) and limonene (1.08%) (Table 33). Out of the 47 proposed compounds, 25 were confirmed by GC coinjection with standards. However, 2 major (≥ 1%) compounds (β-selinene and α-caryophyllene) could not be confirmed by coinjection due to the absence of standards.

Table 33: Chemical composition of *Plectranthus longipes* essential oil

Components	Rt (min)	RE%	Components	Rt (min)	RE%
1. α-Thujene	19.28	0.74	25. Thymyl acetate*	45.28	0.73
2. α-Pinene*	19.75	0.41	26. Methyleugenol	46.98	0.05
3. Camphene*	20.48	0.08	27. α-Ylangene*	47.75	0.82
4. Sabinene*	21.45	0.05	28. β-Elemene	48.40	0.12
5. β-Pinene*	21.83	0.05	29. Isocaryophyllene*	50.88	8.33
6. β-Myrcene*	22.00	1.71	30. Unknown	51.13	0.06
7. α-Phellandrene*	23.08	0.41	31. Alloaromadendrene*	52.30	0.06
8. Unknown	23.53	0.06	32. α-Caryophyllene	52.73	3.04
9. α-Terpinene*	23.75	1.57	33. Germacrene D	53.53	0.06
10. *p*-Cymene*	24.10	9.83	34. β-Selinene	54.48	4.24
11. Limonene*	24.50	1.08	35. α-Selinene	54.83	0.81
12. (E)- β-Ocimine*	25.05	0.13	36. δ-Amorphene	55.73	0.69
13. γ-Terpinene*	26.15	9.16	37. Unknown	57.10	0.07
14. *Trans*-sabinene hydrate*	26.40	0.13	38. Unknown	58.31	0.32
15. Fenchone*	27.43	0.76	39. Caryophyllene oxide*	58.68	2.73
16. Terpinolene*	27.83	0.07	40. Unknown	59.13	0.06
17. Linalool*	27.98	0.05	41. Humuladienone	59.60	0.65
18. Unknown	31.05	0.05	42. Unknown	60.70	0.17
19. Camphor*	31.48	0.05	43. 3-Unknowns	60.78	0.12
20. Terpene-4-ol*	33.43	1.22	44. α-Cadinol	61.13	0.05
21. α-Terpineol*	34.60	0.07	45. Valencene	61.78	0.21
22. Unknown	36.15	0.20	46. Unknown	62.18	0.37
23. Thymol*	39.93	0.28	47. Unknown	70.30	0.09
24. Carvacrol*	41.68	47.17			

*confirmed through co-injection with standards

Figure 9: GC profile of *Plectranthus longipes* essential oil.



The main components of *Plectranthus marrubioides*, a plant from the same genus, have been reported as camphor (48.8%), 1, 8-cineole (9.0%), *p*-cymene (3.1%), α-terpinene (2.6%), fenchone (1.8%), isocaryophyllene (1.7%), viridiflorol (1.6%), camphene (1.6%), β-selinene (1.5%), *trans*-sabinene hydrate (1.2%), caryophyllene oxide (1.1%) and terpene-4-ol (1.1%) (Omolo, 2002). This suggests that plants of this genus exhibit qualitative as well as quantitative differences.

The components, which were positively identified through coinjections, were subjected to bio-assays to determine the active principles in the oils. The components whose bio-activity against the vector had earlier been reported (Omolo, 2002) were not evaluated singly, but only in blends so as to determine their contribution in the activity of the essential oils.

CHAPTER FIVE

BIO-ASSAY OF ESSENTIAL OIL CONSTITUENTS OF ANTI-INSECT PLANTS

The identified constituents of the essential oils from 7 plants, which had not been evaluated against the vector as repellents or adulticides, were evaluated according to the WHO (1996) protocol.

5.1 Fumigant mosquitocidal assays

Most of the compounds, which were identified in the essential oils, had been previously evaluated against the vector and activity reported (Omolo, 2002). The identified components (carvacrol, hexanal, thymol, 4-isopropylbenzenemethanol and thymylacetate) in the insecticidal plant essential oils were evaluated against the vector with the highest concentration tested being 1%. The only insecticidal compound was carvacrol. The durational activity of the compound at varying concentrations revealed the superiority of the compound at higher concentrations and longer duration (Table 34).

Table 34: Mosquitocidal activity data of carvacrol

	% Mortality				
Time (h)/Dose	1.408	2.817	4.225	5.634	7.042
1	0^{p}	0^{p}	0^{p}	1.33 ± 0.677^{o}	7.00 ± 1.125^{m}
2	0^{p}	0^{p}	3.00 ± 0.856^{n}	3.67 ± 0.615^{n}	10.00 ± 1.789^{l}
3	0^{p}	3.33 ± 0.667^{n}	6.00 ± 0.730^{m}	5.00 ± 0.856^{m}	14.33 ± 2.155^{jk}
4	0^{p}	3.67 ± 0.615^{n}	8.33 ± 0.955^{m}	32.33 ± 2.155^{g}	63.00 ± 7.620^{d}
5	3.67 ± 0.615^{n}	8.67 ± 1.430^{l}	15.33 ± 1.687^{j}	51.00 ± 2.620^{e}	75.00 ± 6.807^{c}
6	12.33 ± 1.745^{jk}	19.00 ± 1.528^{i}	39.33 ± 1.764^{f}	67.33 ± 3.252^{d}	82.67 ± 5.506^{b}

Dose x 10^{-3} mg/cm^3; Means followed by the same letter are not significantly different at $p < 0.05$

The results were subjected to probit analysis (Table 35) and the regression equation derived. The LC_{50} value for carvacrol was found to be 4.237×10^{-3} mg/cm^3. The other principles did not show activity at the highest concentration tested.

Table 35: Probit analysis of *An. gambiae* fumigant mosquitocidal assay data for carvacrol after 6 hours

Dose x 10^{-3} (mg/cm^3)	% Mortality (Corrected)	Log Dose + 5	Emperical Probit	Expected Probit	Working Probit
1.408	12.33	2.149	3.82	3.5	3.93
2.817	19.00	2.450	4.12	4.5	4.16
4.225	39.33	2.626	4.72	5.0	4.74
5.634	67.33	2.751	5.44.	5.4	5.45
7.042	82.67	2.848	5.95	5.7	5.92

Regression equation, Y= 3.038X – 2.981

5.2 Repellency assays

In this test, the compounds that were confirmed by GC peak enhancement were evaluated except those that had been assayed earlier (Omolo, 2002). A total of 15 compounds were tested individually for their repellency against *An. gambiae* (Table 36).

Table 36: *An. gambiae* repellency assay data for essential oil standards

	% Protective efficacy			
Concentration (g/ml)	10^{-4}	10^{-3}	10^{-2}	10^{-1}
4-Isopropylbenzenemethanol	36.1 ± 3.15^{uvw}	67.3 ± 3.62^{klm}	81.0 ± 3.30^{fg}	100^{a}
Thymol	30.8 ± 4.30^{wxy}	57.3 ± 5.25^{pqr}	83.4 ± 2.48^{def}	100^{a}
Carvacrol	37.4 ± 6.55^{uv}	74.5 ± 2.44^{hi}	93.1 ± 1.57^{bc}	100^{a}
Phytol	41.4 ± 3.67^{uv}	54.5 ± 3.97^{pqr}	82.8 ± 4.19^{efg}	91.4 ± 2.99^{c}
1-Octen-3-ol	37.5 ± 6.70^{uv}	39.6 ± 6.01^{uv}	55.3 ± 3.79^{pqr}	69.7 ± 4.53^{jkl}
1-Methylpyrole	33.0 ± 4.94^{wxy}	55.8 ± 3.32^{pqr}	62.3 ± 4.02^{nop}	77.1 ± 3.94^{ghi}
Myrcene	34.1 ± 5.17^{wxy}	56.3 ± 4.82^{pqr}	66.1 ± 4.24^{klm}	75.1 ± 3.01^{hi}
Hexanal	19.5 ± 3.33^{z}	32.5 ± 2.39^{wxy}	49.9 ± 2.19^{rst}	61.9 ± 3.54^{nop}
2-Carene	23.8 ± 1.72^{z}	34.6 ± 2.30^{wxy}	45.7 ± 1.18^{tu}	56.3 ± 2.09^{pqr}
Thymyl acetate	19.3 ± 4.00^{z}	29.9 ± 1.77^{y}	48.1 ± 4.36^{rst}	71.4 ± 2.23^{ijk}
3-Hexanyl acetate	25.7 ± 2.14^{y}	34.5 ± 2.11^{wxy}	55.7 ± 1.41^{pqr}	64.7 ± 1.46^{lmn}
Benzaldehyde	19.3 ± 4.00^{z}	29.9 ± 1.77^{y}	48.13 ± 4.36^{rst}	71.4 ± 2.23^{ijk}
γ-Gurjunene	33.4 ± 1.63^{wxy}	46.9 ± 0.75^{stu}	55.4 ± 1.84^{pqr}	68.7 ± 0.90^{klm}
Linalool oxide	58.6 ± 2.23^{pq}	62.6 ± 2.6^{nop}	62.9 ± 6.20^{nop}	70.1 ± 2.78^{jkl}
3-Carene	46.8 ± 2.56^{stu}	51.2 ± 6.20^{qrs}	57.0 ± 2.23^{pqr}	66.4 ± 2.79^{klm}

Means followed by the same letter are not significantly different at $p < 0.05$

The major compounds, which were considered to be responsible for the repellent activity of the essential oils, were blended in the ratio they exist in the essential oils and assayed for their repellent activity against *An. gambiae* (Table 37).

Table 37. *An. gambiae* repellency assay data for blends of essential oil standards

	% Protective efficacy			
Concentration (g/ml)	10^{-4}	10^{-3}	10^{-2}	10^{-1}
MF_1	39.3 ± 3.51^{uv}	58.7 ± 2.34^{pq}	93.7 ± 1.79^{bc}	100^{a}
MF_2	31.1 ± 3.82^{wxy}	61.0 ± 4.21^{nop}	78.6 ± 3.71^{ghi}	99.2 ± 0.56^{a}
MF_3	29.9 ± 2.78^{xy}	56.7 ± 3.28^{pqr}	87.8 ± 3.13^{d}	100^{a}
MF_4	26.1 ± 3.85^{y}	56.8 ± 3.48^{pqr}	81.9 ± 2.25^{efg}	97.9 ± 1.22^{a}
MF_5	50.6 ± 3.34^{rst}	58.3 ± 3.23^{pq}	84.3 ± 4.29^{def}	98.7 ± 1.33^{a}
MF_6	24.3 ± 3.87^{z}	44.1 ± 3.43^{u}	84.0 ± 2.46^{def}	100^{a}
CP_1	48.5 ± 4.15^{rst}	67.3 ± 3.32^{klm}	77.4 ± 2.99^{ghi}	100^{a}
CP_2	64.9 ± 2.60^{lmn}	70.5 ± 2.02^{jkl}	78.7 ± 2.92^{ghi}	94.5 ± 1.48^{bc}
CP_3	29.7 ± 3.95^{y}	55.0 ± 3.72^{pqr}	71.5 ± 4.41^{ijk}	96.7 ± 1.48^{ab}
CP_4	31.2 ± 2.91^{wxy}	55.8 ± 4.70^{pqr}	81.4 ± 1.94^{fg}	99.5 ± 0.49^{a}
ET_1	48.1 ± 3.23^{rst}	55.2 ± 2.84^{pqr}	76.7 ± 1.31^{ghi}	91.3 ± 1.12^{c}
ET_2	33.8 ± 4.11^{wxy}	55.9 ± 3.06^{pqr}	69.5 ± 3.53^{klm}	89.2 ± 2.76^{cd}
ET_3	36.8 ± 1.98^{uvw}	45.4 ± 3.04^{stu}	62.7 ± 2.95^{nop}	73.4 ± 2.46^{hij}
OFI_1	43.99 ± 4.33^{uv}	67.1 ± 2.83^{klm}	79.4 ± 2.90^{fgh}	98.4 ± 0.83^{a}
OFI_2	30.7 ± 3.44^{wxy}	59.9 ± 5.65^{opq}	78.6 ± 2.87^{ghi}	80.0 ± 3.58^{fgh}
OFI_3	35.1 ± 3.1^{vwx}	59.3 ± 2.19^{opq}	70.0 ± 3.42^{jkl}	85.2 ± 3.71^{de}
OFI_4	28.6 ± 4.52^{y}	41.5 ± 3.81^{uvw}	57.3 ± 2.13^{pq}	82.4 ± 1.79^{efg}
OFO_1	51.0 ± 3.69^{qrs}	62.0 ± 3.41^{nop}	74.6 ± 1.85^{hi}	78.3 ± 2.19^{ghi}
OFO_2	37.4 ± 5.54^{uvw}	64.9 ± 3.71^{mno}	66.5 ± 2.39^{klm}	69.3 ± 1.99^{klm}
OFO_3	$28.4 \pm .69^{y}$	45.3 ± 3.24^{stu}	60.7 ± 2.91^{op}	69.4 ± 1.88^{jkl}
OFO_4	43.6 ± 3.88^{uv}	60.2 ± 2.76^{op}	69.0 ± 2.74^{klm}	68.1 ± 2.85^{klm}
PL_1	53.3 ± 5.09^{pqr}	64.1 ± 4.22^{mno}	82.7 ± 2.88^{efg}	100^{a}
PL_2	45.0 ± 2.70^{tu}	65.5 ± 2.42^{lmn}	77.2 ± 2.79^{ghi}	97.1 ± 1.44^{a}
PL_3	34.6 ± 3.50^{vwx}	70.4 ± 3.42^{jkl}	86.2 ± 2.69^{de}	95.2 ± 1.53^{b}
PL_4	31.6 ± 4.72^{wxy}	56.6 ± 2.61^{pqr}	81.4 ± 2.74^{fg}	100^{a}

Means followed by the same letter are insignificantly different at $p < 0.05$

5.2.1 *Mkilua fragrans*

From *M. fragrans*, the components evaluated individually were hexanal, 2-carene and 4-isopropylbenzenemethanol (Table 36). The component which had promising activity was 4-isopropylbenzenemethanol (100%) at 10% concentration. The other two principles (hexanal and 2-carene) had protective efficacy of 61.9 and 56.3 %, respectively at the same concentration.

The principles of the oil which might be responsible for the demonstrated activity of the oil are linalool, camphor, 4-isopropylbenzenemethanol, p-cymen-8-ol, carvone and caryophyllene oxide. Six blends which were evaluated did not show any significant difference in activity at 10% concentaration (Table 37) revealing the importance of the constituting principles towards the

repellent activity of the oil. The blends were MF_1 (2, 1, 12, 8 and 77% of linalool, camphor, 4-isopropylbenzenemethanol, carvone and caryophyllene oxide, respectively), MF_2 (8, 6, 50 and 36% of linalool, camphor, 4-isopropylbenzenemethanol and carvone respectively), MF_3 (2, 1, 13 and 84% of linalool, camphor, 4-isopropylbenzenemethanol and caryophyllene oxide respectively), MF_4 (2, 2, 9 and 87% of linalool, camphor, carvone and caryophyllene respectively), MF_5 (2, 12, 8 and 78% of linalool, 4-isopropylbenzenemethanol carvone and caryophyllene oxide) and MF_6 (1, 12, 9 and 78% of camphor, 4-isopropylbenzenemethanol carvone and caryophyllene oxide respectively). This demonstrated the important role of each compound in the blend and the synergistic nature of the activity. MF_1 exhibited significantly higher repellent activity than all the other blends at 0.1% concentration thus confirming the important role of caryophyllene oxide. Carvone, caryophyllene oxide, linalool and camphor have previously been reported to have individual repellent activity against *An. gambiae* (Omolo, 2002).

5.2.2 *Croton menyharthii*

The components of *C. menyharthii* essential oil that were evaluated individually included 1-methylpyrole, 1-octen-3-ol, β-myrcene, 2-carene and γ-gurjunene with protective efficacies of 77.1, 69.7, 75.1, 56.3 and 68.7%, respectively at 10% concentration (Table 36). There was no blend evaluated for the plant oil constituents as the main components were not commercially available. It was difficult to assign the activity of the oil to a particular or group of constituents as the main components were not evaluated against *An. gambiae*.

5.2.3 *Croton pseudopulchellus*

The componets of *C. pseudopulchellus* evaluated individually were β-myrcene, 1-methylpyrole and linalool oxide with protective efficacy of 75.1, 77.1 and 70.1%, respectively, at the 10% concentration (Table 36). Four blends were evaluated against the vector. Two blends, CP_1 (45, 39, 8 and 8% of linalool, caryophyllene oxide, γ-terpinene and 1-methylpyrole, respectively) and CP_2 (54 and 46% of linalool and caryophyllene oxide), had significantly different protective efficacies (100 and 94.5%, respectively) at 10% concentration. The superiority of CP_1 over CP_2 demonstrates the significant contribution of γ-terpinene and 1-methylpyrole towards the activity of the whole oil. The other two blends CP_3 (71, 15 and 14% of caryophyllene oxide, γ-terpinene and 1-methylpyrole, respectively) and CP_4 (74, 13 and 13% of linalool, γ-terpinene and 1-methylpyrole, respectively) also showed significant superior activity over that of the oil at 10% concentration. This observation also

confirms the importance of linalool and caryophyllene oxide towards the overall repellent activity of the oil.

5.2.4 *Endostemon tereticaulis*

The components of *E. tereticaulis* that were individually assayed included 1-octen-3-ol and 2-carene with protective efficacy of 69.7% and 56.3%, respectively at 10% concentration (Table 36). Three blends, which consisted of the most probable repellent principles, were evaluated against *An. gambiae*. A blend ET_1 (40, 26, 21 and 13% of terpene-4-ol, fenchone, γ-terpinene and terpinolene, respectively) had comparable activity (91.3%) to that of the whole essential oil (95.7%) at 10% concentration suggesting that the components had major contribution to the observed overall repellency. A blend ET_2 (43, 35 and 22% of fenchone, γ-terpinene and terpinolene) was close in repellency (89.2%) to ET_1 (91.3%) and comparable to the whole oil (95.78%). The blend ET_3 (54, 28 and 17% of terpene-4-ol, γ-terpinene and terpinolene) was of inferior activity (73.4%) as compared to that of the whole oil ET_1 and ET_2. However, the exclusion of terpene-4-ol does not lead to a significant drop in repellency. This suggests that the presence of fenchone but not terpene-4-ol is crucial for the overall activity of the oil. 1-Octen-3-ol, which has been reported as an attractant for *Aedes taeniorhynchus* and *Anopheles* spp. (Takken and Kline, 1989) did not demonstrate this kind of activity over the concentration range tested. It has been demonstrated that insect repellent materials can also behave like attractants at very low concentrations (Mehr *et al.*, 1990). This could explain the repellent activity of 1-octen-3-ol.

5.2.5 *Ocimum fischeri*

The compounds from this plant that that were evaluated individually for their repellent activity included benzaldehyde, 1-octen-3-ol, β-myrcene, 3-hexanyl acetate and 3-carene with protective efficacy of 71.4, 69.7, 75.1, 64.7 and 66.4 %, respectively at 10% concentration (Table 36).

Four blends, OFI_1 (89, 6 and 5% of eugenol, terpinolene and β-myrcene, respectively); OFI_2 (54 and 46% of terpinolene and β-myrcene, respectively); OFI_3 (95 and 5% of eugenol and β-myrcene, respectively) and OFI_4 (94 and 6% of eugenol and terpinolene, respectively), were evaluated against *An. gambiae* (Table 37). The OFI_1 was a superior repellent (98.4%) over the concentration range tested showing that eugenol is an important component for the activity of the oil. There was no significant difference in the repellency of OFI_1 (98.4%) and the essential oil (100%) at 10% concentration. There were significant differences in the repellency of OFI_1 (98.4%), OFI_2 (80.0%),

OFI_3 (85.2%) and OFI_4 (82.4%) demonstrating that the three compounds are crucial for the repellency of the essential oil. The significant contribution of eugenol is also demonstrated by the enhancement factor in the various blends in which it is included (Table 38), calculated according to Ladd (1980).

$$\text{Enhancement factor} = \frac{\text{Repellency of blend}}{\text{Mean repellency of the constituting component}}$$

Table 38: *An. gambiae* essential oil repellent blend effects for *Ocimum fischeri* at 10% concentration

Blend/compound	%Repellency	Relative repellency	Enhancement factor*
OFI_1	98.4	111.2	1.40
OFI_3	85.2	96.3	1.04
OFI_4	82.4	93.1	1.01
OFI_2	80.0	90.4	___
Eugenol	88.5	100	
Myrcene	75.1	84.9	
Terpinolene	74.3	84.0	

*-With respect to eugenol

However, the contributions of terpinolene and β-myrcene are also important since their exclusion leads to significant drop in repellency. It is interesting to note that the repellency of OFI_1 (98.4%) is not significantly different from that of the whole oil mixture (100.0%) suggesting that the other minor components may not have a significant contribution.

5.2.6 *Ocimum forskolei*

Two of the identified compounds β-myrcene and 2-carene were tested for their repellent activity individually. The activity of the other major components like fenchone and camphor are already reported (Omolo, 2002). β-Myrcene and 2-carene had protective efficacy of 75.1 and 56.3%, respectively at 10% (Table 36). These values are significantly different from that of the whole essential oil mixture.

The repellent activity of the plant oil could be due to the major active principles (β-myrcene, fenchone, α-pinene and camphor) and as demonstrated by four different blends, OFO_1 (83, 10, 2 and 5% of fenchone, camphor, α-pinene and β-myrcene, respectively), OFO_2 (87, 10 and 3% of fenchone, camphor and α-pinene, respectively), OFO_3 (89 and 11% of fenchone and camphor, respectively) and OFO_4 (78 and 22% of fenchone and linalool, respectively). The repellent activity of the blends OFO_2, OFO_3 and OFO_4 were lower than that of the essential oil at corresponding concentrations suggesting that the components of the blends; fenchone, camphor, β-myrcene and α-pinene were not totally responsible for the repellent activity of the essential oil. The inclusion of α-pinene in OFO_1 blend did not show any remarkable improvement in the activity as compared to OFO_3 (Table 37). Inclusion of β-myrcene in OFO_1 increases repellency. OFO_1 had repellent activity close to that of the essential oil at the corresponding concentrations except at 10%. The observations suggest that there may be minor components of the oil, which contribute towards the repellency at the high concentrations.

5.2.7 *Plectranthus longipes*

Most of the identified components of the essential oil of this plant had been evaluated for repellency against *An. gambiae*. These included α-pinene, camphene, β-pinene, α-terpinene, *p*-cymene, limonene, γ-terpinene, fenchone, terpinolene, linalool, camphor, terpinen-4-ol, α-terpineol, isocaryophyllene and caryophyllene oxide (Omolo, 2002). In the present study the components assayed were thymol, carvacrol, thymyl acetate and β-myrcene. Thymol and carvacrol, which is the major compound, had protective efficacy of 100% at 10% concentration, whereas thymyl acetate and β-myrcene had relatively lower activity (71.4 and 75.1%, respectively) at the same concentration.

The constituents of the oil that are most likely responsible for the demonstrated repellent activity of the oil are α-terpinene, γ-terpinene, terpinolene, linalool, terpenen-4-ol, camphor, α-terpeneol, thymol, carvacrol, thymyl acetate and caryophyllene oxide. The repellency of four blends PL_1 (75, 4, 2, 2, 15 and 2% of carvacrol, caryophyllene oxide, terpene-4-ol, β-myrcene, γ-terpinene and α-pinene), PL_2 (17, 8, 7, 58 and 10% of caryophyllene oxide, terpene-4-ol, β-myrcene, γ-terpinene and α-terpinene), PL_3 (78, 2, 2, 15 and 3% of carvacrol, terpene-4-ol, β-myrcene, γ-terpinene and α-terpinene) and PL_4 (88, 5, 2, 2 and 3% of carvacrol, caryophyllene oxide, terpene-4-ol, β-myrcene and α-terpinene, respectively) were evaluated. The results indicated that PL_1, PL_2 and PL_4 had similar level of activity as that of the oil at 1 and 10% concentrations (Table 13 and 37). This suggests that the other major components of the oil like *p*-cymene, limonene, isocaryophyllene, α-

caryophyllene and β-selinene may not contribute significantly to the overall repellency of the oil. Infact, it has been reported that *p*-cymene, limonene and isocaryophyllene exhibit decreasing repellent activity against, *An. gambiae*, with increasing concentration (Omolo, 2002). The relatively low activity of PL_3 as compared to that of the oil and the three other blends demonstrates the importance of caryophyllene oxide and carvacrol towards the repellent activity of the whole essential oil of *Plectranthus longipes*. It is also interesting to note that the exclusion of the major compound, carvacrol as in PL_2 does not lead to any significant reduction in repellency suggesting that the components act synergistically.

The bioassay data for the 4 most active compounds was subjected to probit analysis (Finney, 1971) (Table 39-41) and the regression equations derived.

Table 39: Probit analysis of *An. gambiae* repellency data for thymol

Dose x 1.78 (mg/cm^2)	% Repellency	Log Dose + 5	Emperical Probit	Expected Probit	Working Probit
10^{-4}	30.8	1.232	4.50	4.5	4.49
10^{-3}	57.3	2.232	5.18	5.2	5.19
10^{-2}	83.4	3.232	5.95	5.9	5.97
10^{-1}	100	4.232	----	----	----

The regression equation derived for thymol was $Y = 0.725X + 3.5918$

Table 40: Probit analysis of *An. gambiae* repellency data for carvacrol

Dose x 1.78 (mg/cm^2)	% Repellency	Log Dose + 5	Emperical Probit	Expected Probit	Working Probit
10^{-4}	37.4	1.232	4.67	4.7	4.68
10^{-3}	74.5	2.232	5.67	5.6	5.66
10^{-2}	93.1	3.232	6.48	6.5	6.01
10^{-1}	100	4.232	----	----	----

The regression equation derived for carvacrol was $Y = 0.905X + 3.5867$

Table 41: Probit analysis of *An. gambiae* repellency data for 4-isopropylbenzenemethanol

Dose x 1.78 (mg/cm^2)	% Repellency	Log Dose + 5	Emperical Probit	Expected Probit	Working Probit
10^{-4}	36.1	1.232	4.64	4.7	4.65
10^{-3}	67.3	2.232	5.44	5.3	5.44
10^{-2}	92.31	3.232	5.88	5.9	5.88
10^{-1}	100	4.232	----	----	----

The regression equation derived for 4-isopropylbenzenemethanol was $Y= 0.620X + 3.9362$

Table 42: Probit analysis of *An. gambiae* repellency data for phytol

Dose x 1.78 (mg/cm^2)	% Repellency	Log Dose + 5	Emperical Probit	Expected Probit	Working Probit
10^{-4}	41.4	1.232	4.77	4.7	4.78
10^{-3}	54.5	2.232	5.13	5.3	5.11
10^{-2}	82.8	3.232	5.95	5.8	5.94
10^{-1}	91.4	4.232	6.34	6.4	6.37

The regression equation derived for phytol was $Y= 0.553X + 4.0367$

From the regression equations, the concentrations that would repel 50% of insects (RC_{50}) for the 4 most active principles were determined (Table 43).

Table 43: *An. gambiae* RC_{50} of essential oil standards

Compound	$RC_{50} \times 10^{-4}$ (mg/cm^2)
Thymol	8.750
Carvacrol	3.648
4-Isopropylbenzenemethanol	5.198
Phytol	5.521

From the RC_{50} values the order of activity of the repellents is carvacrol > 4-isopropylbenzenemethanol > phytol > thymol. The activity of these compounds may be attributed to the presence of hydroxyl and unsaturation in the molecules. Infact, menthol, with a hydroxyl function at the same position as that of thymol save for the saturation of the ring has been reported to have no repellent activity against *An. gambiae* (Barasa *et al.*, 2002). During the course of the study carvacrol and thymol identified in *Thymus vulgaris* were reported to have equivalent repellent activity against *Culex pipiens* Pallens (Choi *et al.*, 2002). The activity of these two compounds against *An. gambiae* is being reported for the first time. The repellent activity of 4-

isopropylbenzenemethanol and phytol against mosquitoes especially *An. gambiae* is also reported for the first time in this work. Even though these compounds are of good activity, the potency of DEET ($RC_{50} = 2.6 \times 10^{-4}$ mg/cm^2) is still better (Jerome and Mustapha, 2000).

5.2.8 Bio-assay of formulated compounds

The 4 compounds that showed good repellent activity at 10% concentration against *An. gambiae* (Table 36) were dispersed in aqueous base, petroleum jelly and emulsificant base (10%) and assayed against the vector to evaluate their duration of protection. The activities of the formulations were evaluated at intervals of two hours for up to 8 hours. The protective efficacies were determined as described earlier (Table 44).

Table 44. Durational repellency assay of formulated compounds

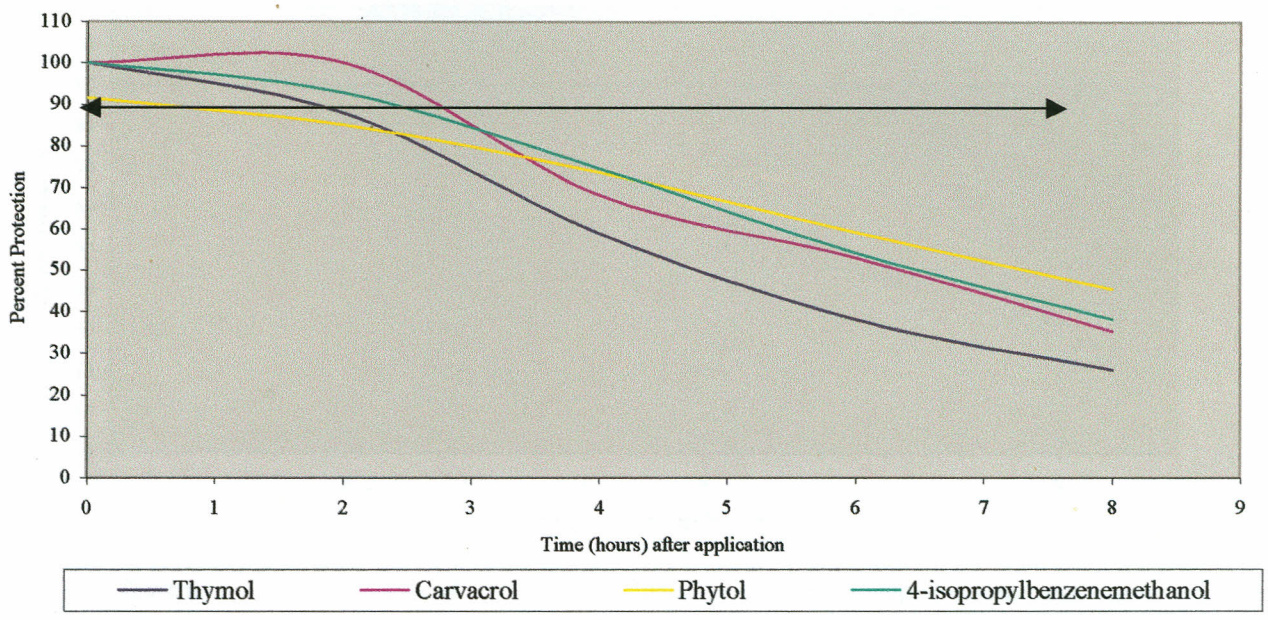
Duration (hours)	0	2	4	6	8
Thymol[1]	100^{a}	87.8 ± 2.04^{d}	58.8 ± 3.94^{pq}	38.1 ± 3.73^{uvw}	26.0 ± 1.59^{y}
Thymol[2]	100^{a}	92.6 ± 1.41^{cd}	71.9 ± 3.93^{ijk}	56.9 ± 3.90^{pqr}	38.2 ± 2.15^{uvw}
Thymol[3]	100^{a}	96.0 ± 1.82^{b}	71.6 ± 2.73^{ijk}	47.2 ± 4.21^{rst}	32.1 ± 1.84^{wxy}
Carvacrol[1]	100^{a}	100^{a}	68.1 ± 4.33^{klm}	53.0 ± 4.25^{pqr}	35.2 ± 3.32^{uvw}
Carvacrol[2]	100^{a}	81.5 ± 7.51^{fg}	69.9 ± 3.65^{jkl}	50.8 ± 4.42^{rst}	31.6 ± 2.32^{wxy}
Carvacrol[3]	100^{a}	94.7 ± 3.50^{bc}	74.0 ± 6.65^{hi}	49.5 ± 7.97^{rst}	29.0 ± 3.45^{wxy}
Phytol[1]	91.5 ± 1.46^{cd}	84.9 ± 2.35^{def}	73.6 ± 2.57^{hij}	59.1 ± 6.59^{pq}	45.4 ± 6.84^{stu}
Phytol[2]	89.5 ± 1.72^{cd}	83.1 ± 2.77^{ef}	78.9 ± 4.41^{ghi}	43.3 ± 2.52^{uv}	27.2 ± 3.37^{y}
Phytol[3]	93.6 ± 3.21^{cd}	86.3 ± 2.39^{de}	73.9 ± 3.84^{hij}	65.1 ± 4.89^{imn}	42.7 ± 5.31^{uv}
Isopropylbenzenemethanol[1]	100^{a}	92.7 ± 2.93^{c}	76.9 ± 3.83^{ghi}	53.8 ± 3.84^{pqr}	38.1 ± 2.66^{wxy}
Isopropylbenzenemethanol[2]	100^{a}	90.1 ± 2.31^{c}	70.3 ± 3.41^{jkl}	51.2 ± 2.71^{qrs}	36.3 ± 3.37^{uvw}
Isopropylbenzenemethanol[3]	100^{a}	89.3 ± 3.13^{cd}	69.4 ± 2.32^{klm}	49.1 ± 1.31^{rst}	34.1 ± 2.39^{wxy}

Means marked by same letter are not significantly different at $P < 0.05$. The compounds marked with the superscript 1, 2 and 3 were dispersed in aqueous base, petroleum jelly and emulsificant base respectively.

Formulations of carvacrol and 4-isopropylbenzenemethanol in aqueous base demonstrated similar durational repellent activity whereas thymol had lower activity at the corresponding time intervals (Table 44).

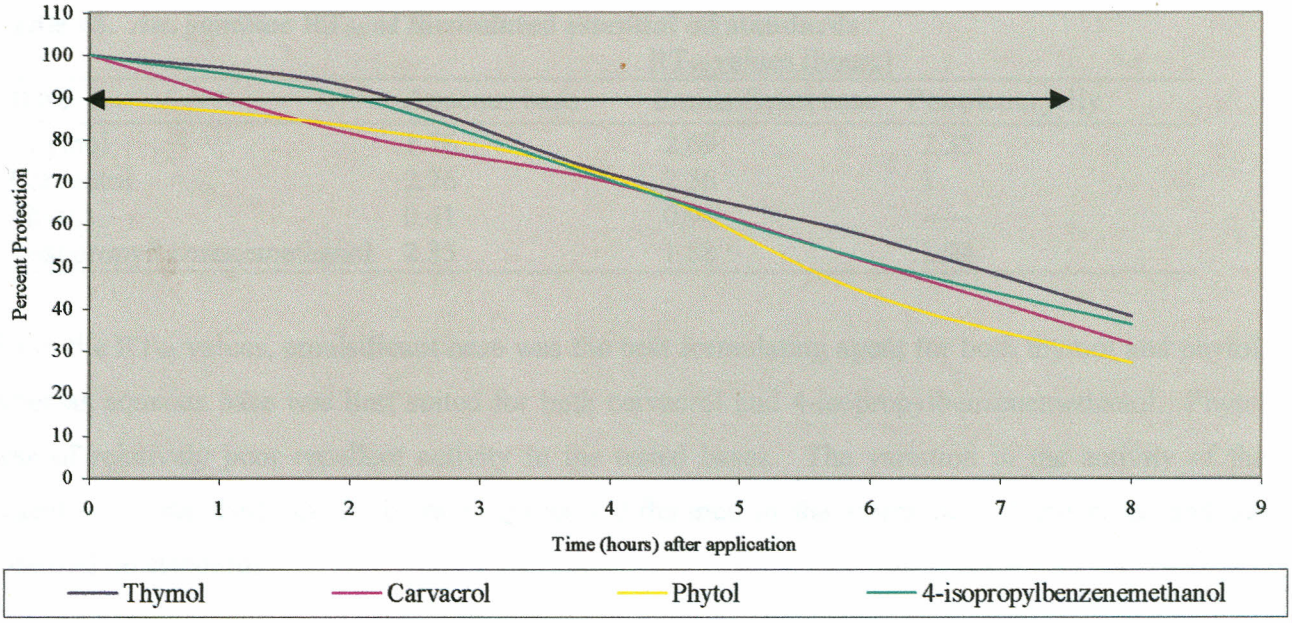
Regression analysis was done and the theoretical decay lines estimated (Figure 10-12) (Hassanali *et al*, 1990).

Figure 10: Decay curves of aqueous base formulation of essential oil standards



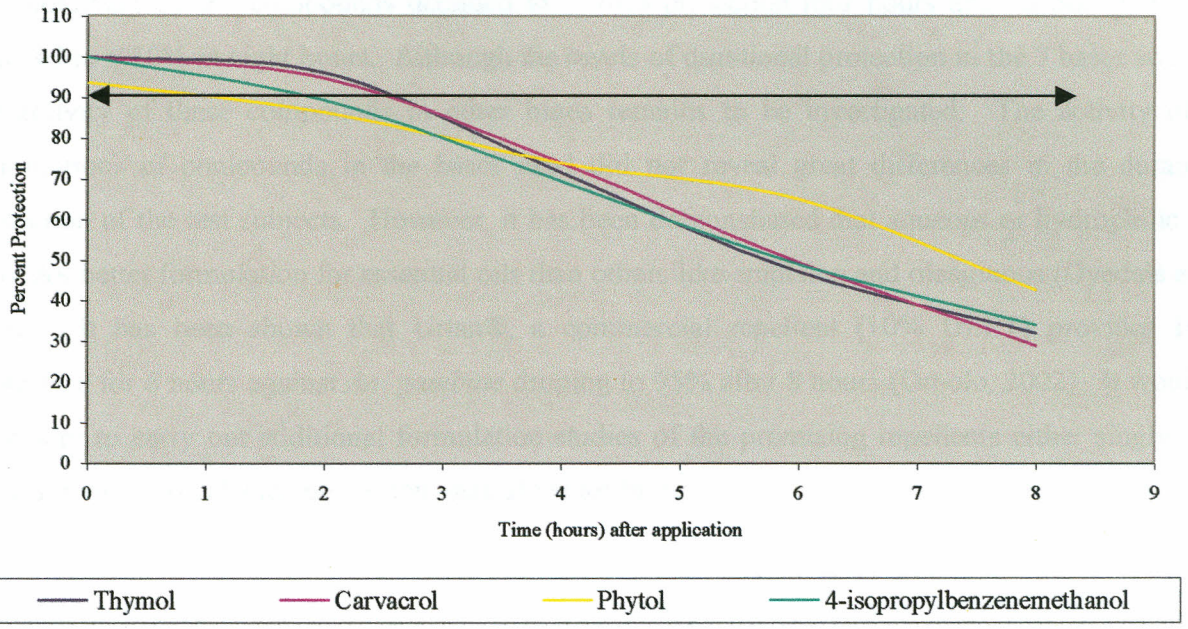
Concentration on skin 0.17 mg/cm^2. A bold longitudinal line indicates the 90% protection level

Figure 11: Decay curves of petroleum jelly formulation of essential oil standards



Concentration on skin 0.17 mg/cm^2. A bold longitudinal line indicates the 90% protection level

Figure 12: Decay curves of emulsificant base formulation of essential oil standards



Concentration on skin 0.17 mg/cm^2. A bold longitudinal line indicates the 90% protection level

From the decay curves, the time at which 90% of insect test population would be repelled (RT_{90}) for the tested formulations were determined (Table 45).

Table 45. *An. gambiae* RT_{90} of formulated essential oil standards

	RT_{90} values (hours)		
Base	Aqueous base	Emulsificant base	Petroleum jelly
Thymol	1.76	2.69	2.35
Carvacrol	2.76	2.56	1
Phytol	0.41	0.94	-----
4-isopropylbenzenemethanol	2.35	1.88	1.94

From the RT_{90} values, emulsificant base was the best formulating agent for both thymol and phytol, whereas aqueous base was best suited for both carvacrol and 4-isopropylbenzenemethanol. Phytol was of relatively poor repellent activity in the tested bases. The variation in the activity of the essential oil standards in the bases suggests a difference in the interaction of the bases and the essential oil standards.

Carvacrol in aqueous and emulsificant base; thymol in petroleum jelly and emulsificant base; and 4-isopropylbenzenemethanol in aqueous base and petroleum jelly provided > 90% reduction in

mosquito bites on subjects for up to two hours after treatment (Figure 10-12; Table 45). However, the protection by the compounds declined to ≈ 70% protection four hours after application which reduced to ≤ 50% at eight hours. Although the levels of durational protection in the 3 bases was low, the activity of these compounds in other bases remains to be investigated. The activity of the formulations of compounds in the bases used did not reveal great differences in the durational protection of the test subjects. However, it has been demonstrated that aqueous or hydrophilic base provides better formulation for essential oils than others like emulsion and oleaginous (Oyedele *et al.*, 2002). It has been shown that Urtan®, a commercial repellent (10% DEET) provides 100% protection for 6 hours against *An. gambiae* droping to 95% after 8 hours (Omolo, 2002). It would be necessary to carry out additional formulation studies of the promising repellents either singly or as blends in order to enhance protection against vector bites.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusions

Bio-evaluation of water, organic solvent and essential oil extracts showed remarkable quantitative differences in their repellent activity against *An. gambiae*. GC and GC-MS analysis provided an insight into the qualitative and quantitative differences in the components of the individual extracts. The repellency assays revealed the order of activity as; water extracts< solvent extracts< essential oils. This trend can be attributed to the volatility of the components of extracts. A good repellent material should have high vapour pressure to ensure adequate saturation of the environment it is supposed to protect.

Even though the water extracts of the plants tested had significantly different repellency, their activities were generally low (Table 3) and cannot provide adequate protection against *An. gambiae*. The organic solvent extracts (Table 4-5) were not any better than the water extracts.

The tarsal contact mosquitocidal assays did not reveal any activity in the tested extracts. Out of the oils tested, only three (*Ocimum forskolei*, *Mkilua fragrans* and *Plectranthus longipes)* showed activity by fumigation (Table 22). *O. forskolei* was the most and *M. fragrans* the least active. This shows that high-level volatility is of importance for fumigant action of the oils.

The most active essential oils in the repellency tests were *Plectranthus longipes*, *M. fragrans*, *O. fischeri*, *O. forskolei*, *Croton pseudopulchellus*, *C. menyharthii* and *Endostemon teriticaulis* (Table 12). The detailed repellency assays revealed the superiority of *P. longipes* and *M. fragrans* at 10% concentration (Table 13). The essential oils of the two plants had the highest repellent activity among all the tested materials, with a protective efficacy of 100% for both, at 10% concentration. It has been observed that a relationship exists between dosage and the number of tests insects used in repellency tests. Repellents have been found to be more effective at low than high insect densities (Schreck, 1985). This observation may explain why the high activity observed during preliminary repellency assays, in which fewer insects were used, was not reproduced in the detailed assays for some of the essential oils. Although the essential oils from *C. pseudopulchellus* and *C. menyharthii* showed relative superiority at lower concentrations, they exhibited low activity at 10% concentration compared to the other plants (Table 7). It has already been reported that there is an optimum

concentration that can effectively repel a given mosquito species (Schreck, 1985). For DEET, it has been demonstrated that 25 and 75% concentrations exhibit no significant repellent activity against *An. albimanus*, whereas 100% concentration provided sufficient protection (Schreck, 1985). This observation conforms to the repellent activity observed for the essential oils of the seven plants (Table 12-13).

The chemical characterization of the essential oil of the seven selected plants showed that they contained many components (Table 27-33), the majority of which are already listed in litrature as components of mosquito repellent oils (Omolo, 2002). These included α-pinene (0.41-5.51%), limonene (1.08-14.08%), γ-terpinene (0.02-9.16%) and α-ylangene (0.21-3.06%) (Tables 27-33) which were common to all the plants. The others which were commonly found in most of the essential oils of the plants analysed were camphene (0.08-3.01%), sabinene (0.05-6.85%), β-pinene (0.05-5.07%), β-myrcene (0.66-8.22%), 2-carene (0.20-1.61%), α-phellandrene (0.08-6.70%), *p*-cymene (0.17-9.83%), *E*-β-ocimene (0.13-11.97%), *trans*-sabinene hydrate (0.06-0.17%), fenchone (0.28-49.86%), terpinolene (0.07-1.99%), linalool (0.05-6.33%), 3, 4-dimethyl-2, 4, 6-octatriene (0.11-2.27%), camphor (0.05-5.93%), terpinen-4-ol (0.24-6.23%), α-terpineol (0.07-0.70%), α-cubebene (0.08-0.99%), methyleugenol (0.05-0.34%), β-elemene (0.12-7.10%), β-caryophyllene (0.25-14.95%), α-caryophyllene (2.69-4.69%), germacrene D (0.06-2.73%), δ-amorphene (0.64-4.29%), hedycaryol (0.05-1.47%), caryophyllene oxide (0.38-8.63%), humuladione (0.23-2.32%) and α-cadinol (0.05-8.63%) (Table 27-33). The qualitative differences in the composition of essential oils may explain the quantitative differences in the repellent activity.

Activity of the most common principles in the plants against *An. gambiae* has already been reported. α-Pinene, a common constituent of all the essential oils, has been reported to be of low repellent activity to *An. gambiae* (Omolo, 2002). Indeed, the low repellent activity is manifested in the relatively low activity of *E. tereticaulis* essential oil in which it is present in higher concentration than in the most active plant essential oils like *M. fragrans* and *P. longipes*. The contribution of α-pinene to the mosquito repellency of the essential oils might thus be one amongst several other compounds. Limonene, a constituent of all the essential oils of the plants studied, has been shown to be of insignificant activity at higher concentrations (Omolo, 2002). Infact, its repellency decreases with increasing concentration. Its abundance in *O. forskolei, E. teriticaulis* and *C. pseudopulchellus* may

explain the low activity (93.74, 95.78 and 90.17%) of these essential oils at the targeted effective concentration (10%). Infact, it could be acting as an antagonist to the active principles in these oils.

γ-Terpinene is reported to have good repellent activity against *An. gambiae* (Omolo, 2002). Its abundance (9.16%) in *P. longipes*, besides other active principles may explain the high repellent activity of the oil. Activity of α-ylangene against the vector is yet to be determined when enough samples become available.

Fenchone which was found in very high amounts (49.86%) in *O. forskolei* exhibits low repellent activity against *An. gambiae* individually. The repellent nature of the essential oil may be as a result of synergistic contribution of other constituents like camphor, α-pinene, β-myrcene and terpinene-4-ol which have been shown to exhibit reasonable activity singly.

β-Pinene, which exhibits repellent activity against *An. gambiae*, could be one of the contributing principles in *E. teriticaulis* and *C. pseudopulchellus*.

p-Cymene exhibits decreasing activity with increasing concentration against *An. gambiae* (Omolo 2002), and its abundance in *P. longipes* and *C. pseudopulchellus* cannot account for the active nature of the oils.

It would be difficult to demonstrate the contribution of *E*-β-ocimene to the activity of the oils since i is air sensitive and turns into a resin once exposed.

Caryophyllene oxide, which has been shown to have remarkable protection time (4 hours) against *An gambiae*, is a major contributor to the repellent activity of the essential oils of *M. fragrans* and *C pseudopulchellus* where it appears to be the major active principle. This was demonstrated ir artificial blends that included it.

The repellent activity of the other 15 principles, which had not been reported against *An. gambiae* are presented in table 36. β-Myrcene, a non-oxygenated monoterpene, showed appreciable repellen activity against *An. gambiae* (Table 36).

1-Methylpyrole, which is a unique constituent of *C. pseudopulchellus* and *C. menyharthii*, also showed significant repellent activity (Table 36). It would be interesting to investigate other *Croton* species to find out if this is a chemotaxonomic characteristic.

The GLVs did not exhibit appreciable repellent activity (Table 36). This could suggest that the GLVS are not important anti-pest materials in plants. Moreover, they are generated from damaged plant tissues during storage or processing.

Thymol, carvacrol and 4-isopropylbenzylmethanol, which are structural isomers demonstrated similar levels of activity in the repellency tests against *An. gambiae*. They all had good repellent activity (100%) at 10% concentration. This indicates no difference in the sensory physiology (chemoreceptors) of the insect to the set of compounds and suggesting that the position of the hydroxyl group within the molecule is not an important feature for the repellent activity of these isomers. *p*-Cymen-8-ol, another isomer in this category is yet to be evaluated as mosquito repellent and more so against *An. gambiae*. A related compound, 4-isopropylbenzaldehyde exhibits low repellent activity (Omolo, 2002). The compound only differs from 4-isopropylbenzylmethanol by the subtitution of the aldehyde with the hydroxymethylene moiety suggesting that the presence of the hydroxyl group is of great importance in the repellent activity of the molecules against *An. gambiae*. However, more analogues need to be assayed to determine the precise structural requirements for optimal repellency of *An. gambiae*. The tests also revealed the potency of phytol as a repellent. This further demonstrates the importance of hydroxyl group for mosquito repellency.

The fumigation tests revealed appreciable insecticidal activity of carvacrol against *An. gambiae* suggesting that it is the constituent that contributed towards the activity of the *P. longipes* essential oil in which it is also the main component (47.17%). It is interesting to note that carvacrol has both insecticidal and repellent activity, a desirable trait for commercial exploitation in pest or vector control. It is also a tick repellent (Ndungu *et al.*, 1995; Lwande *et al.*, 1999).

It might be difficult to claim only one or a few compounds in a natural product as responsible for a given biological activity such as repellency, attraction or toxicity as in this case. It is possible that special blends of compounds, qualitative or quantitative, are of importance for bioactivity of a given natural extract. The fumigant insecticidal activity of *M. fragrans* and *O. forskolei* might therefore be

much more complex than the effect of only a few components. The most probable bioactive components tested negative or had been shown earlier to elicit no fumigation activity against *An. gambiae* (Omolo, 2002). Indeed, the activity of these oils may be influenced by the percentage constitution of the different components and their complex interactions with the test organism.

Natural principles like eugenol have been demonstrated to enhance activity of bioactive principles (Ladd, 1980), suggesting a synergistic action of some natural blends. In this study, various blends were formulated to determine if the constituents had any synergistic activity. Data from those tests in which repellents were exposed alone and in blends appear in table 36-37. Table 38 shows percentage repellency, relative repellency and calculated enhancement factor with respect to eugenol. Repellency of *An. gambiae* in the tests demonstrated the efficacy of eugenol in enhancing the activity of the other constituents. Repellency of the blends containing eugenol were higher than the individual components.

One way of comparing activity of a mixture against a test subject is by computation of an enhancement factor, or index (Ladd, 1980). This index in which a value of one indicates parity between different materials, and which reduces relative repellent capability to a pertest basis, permit comparisons both within and between tests. Factors calculated for OFI_1, OFI_3 and OFI_4 (1.40, 1.04 and 1.01, respectively) generally correspond to the constitution of the blends. Thus OFI_1, the superior repellent blend is 1.40 times more effective, on a pertest basis, than its components. In all the cases, the enhancement imparted by eugenol to the other repellent components indicates true synergism since the activity of all the repellent blends at 10% solution (as indicated by their enhancement factors) were far much greater (82.4-98.4%) than would be expected from the individual components. Our findings demonstrate the ability of eugenol to quantitatively and dramatically enhance the repellency of certain compounds against *An. gambiae*. The unique synergistic effect may well give rise to other highly repellent combinations as research uncovers the basic knowledge of substances that repel *An. gambiae*. It is evident from our investigations that the insect repellent activity of essential oils is not due to any single component but a result of synergistic action of the major components. This may open ways of using essential oils as repellents since the number of components are reduced and toxicological studies can be undertaken.

6.2 Recommendations

Several major and minor components of the essential oils were not identified due to lack of authentic standards. Further efforts should be made to acquire these to confirm their presence in the oils and also to investigate their activity. Particular emphasis is put on p-cymen-8-ol which is a structural isomer of carvacrol, thymol and 4-isopropylbenzenemethanol which have been found to be of anti-mosquito activity. Efforts should be made to access this compound in large quantities through synthesis to enable confirmation of its presence in essential oil and for bioassay.

It is evident from this study that there are several natural products which could protect against mosquitoes like *Anopheles* species. Available toxicological data indicates that some of the oil components posses less favourable properties (Thorsel *et al.*, 1998). An insect repellent ought to be well defined and harmless to exposed subjects. The tested natural products contain single components some with strong activity against the vector and can be used in the development of potent mosquito repellents. However, they are not yet as well described or non-toxic as could be desired. Therefore, toxicological evaluation of these molecules should be carried out to determine their desirability and the concentrations which are safe for use as topical repellents. This is mandatory before any commercial products are developed.

Behavioural differences may occur between the laboratory reared and natural populations of *An. gambiae* and perhaps regional differences among natural populations also occur. Additional testing of the promising repellent compounds against *An. gambiae* under the natural field conditions and at several locations within its ecological range is required to determine if they can provide adequate protection from bites of the vector.

Synthetic analogues of the repellent compounds should be modeled on the active moieties in order to come up with more potent and desirable principles that can protect against vector bites. It has been demonstrated that stereochemical configuration (chirality) of a molecule may influence biological potency and selectivity of insect responses to natural products and synthetic chemicals (Kurithara and Miyamoto, 1998; Klun *et al.*, 2001). Indeed individual isomers generally have different level of biological activity (Testa, 1990). Based on these revelations, it will be of good value to determine the stereochemistry of the isomers of the active molecules and corresponding potency against *An. gambiae*.

It has been demonstrated that live intact plants can reduce domestic exposure to malaria vectors (Seyoum *et al.*, 2002). It would be interesting to carry out an investigation on the potency of live plants like *O. fischeri*, *O. forskolei* and *E. tereticaulis* as repellents for the malaria vector. The potency of these plants by thermal expulsion and direct burning should also be investigated.

CHAPTER 7

EXPERIMENTAL

7.1 Glassware

General purpose glassware was well cleaned with concentrated HNO_3 acid (6 M), rinsed with water and finally acetone before drying them in an oven at 110 °C for 18 hours. The glassware for storage of the sample extracts was cleaned by soaking in freshly prepared chromic acid for 24 hours, cleaned with distilled water and finally rinsed with acetone before being dried in the oven as described earlier.

7.1.1 Solvents and essential oil standards

The solvents (acetone, chloroform and dichloromethane) used were pure analytical HPLC grade obtained from Sigma-Aldrich Chemical Company. The essential oil standards used for the GC coinjection and bio-assay were obtained from Sigma-Aldrich Chemical Company and Fluka Chemika Company.

7.2 Plant collection and identification.

The samples were collected from plants growing in the coastal region of Kenya at altitudes of 0-1829 m in June and December 2001. Selection of plants was based on chemotaxonomic (phytochemical) approach, ethno-medical and ethno-botanical information.

Mkilua fragrans (SGM-2001/1) were collected from Shimba Hills National Reserve at Mwele-Mkubwa. *Croton pseudopulchellus* (SGM-2001/2) and *C. menyharthii* (SGM-2001/14) leaves were collected from Arabuko at Mida Creek (main entrance) and Taru (on the way to the KBC transmitter station), respectively.

Plectranthus longipes (SGM-2001/15), *Ocimum forskolei* (SGM-2001/16) (formerly *O. ladiensis*) and *Endostemon tereticaulis* (SGM-2001/17) aerial parts were collected from Taru (on the way to the KBC transmitter station).

Ocimum fischeri (formerly *Hermazizygia fischeri*) (SGM-2001/18) was collected from Tsavo West National Park along the road to Ngulia Lodge next to Mombasa-Nairobi oil pipeline through Man Eaters Gate.

The collected plants were identified by Mr Simon Mathenge from Botany Department, University of Nairobi (UoN), Kenya. Voucher specimens of the plant materials were deposited in the Herbarium at the Department of Botany, UoN.

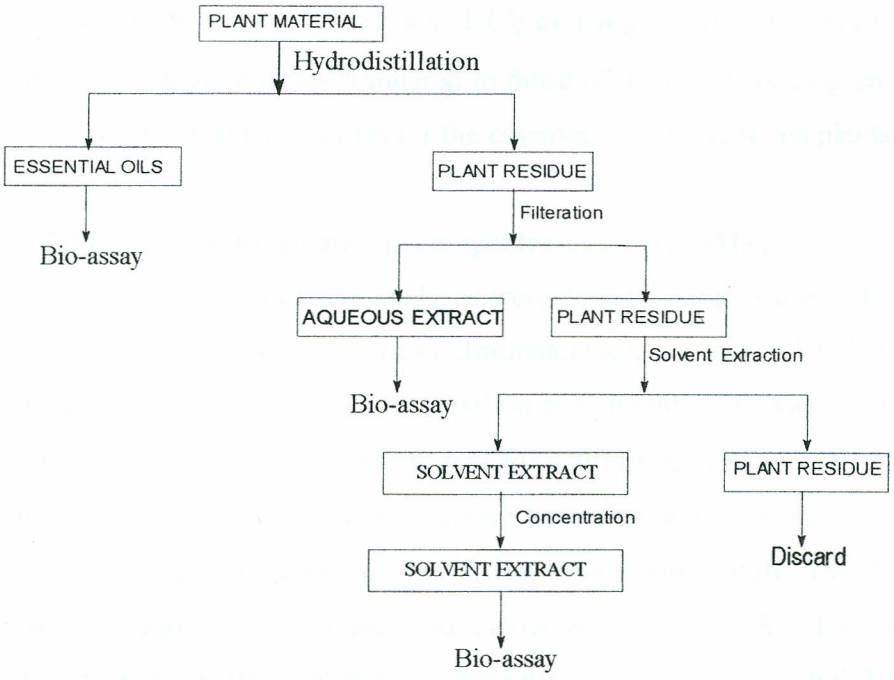
7.3 Sample handling

Semi-dried sample materials were used; the plant materials were air-dried under shade during harvest period, but where possible freshly harvested aerial parts were preferred. Labeling and packaging was done to allow for the materials to be in usable condition at the destination through the use of aerated netting bags. Two plant parts (flowers and/or leaves) were used. The plants which grow as herbs were harvested at full bloom. Harvest date and locality was recorded to allow for any effects of seasonal or geographical variation on the chemical composition in cases where re-collection was necessary.

7.4 Extraction

Selective extraction procedures were used. The volatile fractions were isolated by hydrodistillation of 600 g of the plant material in 5 l round-bottom flask with 2.4 l of tap water. Modified Clavenger apparatus was used for oil collection. The hydrodistillation temperature was thermostatically maintained at 80 °C. The distillation period was 3-6 hours depending on whether more oil could be collected. Plant materials that initially showed low essential oil content were flaked before hydrodistillation. The resulting essential oils were dried over anhydrous sodium sulphate and stored at 4 °C in the dark for further use. The amount and percentage yield of essential oil from each plant is summarised in table 2.

The water extract left in the flask was carefully decanted into a sample bottle and stored in the refrigirator or cold room for bio-assay. Non-volatile organic components left in the plant residue after steam distillations were extracted with cold chloroform or dichloromethane for 48 hours. The chloroform or dichloromethane extracts were concentrated under reduced pressures using a rotary evaporator. The amounts and percentage yield of chloroform and dichloromethane extracts are summarised in table 2. The extraction process is summarised in scheme 3.



Scheme 3. The flow chart of the extraction sequence

7.5 Purification and chemical identification.

Characterization, identification and determination of relative amounts of the components of the essential oils from the selected plants was done by gas chromatography (GC), gas chromatography-mass spectrometry (GC-MS), and GC-coinjection of the essential oils with standards (Waller, 1972; Adams, 1989).

7.5.1 Gas chromatography (GC)

GC analysis was carried out on a Hewlett Packard (HP) 5890 GC fitted with a flame ionization detector, a 3393A integrator and a HP cross-linked methylsilicone fused-silica capillary column, 50 m x 0.20 mm (i.d) x 0.33 μm (film thickness). Analytical conditions were: split ratio, 1:60; injector and detector temperature at 250 °C and oven temperature programmed from 50-250 °C [50 (5) @10-100 @2-180 @5 °C/min-250 °C (2 min)]. The carrier gas was nitrogen at a flow rate of 0.84 ml/min. The flow rates of air and hydrogen was 400 and 30.5 ml/min, respectively.

For the GC analysis of essential oils, 5 μl of neat sample was diluted in 1 ml of CH_2Cl_2 and 2 μl of the resulting solution injected.

For standards, 0.5 µl in 1 ml of CH_2Cl_2 or 1 mg of solid in 2 ml of CH_2Cl_2 were similarly analyzed and their retention times compared to those of the corresponding components of the essential oils of the 7 plants. The GC profiles for the essential oils of the seven plants are given in figures 3-9.

7.5.2 Gas chromatography-mass spectrometry (GC-MS)

Qualitative and quantitative analyses were done by combined gas chromatography mass spectrometry (GC-MS). HP 8060 Series II gas chromatograph coupled to VG Platform II Mass Spectrometer was used for identification of the essential oil constituents. The GC column used was the same as the one described for the GC analysis except for a film thickness of 0.5 µm. The GC operating conditions were also the same as described above but using helium as the carrier gas. MS conditions were as follows: Ionization potential, 70 eV; ion source temperature, 180 °C; resolution, 1000; scan time, 1 sec; interscan delay, 0.5 sec. and ionization current, 1 A. The preliminary identification of the constituents was based on the computer matching of mass spectral data of the components against the Wiley and NIST library spectra constituted from spectra of pure substances and components of the known essential oils, and MS literature data (Formacek and Kubeczka, 1982). These were confirmed, whenever possible, by their GC retention time comparison as well as coinjection, co-chromatography or co-elution with standards. The percentage composition of the essential oil was computed in each case from GC peak areas without using correction factors. For the GC-MS analysis of essential oils, 10 µl of neat sample was diluted in 1ml of CH_2Cl_2 and 5 µl of the resulting solution injected.

7.5.3 GC-coinjection

Identity of the components of the essential oils was confirmed by peak enhancement (co-elution) upon GC coinjection of the obtained essential oils with standards. Lists of the essential oil components identified in the essential oils from repellent or insecticidal plants is given together with their relative amounts (% peak areas) in tables 27-33.

7.6 Biological evaluation of the extracts

This was conducted at ICIPE, Duduvile, Nairobi. The essential oils, chloroform or DCM and water extracts were assayed for repellency and mosquitocidal activity against female *An. gambiae* mosquitoes. The insects were reared at ICIPE and the bio-assays based on the WHO (1996) protocol for the laboratory and field evaluation of insecticides and repellents.

7.6.1 Repellency assays

The repellency assays were carried out in a dark room with red light as the only source of illumination. The room conditions (temperature and humidity) were controlled to mimic the feeding conditions for female *An. gambiae*. Repellency assays were carried out on starved but previously glucose (6% solution) fed female adult (5-6 days old) *An. gambiae*. Six (6) human volunteers (3 males and 3 females) were used in repellency assays. They did not apply any lotion, perfume, oil or perfumed soap on the day of the bio-assay.

7.6.1.1 Preliminary screening

Preliminary screening of the essential oils was carried out at 0.001, 0.1, 1 and 10% solution in acetone depending on the yield of the oil while the chloroform or DCM extracts were tested at 0.0005, 0.05 and 5%. The solutions were screened using six different people (3 males and 3 females), who showed mild or no allergic reaction to experimental mosquito bites. The evaluation of the repellency was carried out using human-bait technique to simulate the condition of human skin to which repellents will be eventually applied (Schreck and McGovern, 1989; WHO, 1996). Aluminium frame cages (50 x 50 x 50 cm) with a sheet metal bottom, window screen (mesh size 256) on top and back, clear acrylic (for viewing) on the right and left sides, and a cotton stockinet sleeve for access on the front was used. A total of 18 cages, each containing 25 female *An. gambiae* mosquitoes (5-6 days old, starved for 15 hours), were used. The test cages were positioned securely on a bench, at chest height of volunteers while seated. The volunteers sat 1.5 m away from each other. Treatment comprised of 1 ml of test material solution in acetone applied evenly to approximately 540 cm^2 (approximated from the area of paper wrapped on volunteers arm) of the forearm skin between the wrist and elbow of a volunteer. The solvent (acetone) was dispensed on the other arm to act as a control. The rest of the hands were covered with gloves. The control hand was introduced into the cage immediately after releasing the insects and kept there for two minutes. The mosquitoes that landed were recorded and then shaken off before they had a chance to imbibe any

blood. The test hand was then introduced in the cage and kept for the same duration and the number of mosquitoes that landed recorded.

Volunteers' positions were rotated on each test occasion to allow for any variation among the positions. The bio-assays were done with progressively increasing concentrations of test substance. Each concentration was screened with a new batch of mosquitoes. After bio-assay of each concentration, the hands were washed using a non-perfumed soap and tap water and allowed to dry naturally for a period not less than 20 minutes before dispensing the subsequent concentration. The tests of each oil against the vector were conducted on separate days.

The percentage protective efficacy was calculated using the formula:

$PE = \left(\frac{C - T}{T} \right) X\ 100\%$. Where C and T are mean number of mosquitoes that landed on control an test hands, respectively (Sharma and Ansari, 1994; Matsuda *et al.*, 1996; Yap *et al,* 1998).

The formula is similar to that described earlier by Weaving and Sylvester (1967). No skin irritation, hot sensations or rashes were observed on the arms of the test volunteers treated with the volatile oils or extracts during the 14 months of the study period.

The results of the preliminary repellency assay of chloroform and essential oils extracts are summarized in tables 4 and 12, respectively.

The aqueous extracts (1.0 ml) were similarly tested with water acting as the control and protective efficacy determined as earlier described. The results are summarized in table 3.

7.6.1.2 Detailed repellency assay of essential oils

Detailed repellent test comprised the determination of mosquito repellency for five concentrations of essential oils that had shown remarkable repellency in the preliminary screening. The repellent activity was assessed as earlier described. Neat oil (1 g) from each plant sample was dissolved in 10 ml of acetone to give 10% solution. By sequential dilution 1, 0.1, 0.01 and 0.001% solutions were prepared. The surface area of the arms of volunteers were estimated using paper sheets and found to be 550.37, 585.50, 497.68, 468.40, 526.95 and 556.25 cm^2, respectively. The volume of the sample solution dispensed on each volunteer arm was 0.94, 1, 0.85, 0.8, 0.90, 0.95 ml, respectively. All the solutions were bio-assayed with each being subjected to a fresh batch of 50 adult female *An. gambiae* mosquitoes in each cage. The percentage protective efficacy was determined as earlier stated. The test procedure was replicated six times using different human volunteers for each concentration and

statistically reliable estimates of their median effective repellent concentrations (RC_{50}) obtained by probit-log concentration analysis and the derived regression equations determined (Finney, 1971).

7.6.2 Repellency assay of identified components of the oils

The test solutions of the identified components were prepared using acetone as already described. The concentrations used in this case were 10, 1, 0.1, 0.01%. The results are outlined in table 36.

The RC_{50} values for standards which had good activity were calculated by probit analysis (Finney, 1971). The results are summarized in table 43.

7.6.3 Repellency assay of the blends of identified compounds

The major compounds identified from the leaf oils of the repellent plants were taken in the ratio in which they are present in the essential oils and assayed against *An. gambiae* as detailed above. The results are summarized in table 37.

7.6.4 Mosquito repellency assay of formulated compounds

The compounds that showed good repellent activity were formulated in various carrier media and assayed once again to ascertain the longevity of protection. Aqueous base, emulsificant base and petroleum jelly were investigated. Formulated compounds (10%) were assayed for repellent activity against *An. gambiae* after 0, 2, 4, 6 and 8 hours after application and the protective efficacies (PE) calculated as previously explained. The oils were eventually formulated in an aqueous base. The results obtained are summarised in table 44. The durational repellency data was subjected to regression analysis and the best fitting decay curves realized (Figure 10-12).

7.6.6 Mosquitocidal assays

Two set-ups were made for adulticidal assay. These were fumigation and tarsal contact mosquitocidal assay.

7.6.6.1 Fumigant mosquitocidal assays of essential oils

For preliminary screening, 25 adult female *An. gambiae* mosquitoes were introduced into a small cage measuring 20 x 20 x 35.5 cm and fumigated with 0.1 g/ml (10%) solution of a plant essential oil on a filter paper (7 cm diameter) placed in a petri dish covered with wire mesh to prevent direct

contact with the insects. In a separate cage, 25 adult female *An. gambiae* were fumigated with same volume of acetone to act as a control. Glucose solution in a small bottle with a rolled filter paper, 9.5 x 14.5 cm in it, was provided to the insects in each cage to serve as food. The number of dead mosquitoes in each cage was recorded, separately, at interval of 30 minutes for 6 hours depending on activity of the oil. Oils that showed insecticidal activity were subjected to detailed bio-assay where 6 replicates were obtained and percentage insecticidal activity (PI) determined according to WHO (1996).

$$PI = \frac{N}{T} \times 100\%$$

Where N represents the number of dead mosquitoes in the cage minus the number dead in control cage, while T is the total number of mosquitoes introduced in the test cage

7.6.6.2 Fumigant mosquitocidal assay of identified components

The pure compounds identified from the essential oils of the 3 insecticidal plants (*Plectranthus longipes*, *Mkilua fragrans* and *Ocimum forskolei*) were subjected to mosquitocidal assay by fumigation method as detailed above. The bio-assay data was subjected to probit-log concentration analysis (Finney, 1971) and lethal concentration (LC) levels determined from the derived regression equation.

7.6.6.3 Tarsal contact mosquitocidal assay of essential oils

In this assay two petri-dishes (different sizes) were used. A hole ($\approx$ 1 cm in diameter) was made at the centre of the big petri dish (9.5 cm diameter) for introducing the insects. A filter paper (8 cm diameter) was placed in the small dish (9.0 cm diameter) and 1 ml of 1 mg/ml (0.1%) solution of the oil spread evenly on it. The paper was allowed to dry for 18 hours, before covering the small petri dish with the big one and introducing 10 non-starved female mosquitoes ($\geq$ 4 days old) in the bio-assay chamber through the small hole. The hole was then covered with cotton wool and the insects kept in the chamber for 30 minutes before being transferred into a netting cage in which they were fed on glucose solution (6%). The number of dead mosquitoes was to be reported within 24 hours. The control experiment was similarly set up but with 1 ml of acetone.

7.6.6.4 Tarsal contact mosquitocidal assay of solvent extracts

The chloroform or dichloromethane extracts were also subjected to tarsal contact mosquitocidal assay as earlier outlined.

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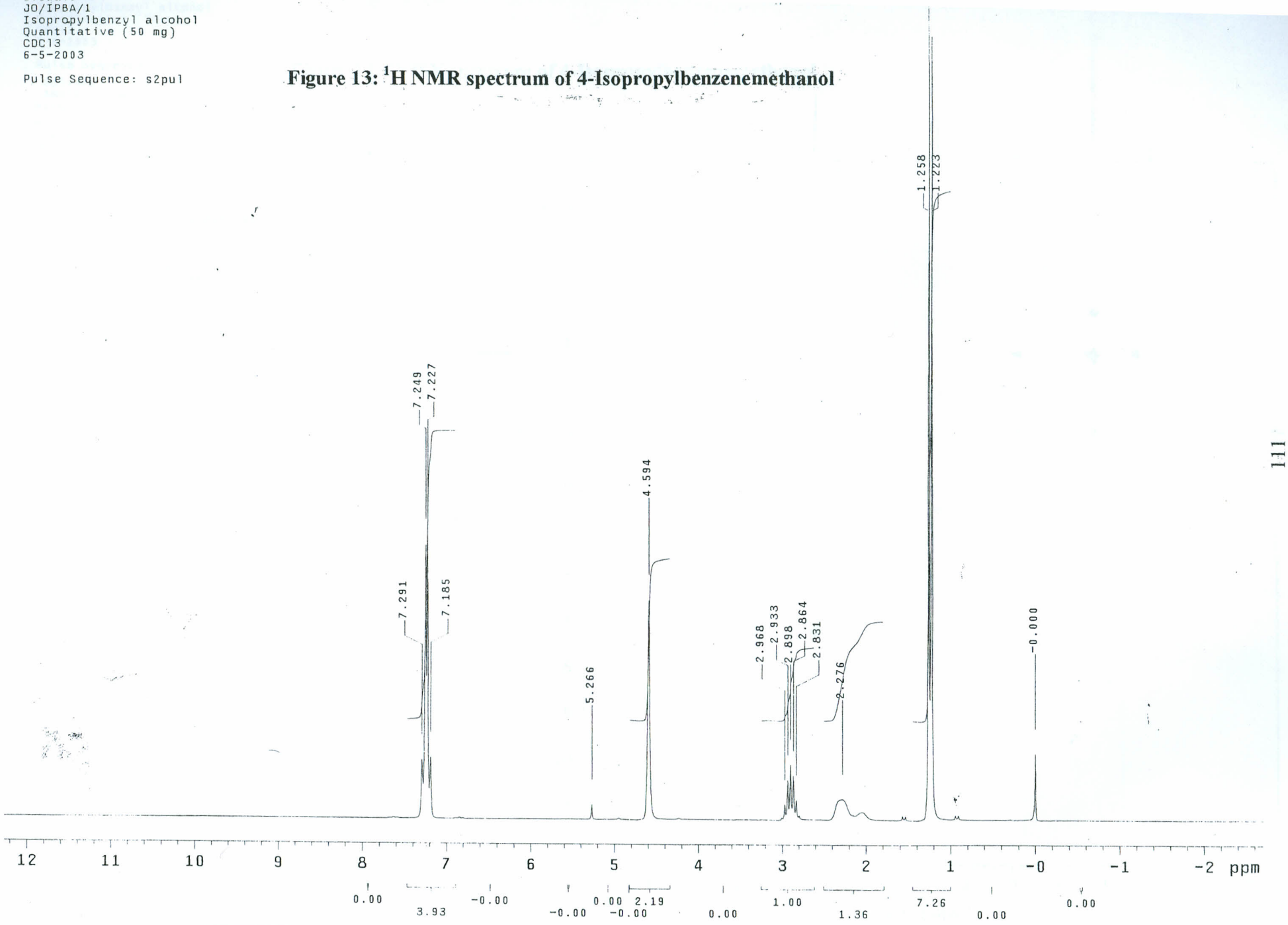
APPENDICES

J.Odalo
JO/IPBA/1
Isopropylbenzyl alcohol
Quantitative (50 mg)
CDCl3
6-5-2003

Pulse Sequence: s2pul

Figure 13: ^{1}H NMR spectrum of 4-Isopropylbenzenemethanol



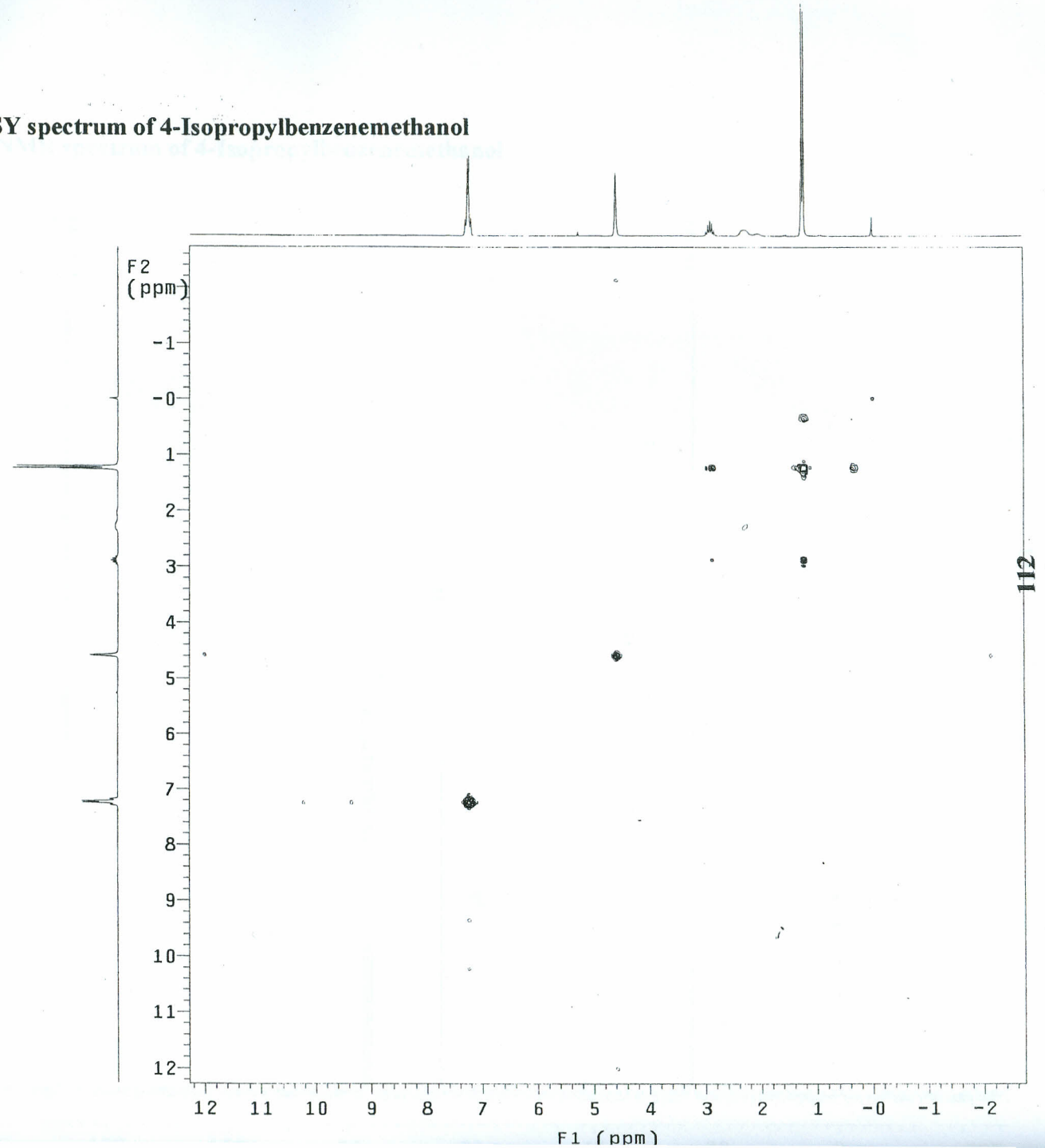
J.Odalo
JO/IPBA/1
Isopropylbenzyl alcohol
Quantitative (50 mg)
CDCl3
6-5-2003

Pulse Sequence: relayh

Solvent: CDCl3
Ambient temperature
Mercury-200 "uonnmr200"

PULSE SEQUENCE: relayh
Relax. delay 1.000 sec
COSY 90-90
Acq. time 0.171 sec
Width 3000.3 Hz
2D Width 3000.3 Hz
64 repetitions
125 increments
OBSERVE H1, 200.0507889 MHz
DATA PROCESSING
F1 DATA PROCESSING
Line broadening 0.3 Hz
FT size 1024 x 1024
Total time 2 hr, 45 min, 42 sec

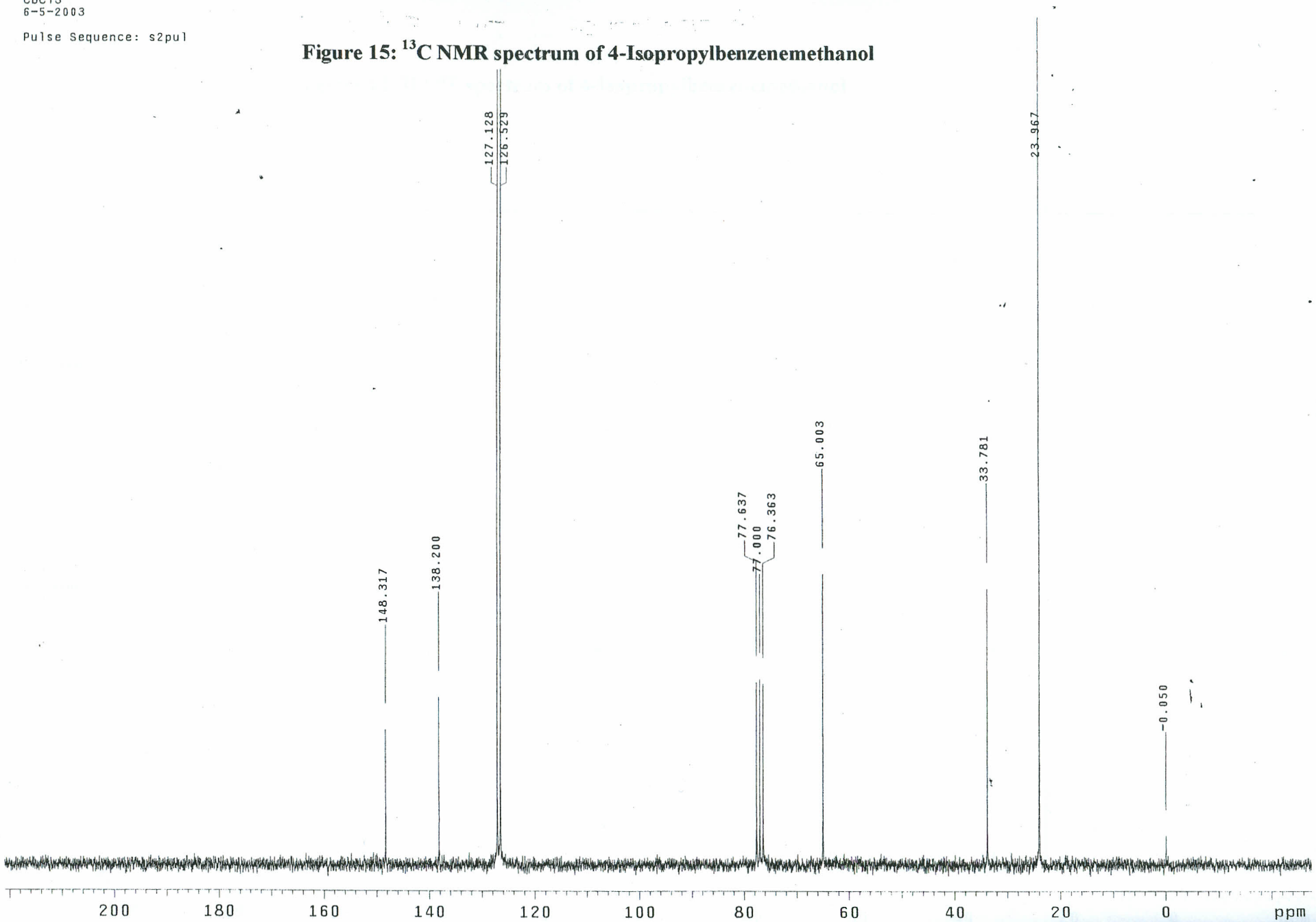
Figure 14: COSY spectrum of 4-Isopropylbenzenemethanol



J.Odalo
JO/IPBA/1
Isopropylbenzyl alcohol
Quantitative (50 mg)
CDCl3
6-5-2003

Pulse Sequence: s2pul

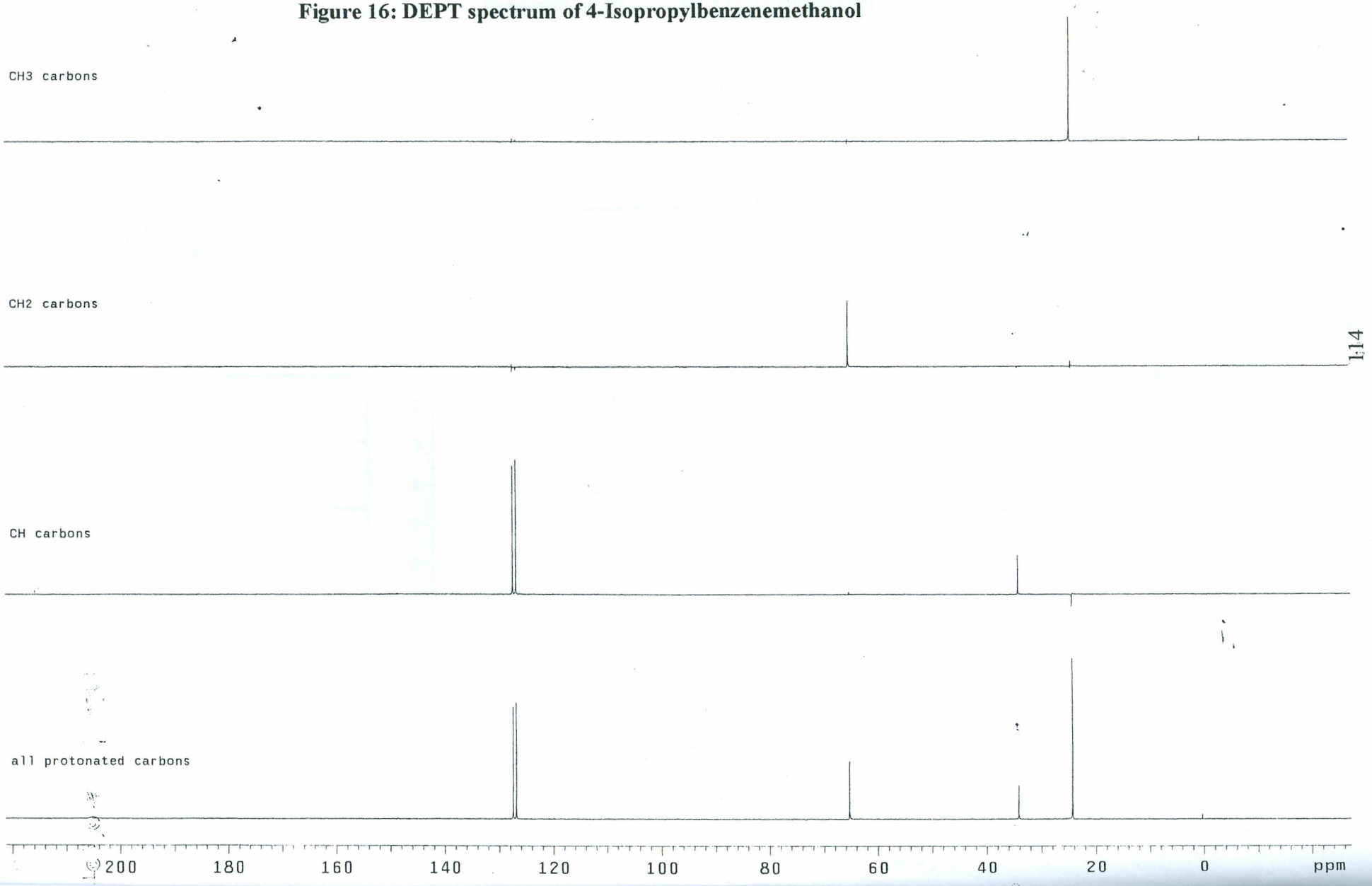
Figure 15: ^{13}C NMR spectrum of 4-Isopropylbenzenemethanol



J.Odalo
JO/IPBA/1
Isopropylbenzyl alcohol
Quantitative (50 mg)
CDCl3
6-5-2003

Pulse Sequence: dept

Figure 16: DEPT spectrum of 4-Isopropylbenzenemethanol



J.Odalo
JO/IPBA/1
Isopropylbenzyl alcohol
Quantitative (50 mg)
CDCl3
6-5-2003

Pulse Sequence: hetcor

Solvent: CDCl3
Ambient temperature
Mercury-200 "uonnmr200"

PULSE SEQUENCE: hetcor
Relax. delay 1.000 sec
Acq. time 0.082 sec
Width 12500.0 Hz
2D Width 3000.3 Hz
256 repetitions
128 increments
OBSERVE C13, 50.3027980 MHz
DECOUPLE H1, 200.0517433 MHz
Power 31 dB
on during acquisition
off during delay
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
F1 DATA PROCESSING
Line broadening 0.3 Hz
FT size 2048 x 512
Total time 10 hr, 39 min, 58 sec

Figure 17: HMQC spectrum of 4-Isopropylbenzenemethanol