BIO-PROSPECTING FOR PHYTOCHEMICAL REPELLENTS/ADULTICIDES OF *ANOPHELES GAMBIAE*

BY

MAURICE OCHILO OMOLO, B.Sc. (Hons.), Kenyatta.

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DECLARATION

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

Signed---------- Date-22/8/2002

MAURICE OCHILO OMOLO,

CHEMISTRY DEPARTMENT, KENYATTA UNIVERSITY.

This thesis has been submitted with our approval as supervisors.

Signed---------- Date-27/08/2002

Dr. ISAIAH OMOLO NDIEGE,

CHEMISTRY DEPARTMENT, KENYATTA UNIVERSITY.

Signed---------- Date-22/8/2002

Dr. WILBER LWANDE,

BEHAVIOURAL AND CHEMICAL ECOLOGY DEPARTMENT,

INTERNATIONAL CENTER OF INSECT PHYSIOLOGY & ECOLOGY.

Signed---------- Date-22/08/02

PROF. AHMED HASSANALI,

BEHAVIOURAL AND CHEMICAL ECOLOGY DEPARTMENT,

INTERNATIONAL CENTER OF INSECT PHYSIOLOGY & ECOLOGY.

DEDICATION

This work is dedicated to my late father Andrew Omolo, my dear wife Lilian A. Omolo and my sons Andrew Omolo and John Vitalis Omolo. It is also dedicated to my mother, Magdaline Okoth Omolo, uncle William Abara, mother in-law Roseline A. Onyiego and mostly to Br. Edward McCarthy (of Patrician Brothers) without whose support I would not have come this far.

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LIST OF ABBREVIATIONS

BA ----- Biological Abstracts

CA ----- Chemical Abstracts

CHMC ----- Cyclohexamethylene carbamide

CN ----- *Conyza newnii*

CO ---- Coinjection

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

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

DEET ----- Diethyl-*m*-toluamide

DMP ----- Dimethyl phthalate

E.A ----- East Africa

FID ----- Flame Ionization Detector

GC ----- Gas Chromatography

HNO_3 ----- Nitric acid

HP ----- Hewlett Packard

IGRs ----- Insect Growth Regulators

IM ----- *Iboza multiflora*

LJ ----- *Lippia javanica*

LU ----- *Lippia ukambensis*

MCU ----- Malaria Control Unit

MFI ----- Malaria Foundation International

MIM ----- Multilateral Initiative on Malaria

MMV ----- Medicines for Malaria Venture

MoH ----- Ministry of Health

MS ----- Mass Spectrometer/spectra

MVI ----- Malaria Vaccine Initiative

NGOs ----- Non-Governmental Organizations

NMCP----- National Malaria Control Programme

PM ----- *Plectranthus marrubioides*

RBM ----- Roll Back Malaria

RITAM ----- Research Initiatives on Traditional Anti-Malarials

TC ----- *Tarchonanthus camphoratus*

TDR ----- Tropical Diseases Research

UoN ----- University of Nairobi

ABSTRACT

Mosquitoes are vectors of vast economic and medical importance. They transmit life threatening human and animal diseases such as malaria, viral encephalitis, dengue fever, filariasis, and dog heart worms. Currently, malaria causes more than 500 million cases of acute illness and > 1 million deaths annually, with 90% of the deaths occurring in Africa. This disease is responsible for 25% of the deaths in children. It leads to miscarriage, low birth weight, and deaths among pregnant women.

The malaria parasites and vectors have developed resistance to the available commercial anti-malarial drugs and insecticides as well as insect repellents respectively. There is no successful malaria vaccine that has been developed. Plant derived new mosquito repellents and mosquitocides, may contribute to the control and the spread of malaria among other mosquito-transmitted diseases.

Our research has covered 33 plants within the families: Labiatae, Compositae, Verbeneceae, Euphorbaceae, and Rutaceae. The selection of the plants was based on the ethno-botanical and chemo-taxonomic information, as well as random sampling of plants with aromatic leaves. Out of all the plants screened, 10 showed mosquito repellent activity, while 6 exhibited both insecticidal and mosquito repellent properties. Six compounds with good mosquito repellency and two with mosquitocidal activity have been discovered. Formulation of these compounds into topical repellents have shown good protective efficacy.

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CHAPTER 1: INTRODUCTION

1.1 History and distribution of malaria

Malaria parasites have been with us since the dawn of time. They probably owe their origin to Africa (along with mankind). Fossils of mosquitoes up to 30 million years old show that the vector for malaria was present well before the earliest known history of man. From their origins in Africa, early trans-pacific voyagers possibly brought *Plasmodium vivax and P. malariae* to the New World, and this trend of imported malaria continues to this day. *P. falciparum* may have come in consignments of slaves bound for the Spanish colonies. The *Plasmodium* parasites are highly specific, with vertebrates as the only hosts and *Anopheles* mosquitoes as the only vectors (Anderson and Morales, 1993).

Hippocrates was the first to describe the manifestations of the disease, and relate them to the time of the year and where the patients lived. Before this, the supernatural was blamed. The association of malaria with stagnant waters led the Romans to begin drainage programs as the first control strategy against malaria. The first recorded treatment dates back to 1600, where the native Peruvian Indians used the bitter bark of the cinchona tree. By 1649, the bark was available in England, as 'Jesuits powder' (Barry *et al.*, 1995) so that those suffering from 'agues' might benefit from the quinine in it. Malaria in UK (known as agues) would have been clustered around stagnant marshes, and the invading Roman soldiers would certainly have brought the disease with them.

It was not until 1889 that Laveran described the protozoan cause responsible for malaria, from his work in Algeria, and in 1897 *Anopheles* mosquito was demonstrated to be the vector for the disease. Epidemiology of malaria was clear at this point and implementation of control measures started.

Global eradication of malaria seemed possible with the discovery of DDT in 1942 and its first use in Italy in 1944. Widespread systematic control measures such as the spraying with DDT, coating marshes with paraffin (to block *Anopheles* mosquito larvae spiracles), draining stagnant water and the use of cheap, effective drugs such as chloroquine were implemented, with impressive results. Despite initial success, there was a complete failure to eradicate malaria in many countries due to many factors. Although technical difficulties such as

insecticide and drug resistance have played a part, the main failure to reduce the disease is probably due to socio-political factors preventing efficient implementation of control measures (Anderson and Morales, 1993).

Malaria occurs in many locations of the tropical world and in some locations of the subtropics. It is most common between the latitudes of 23.5° N. [Tropic of Cancer] and 23.5° S. [Tropic of Capricorn]. It also occurs outside these latitudes in areas such as portions of South Africa [Kruger National Park and surrounding area - 25° S.] and New Delhi, India [28.5° N.].

The disease is common in sub-Saharan Africa, where the predominant species is *Plasmodium falciparum*, which is now resistant to chloroquine. Malaria outbreaks are being reported in some locations of Africa that had previously been thought to be at elevations too high for its transmission (highlands of Kenya).

Malaria is widespread in numerous countries in Asia and Oceania, including India, Pakistan, Bangladesh, Thailand, Vietnam, Lao, Myanmar, Cambodia, Indonesia, and Papua New Guinea. It also occurs in parts of Iran and the Middle East. *P. falciparum* is the main parasite in these countries, however *P. malariae* is also present.

In South America, malaria occurs at altitudes below 1000 m in Brazil, Peru, Colombia, Bolivia, Ecuador, Venezuela, Guyana, Suriname, and French Guyana. *P. vivax* is the most common species in this area, although there is an unfortunate increase in *P. falciparum* cases particularly in regions where control programs have deteriorated or been abandoned since the early 1990's.

Malaria occurs in low altitude areas of the countries in Central America, including Honduras, Nicaragua, and Guatemala. Limited numbers of cases occur in Panama, Costa Rica, and Southern Mexico. *P. vivax* is the dominant species and, fortunately, remains susceptible to chloroquine.

In Caribbean, malaria remains controlled in many countries. However, *P. falciparum* is responsible for the disease in Haiti and the Dominican Republic. European countries affected by the disease include Turkey, Armenia and Azerbaijan. The main parasite here is *P. vivax*. Europe, North Armenia, Central America, Caribbean, Australia and Meexico represent the major success stories of malaria vector control efforts.

Fig. 1. The distribution of malaria in the world (WHO, 1997)

At present, more than 40% of the world's population lives in malaria prone areas (WHO, 1997). Globally, malaria causes more than 500 million cases of acute illness and > 1 million deaths annually, with 90% of the deaths occurring in Africa. In 1998 five times as many malaria cases were reported as tuberculosis, Acquired Immune Deficiency Syndrome (AIDS), measles and leprosy combined (Anonymous, 2000). It is responsible for 25% of the deaths in children below the age of 5 years (WHO, 1998). During epidemics, all age groups are affected (WHO, 1997). Malaria can re-emerge in areas where it has been under control.

The occurrence of clinical malaria cases may depend on the parasite virulence and cytoadherence among other factors (Ferreira *et al.* 1998; Modiano *et al.* 1991; Terrenato *et al.* 1988; Rooth *et al,* 1992; Genton *et al*, 1998). Vector behaviour may also contribute to the variability in human exposure to malaria infections. Large differences may be there in anopheline densities and inoculation rates between localities and households. There may also be innate individual differences among people in attracting mosquitoes (Lindsay *et al,* 1993; Knols *et al,* 1995), which affect the individual inoculation rates. Differences in human behaviour and occupation also affect man's exposure to malaria infections.

1.2 Economic burden

The burden malaria imposes on the economy is substantial. The cost of treatment and prevention alone is vast. Number of hours of work lost each day from those with malaria or those taking care of such patients is unimaginable. Pregnant women suffer severe anaemia; have up to 800,000 infantile mortalities, a substantial number of miscarriages, and very low birth weight (VLBW) babies per year as well as higher risk of death due to the disease (Anonymous, 2000). The economic costs involved as a result of deaths from malaria are extremely high, not to mention the pain and suffering associated with it. In addition, the spread of drug-resistant malaria strains substantially raise the cost of treatment (Anonymous, 2000; http//www.malaria.org.).

Health experts estimate the cost of malaria to African economies to be up to US$ 12 billion annually. This is more than all the foreign aid to the continent, yet it can be controlled for a small fraction of this amount. Those who suffer most are some of the continent's most impoverished and malaria makes them poorer. A poor family living in malaria-affected area may spend up to 25% or more of its annual income on prevention and treatment. The disease has slowed down the economic growth in African countries by up to 1.3 per cent per year. As a result of the compounded effect over 35 years, the Gross Domestic Product (GDP) level for African countries is now up to 32 per cent lower than it would have been in the absence of malaria. Moreover, foreign entrepreneurs are reluctant to invest in countries with high malaria rates, whilst tourists are shunning these areas due to lack of protection from current anti-malarial drugs (Anonymous, 2000). This would put the tourism industry in jeopardy and threaten Ksh. 6.6 billion of annual foreign exchange earning from this sector.

1.3 Resurgence of malaria

Malaria epidemics have been linked to climatic changes like the *El nino* weather phenomenon, global warming and interference of man with the environment (Mouchet *et al.*, 1988; Bouma *et al.*, 1996, 1997; Lindsay, 1996; Jerten *et al.*, 1996), drug and insecticide resistance (WHO, 1999). Thoughtless man-made irrigation schemes, dams, and other development projects such as agroforestry, mining, and road construction have provided new habitats for *Anopheles* mosquitoes and resulted in 'man-made' malaria (Lindsay and Martens, 1998; Sharma *et al.*, 1986).

The extension of urban areas has led to epidemics in the peripheries of the growing cities. Mass migrations of non-immune populations into endemic areas for political reasons have also led to increased transmission of the disease. The growing interchange of populations between malaria-endemic and malaria-free countries is responsible for the continuous increase in the number of imported malaria cases in European countries, and causes serious concern because of possible epidemic focal resurgence in receptive areas such as the Mediterranean. Besides, malaria has re-emerged in certain locations in Africa that had previously had effective control programs, such as Madagascar, South Africa and Zanzibar (Bouma *et al.*, 1996, 1997; Lindsay, 1996).

Since 1976, several new pockets of malaria transmission have evolved, and a WHO report (1984) recommended that countries, which had become malaria-free, should maintain at least one malaria vigilance unit. In many regions, malaria control programs have deteriorated or been abandoned due to high costs of sustaining them. Renewed efforts in malaria control are now needed than ever before.

1.4 Current initiatives on malaria

The global efforts on malaria research over the previous ten years have been lower than the other diseases, and appear to be declining altogether (WHO, 1998). At present, international initiatives on malaria include the Multilateral Initiative on Malaria (MIM), Medicines for Malaria Venture (MMV), Malaria Vaccine Initiative (MVI), Roll Back Malaria (RBM), Research Initiatives on Traditional Anti-malarials (RITAM) and the Malaria Foundation International (MFI) among others (http//www.malaria.org).

MIM is an alliance of organization and individuals concerned with malaria control. It was launched in Dakar in January 1997 when a number of institutions (public and private) joined forces to promote malaria research in Africa. The UNDP/World Bank/WHO special programme on Tropical Diseases (WHO/TDR) has joined the initiative, establishing a task force to address the needs of endemic countries and to fund activities related to strengthening research capacities in malaria. MIM aims at maximizing the impact of scientific research against malaria in Africa, by facilitating global research collaboration and co-ordination (http/www.mim.nih.gov).

MVI aims at accelerating the clinical development of promising malaria vaccines. It also coordinates efforts on malaria vaccine development programmes in various organizations and agencies. It strongly advocates for identification of gaps in the current research efforts and the application of resources to advance promising malaria vaccines.

MMV is a joint public/private sector initiative that aims at developing anti-malarial drugs and drug combinations for distribution in poor countries (WHO, 1999). Support for this venture is being solicited from foundations and other public sources as well as the pharmaceutical industry.

Dr. Mary R. Galinski founded MFI in 1992 with the commitment and dedication of malaria researchers and experts in a variety of professions. It is a private international entity dedicated to the effective prevention, treatment and control of malaria. The Malaria Foundation Inc. was registered in the United States as a tax-exempt 501 (c) (3) non-profit organization in 1993 and was subsequently named MFI (http/www.malaria.org.). MFI was founded with the tenet that much more must be learned about malaria before long-lasting preventive and curative methods can be assured. The ultimate solutions will come through further research and its effective application. This will require strong political will and steadfast dedication, along with enhanced global communication and networking, long-term funding commitments, sustained training programs, and capacity building.

WHO initiated the RBM, in May 1999. It is a global strategy whose goal is to improve health systems with an aim of achieving a 50% reduction in malaria deaths by the year 2010 using techniques which already exist and need wider dissemination or which can be rapidly developed. These include: better treatment of the disease through proper diagnosis; better protection through use of repellent and insecticide treated mosquito nets; control of mosquitoes with environmental development and industrial groups; improved surveillance of disease and mosquito vectors. This could be achieved by: increasing access to effective treatment and means of protection from mosquito bites, thus enabling national authorities and NGO's to combat malaria through intensified efforts, in developing new products for the prevention and treatment of malaria (WHO, 1998).

Following several years of disillusionment and apathy, there is now a more cooperative and positive outlook to the malaria problem. Meetings across the continent of Africa have cemented international commitment to put malaria control back on the global agenda. With the international recognition of the enormity of the fatal, morbid, economic, and social burden posed by malaria, Kenya has re-doubled her control efforts with increased donor support (Anonymous, 2000).

The Department for International Development (DFID) has recently launched a campaign whose aim is to strengthen the Ministry of Health's capacity to coordinate new initiatives in the combat of malaria. The aim is to create further awareness on the threat malaria poses to our nation. Health management, malaria in children and pregnancy, drug procurement, health education and the promotion of provision of community-based health programmes provide the basis of the work carried out by the Ministry of Health (MoH). It is these areas that form a pre-requisite for sustained control of malaria here in Kenya.

Under the auspices of the MoH, the National Malaria Control Programme (NMCP) was devised to control the threat of malaria in Kenya. With the support of the international community, NGO's and researchers, the Malaria Control Unit (MCU) puts the programme into practice. The MCU plays an essential role in the coordination of malaria control activities on the ground. In spite of the spirited efforts to contain it, malaria still remains a major public health problem in Kenya.

1.5 The malaria parasite

Malaria is caused by a protozoan *Plasmodium* (Laveran, 1880). After the first observations of oocysts by Ross (1897) and of all stages of the parasite in a mosquito, Bastianelli *et al.* (1898) described the different developmental stages of the human malaria parasite in *Anopheles claviger* (Meigen). Developmental cycles of *Plasmodium falciparum* Welch and *P. vivax* were described by Grassi *et al.* (1899). Grassi (1890) demonstrated that only *Anopheles* spp are capable of transmitting human malaria.

Malaria in humans is caused through infection by one or more of the four species of malaria parasites: *P. vivax, P. ovale* (Stephens), *P. malariae* (Laveran) and *P. faliciparum*. *P. faliciparum* is the most virulent species and predominates in Africa, eastern Asia, Oceania and the Amazons (WHO, 1997).

The life cycle of *Plasmodium* consists of two phases, the sexual phase (sporogony) in female *Anopheles* mosquito and the asexual phase (schizogony) in man. During sporogony, a mosquito feeding on blood takes up the gametocytes, which would fuse, and form a zygote. The zygote penetrates the stomach of the mosquito to form an oocyst (Ganham, 1966). Within the oocyst, large numbers of sporozoites develop. The sporozoite pass through the body cavity and some enter the salivary glands. Sporozoites are inoculated into a new host when an infective mosquito takes blood. They are inoculated by an infected female mosquito into the host and subsequently disappear from the circulation within one hour (Fairley, 1947). The time from infection until the appearance of parasites in the blood varies with the species of parasites; *P. falciparum*, 5-7 days; *P. vivax*, 6-8 days; *P. ovale*, 9 days; *P. malariae*, 12-16 days, (Manson and Bell, 1987).

The duration of the extrinsic incubation period in the mosquito varies according to the temperature and the *Plasmodium* species. Below temperatures of 16 and 18 °C *P. vivax* and *P. falciparum*, respectively, cannot complete their developmental cycle (Wernsdorfer and Macgregor, 1988).

1.6 Mosquito as vector of malaria

All vectors of human malaria belong to the genus *Anopheles* and the family Culicidae that has two major sub-families (Anophelinae and Culicinae). There are 380 species of *Anopheles* out of which only 60 act as vectors of human malaria. These are found in tropical and sub-tropical regions below 2000-2500 m (Katinka, 1999).

There are twelve geographical zones worldwide each of which has its own dominant vector species. The three most efficient vectors of malaria in the world are; *A. gambiae* s.s. Giles, (Plate **I**), *A. arabiensis* Patton and *A. funestus* Giles. These species together with *A. pharaoensis*, *A. nili* and *A. rufipes* are widely distributed in tropical Africa (Katinka, 1999).

Plate I. *Anopheles gambiae* mosquito feeding on human blood

The potential vectors of malaria in the Mediterranean are *A. labrachiae* and *A. superpictus* while in Europe there are *A. maculipennis* Mg and *A. superpictus* Gr. In Middle East there are *A. maculipennis, A. superpictus* Gr., *A. pulcherimus* Theob among others. In East Asia, *A. hyrcanus* Pall and *A. pattoni* Christ, while in South-East Asia *A. minimus* Theob, *A. maculatus*, *A. sundaicus* Rod (also in India), *A. jeyporiensis* James, *A. leucosphyrus* Don, *A. letifer* Sand, *A. umbrosus* Theob, *A. barbirostris* V.d. Wulp, *A. aconitus* Don and *A. hyrcanus* Pall. (S.I.) are the potential vectors of malaria. *A. maculipennis* Mg (S.I.), *A. superpictus* Gr and *A. stephensi* List are dominant in South West Asia. Australia and New Guinea have *A. farauti* Lav, and *A. punctulators* Don while India has *A. stephensi* List, *A. culicifacies* Giles, *A. fluviatilis* James and *A. philippinensis.* Lull. Far East has *A. dirus*, *A. fluviatilis A. sundaicus, A. aconitus, A. maculatus, A. culicifacies, A. stephensi, A. annularis*, and *A. sinensis.* In North America, we find *A. maculipennis* Mg. (S.I.), *A. sergenti.* Theob, *A. multicolor* Camb and *A. pharaoensis* Theob. South America is dominated by *A. darlingi.* Root and *A. albimanus* Wied (Wigglesworth, 1976).

Anopheles larvae are found in different habitats like fresh and salty water marshes, mangrove swamps, rice fields, edges of streams and ponds. They are also found in small temporary waters like tree holes, puddles, hoof prints, wells, waste containers, tyres and sometimes in water storage containers (Service, 1976).

Larval development takes 7 days for tropical mosquitoes and the pupal stage lasts 2-3 days (Gillies and De Meillon, 1968). Both sexes of adult mosquitoes feed on nectar and other plant fluids. The female ingests blood from vertebrates (Tempelis, 1975). One blood meal is enough for a female mosquito to lay a batch of 30-150 eggs. Among the culicidae, *Anopheles* species have the most regular genotrophic cycle (blood feeding and egg laying). In the tropics, it takes 2-3 days depending on temperature, while in temperate areas it may take several weeks. Each species has its own cycle of activity. Some attack at dusk, during daytime and others at mid-night. Most species are nocturnal. Athropophilic and athropophagic species are those showing strong attraction to and feeding on man's blood, respectively. Zoophilic and zoophagic species prefer animals to man. Endophilic and exophilic species rest indoors and out-door, respectively, while endophagy and exophagy refer to in-door and out-door feeding in that order. Some species feed indoors on man and fly outside to rest before ovoposition (Garett-Jones *et al.*, 1980).

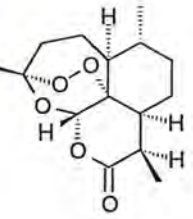
A. gambiae complex is presently considered to consist of six different species. Some of its members are among the most efficient vectors of malaria parasites (Coluzzi, 1964). *A. gambiae* s.s. (Plate **I**) is the principal vector of malaria parasite in East Africa.

1.7 Malaria control strategies

Generally, malaria control has been achieved through chemotherapy and vector control but vaccination has also been attempted and is still under active research.

1.7.1 Chemotherapy and drug resistance

There are a limited number of drugs for the effective treatment of malaria today. The disease has become so difficult to treat because of the worsening problems of drug resistance in many parts of the world (Trigg *et al.*, 1997). Resistance *in vivo* has been reported to all anti-malarial drugs except artemisinin (**1**) and its derivatives (Zucker and Campbell, 1992).



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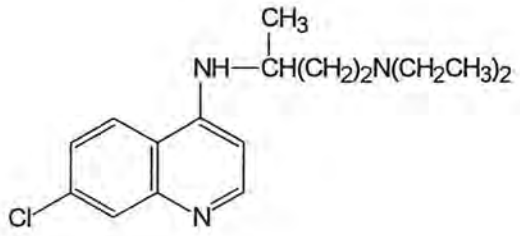
Resistance development forces many malaria patients to use drugs, which are more expensive. This may lead to undesirable side effects. In some parts of the world, artemisinin-based drugs are the first line of treatment of suspected uncomplicated malaria. The implication of this is that we can expect to see malaria forms resistant to artemisinin soon (WHO, 1987). The areas affected most with drug resistance are the Indo-Chinese peninsula and the Amazon region of South America.

The problem of drug resistance may be attributed mainly to increased selection pressures on *P. falciparum*, due to indiscriminate and incomplete doses for self treatment (Zucker and Campbell, 1992). In Thailand and Vietnam, *A. dirus* and *A. minimus* spread the drug resistant parasites. These mosquitoes adapt their biting activity to human behaviour patterns and therefore maintain intense transmission cycles.

Drug resistant *P. falciparum* was first reported in Thailand in 1961. Various *P. falciparum* 'strains' have now attained resistance to all commonly used and generally available anti-malarial drugs (Kevin *et al.*, 1994). In man, the problem of resistance to the common anti-malarial drugs such as chloroquine (**2**) and pyrimethamine (**3**) plus the decreasing effectiveness of quinine (**4**) are mainly limited to *P. falciparum* infection. Chloroquine still remains the treatment of choice for *P. vivax* (Wernsdorfer, 1979). Several mechanisms can account for changes in drug sensitivity of the malaria parasites: physiological adaptations due to non-genetic changes, selection of previously existing drug resistant parasites from a mixed population under drug pressure, spontaneous mutation, mutation of extra-nuclear genes, or the existence of plasmid-like factors.



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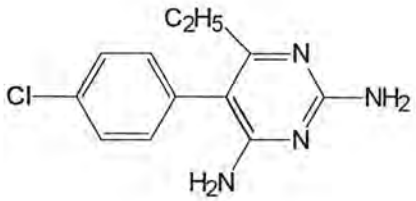

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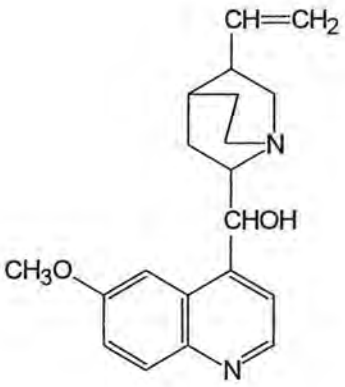

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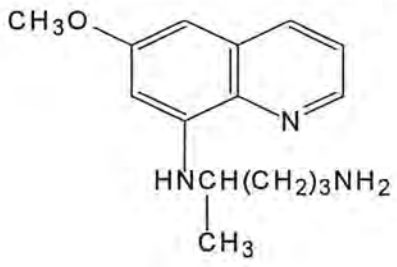
Selection of mutants by the drugs themselves appears to be an important mechanism (WHO, 1987). In an environment with sub-therapeutic levels of the anti-malarial drugs, those parasites, which have resistance through their natural variation or through mutations, clearly have an important biological advantage. This means that even though the drug-resistant forms were initially in the minority, the continued drug mediated elimination of intra-specific competition from the non-resistant forms, has allowed the resistant ones to attain numerical superiority - to the point that drugs like chloroquine are considered useless (Wernsdorfer, 1979). Majority of studies indicate that drug-created selection pressure is to blame for the emergence of resistant malaria (WHO, 1987). Sub-curative plasma levels of drugs found in many areas with uncontrolled and irresponsible prophylactic and treatment regimes will kill the most drug sensitive forms of the parasite, but select the less sensitive ones. Besides, spontaneous mutations in these forms tend to reduce the sensitivity of the parasite to the drug (Yamanda and Sherman, 1979). Fortunately, the problem of irresponsible prophylaxis has been recognized and precautions are being taken. For instance, in Zimbabwe and Kenya it is now illegal to sell chloroquine other than full courses (Bradley and Behrens, 1994).

The rapid spread of drug-resistant malaria may be due to an increasing efficiency of vector. This phenomenon may be explained by the increased efficiency of oocyst formation that has been observed with drug-resistant species (WHO, 1987).

In order to appreciate the physiological nature of resistance, it is necessary to understand the metabolism of the parasites and their mode of action on anti-malarial drugs. Intra-erythrocytic stages of malaria ingest the haemoglobin into food vacuoles. Here exo-peptidases break down haemoglobin into haemozoin (malaria pigment), of which the cytotoxic ferriprotoporphyrin IX is a major component (Wernsdorfer and Trigg, 1984). A parasite synthesized binding protein, 'haembinder', seemed to sequester the membrane-lytic ferriprotoporphyrin IX into the inert haemozoin complex to protect the *Plasmodium* cell membranes from damage. It is now appropriate to discuss a number of anti-malarials and apparent adaptations seen in resistance.

Primaquine (**5**): This drug has been mainly used against gametocytes and hypnozoites. The drug is thought to work by inhibiting the ion transport chain of the parasite, though the precise metabolic interaction is not known. Neither is it certain as to whether it is the drug itself or derived metabolites, which have the desired effects (Merhli and Peters, 1976). There is no evidence that gametocyte resistance exists, but if the drug is used against schizonts, then resistance is rapidly attained (Ferone, 1970). The surviving resistant parasites had increased numbers of mitochondria suggesting that the resistance mechanism involves the production of extra organelles to compensate for the damage caused by the drug (WHO, 1987).



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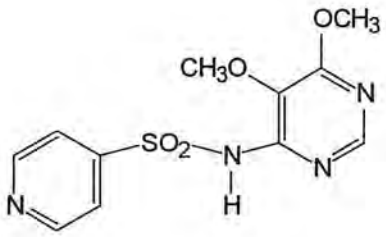
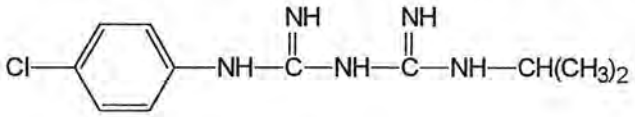
Sulfonamide (**6**): Parasites, which become resistant to sulfonamides, must bypass the metabolic step at which *para*-aminobenzoic acid (*p*ABA) is incorporated into dihydropterate. Sulfonamides work by inhibiting *p*ABA synthesis, which is required to synthesise the dihydropterate - an intermediate compound in the synthesis of tetrahydrofolate.

Tetrahydrofolate derivatives serve as donors of one-carbon compounds in a variety of essential biosynthetic pathways. Little is known about this side of parasite metabolism, or the exact mechanism of resistance - though resistance is clearly stable, transmissible and prolific (WHO, 1987).



6

Proguanil (**7**) and pyrimethamine (**3**) (antifolics): Both of these compounds inhibit the action of dihydrofolate reductase. As with the sulfonamides, resistance occurs in all stages of the life cycle. Dihydrofolate reductase enzymes of resistant strains bind to pyrimethamine 400-800 times less readily than the enzymes of drug sensitive strains (Eckman *et al.*, 1977). Interestingly, high levels of resistance to sulfonamides are associated with hypersensitivity to antifols and vice versa, so combination treatments have had good effects. Unfortunately, resistance to these drug cocktails has now become apparent (WHO, 1987).



7

Chloroquine (**2**) and related compounds: It is known that chloroquine mediates its effects on the haemoglobin metabolism of malaria parasites, perhaps preventing the neutralization of the toxic ferriprotoporphyrin IX. Chloroquine resistant parasites are unable to produce haemozoin, but are still able to digest haemoglobin. In non-resistant forms, most of the ferriprotoporphyrin IX is sequestered in haemozoin, but in the resistant forms, this toxic metabolite becomes available to the host cell haemoxygenase system for elimination (Fitch, 1983). In chloroquine-sensitive parasites, the drug is taken up into food vacuoles, and it is proposed that it competes with the haembinder for the ferriprotoporphyrin IX, to form a destructive compound (Bradley, 1995).

8

Quinine (**4**) and mefloquine (**8**): These cause blabbing of the parasite membranes and aggregations of haemozoin. Parasite-resistance occurs by uncertain mechanisms, but is stable and transmissible (WHO, 1987).

Artemisinins: Are among the newest and most effective of all anti-malarial drugs, and seem to affect protein synthesis. Artemisinins must be protected and used rationally to prevent the emergence of inevitably resistant *P. falciparum* for as long as possible. Schizontocidal activity of artemisinin (**1**) and several of its derivatives against *Plasmodium* strains resistant to all known anti-malarial drugs, with virtually no toxicity, have been well evaluated in clinical tests in China (Klayman, 1993; Kinghorn and Balandrin, 1993; Wolfender and Hostettmann, 1995). However, artemisinin derivatives require long treatment courses and, when used alone, re-crudescence may occur (WHO, 1998). *In vitro*, artemisinin resistant forms have already been demonstrated (WHO, 1987).

Antibiotics: Tetracyclines are often used in conjunction with other drugs to combat chloroquine resistant falciparum malaria. *Plasmodium* protein synthesis appears to be eukaryotic and insensitive to chloramphenicol, but affected by cycloheximide. It has been suggested that antibiotics such as tetracycline act on the mitochondrial ribosomes of the parasite, thus inhibiting protein synthesis. Macrolides such as erythromycin seem to inhibit autophagic vacuole formation, thus potentiating the action of chloroquine (WHO, 1987). Resistance to these compounds is not a current problem.

It is obvious, then that resistance is an ongoing problem. By 1973, sulfadoxine-pyrimethamine cocktails replaced chloroquine, but by 1985, this too was ineffective. Though quinine remains effective, there is a 50% failure rate unless it is supplemented by tetracyclines.

Its compliance with the 7-day regimen is poor. Between 1985 and 1990, the recommended treatment for malaria in Thailand was mefloquine, combined with sulfadoxine-pyrimethamine at a dose of 15/30/1.5 mg/kg body weight, but by 1990 the cure rate had fallen to 71% in adults and 50% in children. This treatment is no longer used due to resistance (Kevin *et al.*, 1994). The future of chloroquine is not clear, although a recent report (IDRC, 1995) suggests that due to the current absence of drug pressure, chloroquine-sensitivity may be regained.

The common prophylactic drugs are, for many areas, obsolete (IDRC, 1995), and the use of advanced drugs such as artemisinin derivatives for uncontrolled prophylaxis, would be downright irresponsible, given the obvious ability of *Plasmodium falciparum* to attain a high degree of resistance in a short period. It has already been suggested that strains resistant to artemisinins will appear by the end of the decade (Zucker and Campbell, 1992), and this seems inevitable. For that reason researchers will still have sleepless nights in their efforts to try other less developed remedies like vaccination therapy. Vaccine development for malaria control, therefore, has a pivotal role in the future.

1.7.2 Malaria vaccine

Over the past decade, substantial progress has been made in search for a malaria vaccine. The three major types of vaccines being developed are: 'anti-sporozoite' vaccine, which is designed to prevent infection (Franke *et al.*, 1999), 'anti-asexual blood state' vaccines, designed to prevent severe manifestations of the disease and the 'transmission - blocking' vaccines designed to arrest the development of the parasite in the mosquito (WHO, 1998).

Although, a vaccine recently developed in Britain and tried in Gambia offered 47% protection (Anonymous, 2001), it is however, the hope of researchers that an effective vaccine will be available within the next 7-15 years. The development of such vaccines has been made complicated by the parasites' ability to change their immunological identity, thereby concealing themselves from the immune responses that might otherwise be stimulated by a vaccine. Mapping of the malaria parasite genome is being done by a consortium of partners (WHO, 1998). It is believed that knowledge of the genome will open more rational ways of discovering new vaccines (Collins *et al.*, 1995).

Vaccine development may still not be easy due to the different set of genes, which are probably switched on at each of the four stages of the parasites' extraordinary complex life cycle.

1.7.3 Vector control

Presently, vector control is thought of as one of the best approaches in the war against malaria, especially in the absence of a vaccine and an effective chemotherapeutic agent. Control of the malaria vector can be achieved using insecticides, larvicides, insect growth regulators (IGRs), traps baited with semiochemicals (attractants and pheromones) and the use of repellents among other methods. In the search for novel techniques in vector control methods, the protection of human targets from infective mosquito bites has been found to be the most effective.

1.7.3.1 Insect growth regulators

Chemicals exhibiting insect growth inhibiting properties have been used to control the growth of most insects at the larval stage. Most of the IGRs so far discovered, can be classified as juvenile hormone mimics or chitin synthesis inhibitors. These compounds are safe to humans but have mild or no toxicity to most non-target organisms. Their activity is against immature stages of mosquitoes, flies, and other insects. However, some IGRs induce sterility among other reproductive disorders in the adult stage (WHO, 1996).

1.7.3.2 Larvicides and bio-larvicides

Many chemicals as well as biological larvicides have been used to control mosquito larvae. One of the earliest reports of the use of plant extracts against mosquito larvae found that plant alkaloids (Katinka, 1999) like, nicotine, anabasine, methylanabasine, and lupinine extracted from the Russian weed, *Anabasis aphylla,* killed the larvae of *Culex pipiens* Linn, *C. territans* Walker, and *C. quinquefasciatus* Say. Extracts from Amur Cork tree fruit and *Phellodenron amurense*, yielded a quick acting mosquito larvicide. In the USA oil sprays on water has been used to control mosquito larvae (Wigglesworth, 1976).

The most widely used biological control agents are the larvivorous fishes like *Gambusia affinis* and *Tilapia* spp (Bay, 1967). Corbat (1986), used dragonfly nymphs to eliminate the larvae of *Aedes aegypti*. Banmgartner (1987) experimented with aquatic carnivorous plants like, *Urticularia vulgaris*, for the control of larvae. *Azollo fulcoides*, a floating water fern was also tested by Lu *et al.* (1996) on *Anopheles* and *Aedes* larvae.

Bio-larvicides include microbial control agents, phytochemicals with pesticidal activity, toxins produced by fungi and bacteria, among other organisms. Some attempts have been made to isolate, identify, and develop spore-forming bacterial agents for vector control. Among these, *Bacillus thuringiensis* and *B. sphaericus* have been commercialised and used for quite some time in the control of disease vectors (WHO, 1996; Collins *et al.*, 1995).

1.7.3.3 Insecticides

Control of the adult mosquito population or bite is only possible by the use of either insecticides or repellents. There has been increasing interest in the use of insecticide treated bed nets for malaria control. They were first used in Russia in the 1930's and by American and German forces during world war II (Curtis and Lines, 1985). Bed nets are widely used against nuisance mosquitoes in China, Thailand, Latin America, Papua New Guinea and Africa. Since most *Anopheles* species bite at night, it has been assumed that nets should reduce the chances of contracting malaria (Lindsay and Gibson, 1988). In the Gambia (Snow *et al.*, 1988; Curtis, 1990; Alonso *et al.*, 1991; 1993), Guinea Bissau (Joenson *et al.*, 1994) and elsewhere (Curtis and Lines, 1985; Curtis *et al.*, 1987), introduction of insecticide-impregnated nets in the communities has remarkably reduced parasite prevalence and malaria cases (Marbiah *et al., 1998*). More information on exploitation of insecticides by man to control insects is discussed in the following chapter.

1.7.3.4 Insect repellents

Perhaps the best and most effective protection against malaria is avoiding being bitten by mosquitoes. Personal protection can be achieved by the use of bed nets, suitable clothing, and repellents (WHO, 1995). The use of repellents by man as a weapon in the bid to gain protection from the painful mosquito bites is discussed in depth in the next chapter.

1.8 Justification

Malaria continues to be the biggest contributor to disease burdens in terms of deaths and suffering (WHO, 1997). An estimated 500 million people catch malaria each year and, of the two million who die, 90% live in sub-Saharan Africa. At present there is no drug that offers foolproof protection against malaria and some of the drugs used to treat the disease have some extremely nasty side effects on a small percentage of people. In Kenya, the parasites have now become 80% resistant to quinine (WHO, 1987). This resistance is said to be one of the highest rates in the world.

Control of malaria can effectively be achieved by controlling the vector itself, yet most *Anopheles* mosquitoes have become resistant to DDT, the most effective general-purpose insecticide available in the market. The available synthetic insecticides are not friendly to the environment as they accumulate in food chains, with serious environmental health repercussions. Important malaria vectors such as *Anopheles pulcherrimus*, *An. albimanus*, *An. arabiensis*, *An. gambiae* and *An. funestus* are less susceptible to DEET, the most potent ingredient of many commercially available repellents. Moreover, DEET attacks paint, varnish and some hard plastics, (WHO, 1996). Alternative repellents must, therefore, be found urgently. It is clear then, that vector control strategies of the future must include potent, more selective and biodegradable insecticides/repellents discovered from natural sources such as higher plants and animals.

1.9 Objectives

The general objective was to investigate the repellency/insecticidal activity of phytochemical extracts from some Kenyan plants on *Anopheles gambiae*.

The specific objectives included:

- To extract and bio-assay the various extracts from some plants used in traditional cultural practices to prevent mosquito bites.
- To isolate, identify and determine the chemical compositions of the active plant extracts.
- To bio-assay the isolated compounds and identify the repellent/insecticidal compounds in the active plant extracts.

CHAPTER 2: LITERATURE REVIEW

2.1 Insecticides

Insecticides are chemicals that are used to control damage or annoyance from insects. Control is achieved by poisoning the insects by oral ingestion of stomach poisons, contact poisons (that penetrate through the cuticle) or fumigants (penetrate through the respiratory system) (Kirk and Orthmer, 1992). Ancillary chemicals are also employed in insect control. These include, attractants and repellents, which influence insect behaviour and chemosterilants, which influence reproduction.

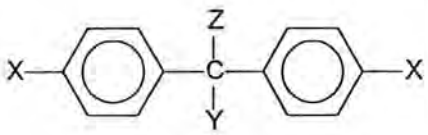
Insecticides may be classified by their chemical nature and source of supply as inorganic, synthetic and natural organic compounds. Inorganic insecticides generally act as stomach poisons, whilst natural products act largely as contact poisons. Synthetic organic insecticides may have contact or stomach poison action and are sometimes used as fumigants (Kirk and Orthmer, 1992).

Stomach poisons are generally applied against insects with chewing mouthparts. They are effective against insects with sponging, siphoning, lapping, or sucking mouthparts. Contact poisons are the principal weapons against insects with sucking mouthparts. Such insects feed beneath the surface and are not affected by the stomach poisons. Contact poisons may penetrate the blood directly through the insect cuticle or the spiracles of the respiratory system into the tracheae. These poisons owe their effectiveness to the extraordinarily efficient absorptive properties of the insect cuticle for organic molecules (Kirk and Orthmer, 1992).

The inorganic insecticides so far used include arsenicals and fluorides. Arsenicals like lead arsenate ($PbHAsO_4$), arsenic trioxide (As_2O_3), calcium arsenate (neutral) $Ca_3(AsO_4)_2$, calcium arsenate (basic) $[Ca_3(AsO_4)_2]_3.Ca(OH)_2$, copper acetoarsenate, $[Cu(C_2H_3O_2)_2.3Cu(AsO_2)_2]$, sodium arsenite ($NaAsO_2$) among others have been used. Fluorides include salts of hydrofluoric acid (HF), fluorosilicic acid (H_2SiF_6) and fluoroaluminic acid (H_3AlF_6). Specific examples are sodium fluoride (NaF), sodium fluorosilicate (Na_2SiF_6), sodium fluoroaluminate (cryolite) (Na_3AlF_6) among many others (Kirk and Orthmer, 1992).

Many synthetic organic insecticides have been used to control insects. 1,1,1-Trichloro-2,2-bis-(*p*-chlorophenyl)ethane (DDT) (**9**) was synthesized in 1874 and its insecticidal properties discovered in 1939 (Kirk and Orthmer, 1992). It has been employed for control of several insects and is still widely used for control of insect vectors of public health importance like mosquitoes. Its efficacy has however reduced due to resistance developed by many insects. DDT is non-biodegradable hence a major environmental pollution problem.

Several DDT analogues have been synthesized and have attained commercial importance as insecticides. They include 1,1-dichloro-2,2-bis-(*p*-chlorophenyl)ethene (DDE) (**10**) and 1,1-dichloro-2,2-bis-(*p*-chlorophenyl)ethane (TDE) (**11**) which are toxic and with the same environmental pollution and resistance problems as DDT.

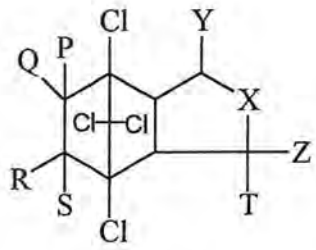


		X	Y	Z
9	DDT	Cl	CCl_3	H
10	DDE	Cl	CCl_2	–
11	TDE	Cl	$CHCl_2$	H
12	Methoxychlor	OCH_3	CCl_3	H

1,1,1-Trichloro-2,2-*bis*-(*p*-methoxyphenyl)ethane (Methoxychlor) (**12**), is related to DDT and has also been used as an insecticide. It gives a rapid knockdown of many insects than DDT. The methoxy groups are readily dealkylated *in vivo* by microsomal oxidases producing phenols that are easily eliminated. Unlike DDT, methoxychlor does not accumulate in nature and so it is favoured for general environmental use. Unfortunately, insects that are resistant to DDT show cross-resistance to methoxychlor.

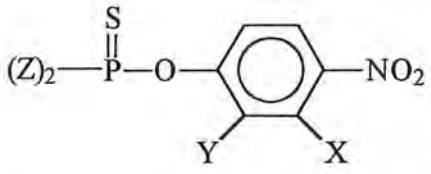
Cyclodienes are polychlorinated cyclic hydrocarbons with endomethylene-bridged structures. Discovery of 2,3,4,5,6,7,8,8-octachloro-2,3,3a,4,7,7a-hexahydro-4,7-methanoindene (chlordane) (**13**) in 1945 resulted in the development of other insecticidal chlorinated cyclodienes. These include 1,3,4,5,6,7,8,8-octachloro-3a,4,7,7a-tetrahydro-4,7-methanophthalon (telodrin) (**14**) and 1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-*endo-exo*-5,8-dimethanonaphthalene (aldrin) (**15**).

These insecticides are effective against a wide range of insect pests. However, they are not bio-degradable, accumulate in food chains and deplete the ozone layer.



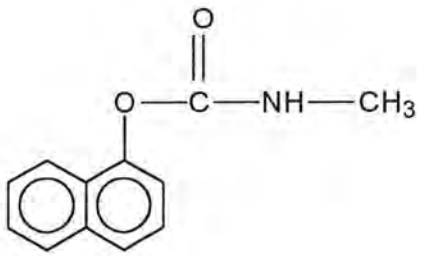
		P	Q	R	S	T	Z	X	Y
13	Chlordane	H	Cl	Cl	H	H	Cl	CHCl	-
14	Telodrin	-	Cl	Cl	-	H	Cl	O	Cl
15	Aldrine	H	Cl	Cl	H	H	CH=CH	CH_2	H

Organophosphorus insecticides include *o,o*-diethyl-*o,p*-nitrophenlyphosphorothionate (parathion) (**16**), *o,o*-dimehtyl-*o,p*-nitrophenylphosphorothionate (methylparathion) (**17**), *o*-3-chloro-4-nitrophenyl-*o,o*-dimethylphosphorothionate (chlorthion) (**18**) among others. This group of insecticides is also not highly selective, though most of them are bio-degradable and less toxic to the mammals.



		X	Y	Z
16	Parathion	H	H	OC_2H_5
17	Methylparathion	H	H	OCH_3
18	Chlorthion	Cl	H	OCH_3

Examples of carbamate insecticides used in mosquito control include 1-naphthyl-N-methyl carbamate (**19**) and 2-isopropyloxyphenyl-N-Methyl carbamate (baygon) (**20**) (Kirk and Orthmer, 1992). Carbamates are less effective in mosquito control, slightly toxic to the mammals and not highly selective. However, they do not accumulate in nature and are friendly to ozone layer.



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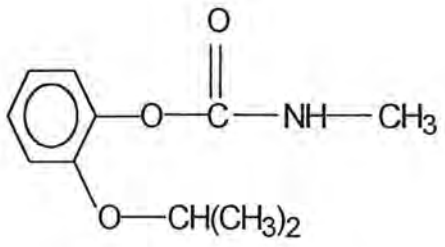
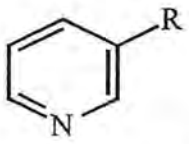


20

Nicotine from tobacco was one of the earliest insecticides. L-methyl-2-(3'-pyridyl) pryrrolidine (nicotine) (**21**) is found in the leaves of *Nicotiana tobacum*, *N. rustica*, *Duboisa hopwoodii* and *Aesclepias syriaca*. It occurs as the main alkaloid along with small amounts of twelve other alkaloids of which 2-(3'-pyridyl) pyrrolidine (nornicotine) (**22**) and

L-2- (3'-pyridyl) piperidine (anabasine) (**23**) are of insecticidal importance (Rappaport, 1992; Kirk and Orthmer, 1992).



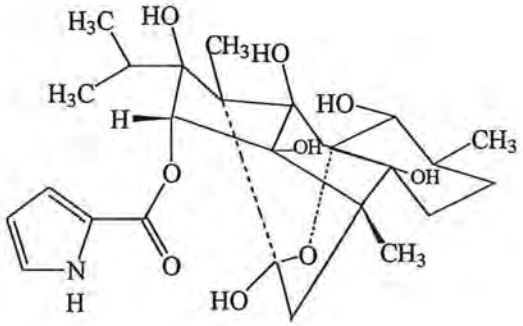
R

21 N–methylpyrolidyl

22 Pyrolidyl

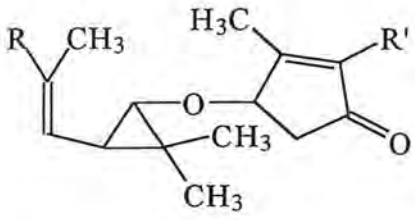
23 Piperidyl

Synthetic analogues of nicotine like, 5'-methylnornicotine have been demonstrated to be effective insecticides. Ryanodine (**24**), an alkaloid from the tropical shrub, *Ryania speciosa* has been used as a commercial insecticide against European corn borer. The high cost, toxicity to mammals and limited efficacy has limited the use of natural alkaloids as insecticides (Duke, 1990).



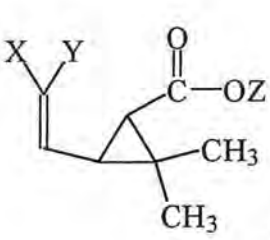
24

The most successful example of a plant-produced insecticide is that of the pyrethroids. Insecticidal properties of several *Chrysanthemum* species were known for centuries in Asia. Even today powders of dried flowers of these plants are sold as insecticides. The insecticidal properties of pyrethrum from the ground flowers of *Chrysanthemum cinerariaefolium* and *C. coccineum* is due to six terpenoids; pyrethrin I (**25**) and II (**26**), cinerin I (**27**) and II (**28**) and jasmolin I (**29**) and II (**30**).

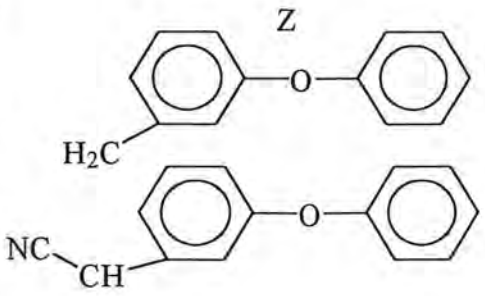


		R	R '
25	Pyrethrin I	CH_3	$CH_2CH{=}CHCH{=}CH_2$
26	Pyrethrin II	$COOCH_3$	$CH_2CH{=}CHCH{=}CH_2$
27	Cinerin I	CH_3	$CH_2CH{=}CHCH_3$
28	Cinerin II	$COOCH_3$	$CH_2CH{=}CHCH_3$
29	Jasmolin I	CH_3	$CH_2CH{=}CHCH_2CH_3$
30	Jasmolin II	$COOCH_3$	$CH_2CH{=}CHCH_2CH_3$

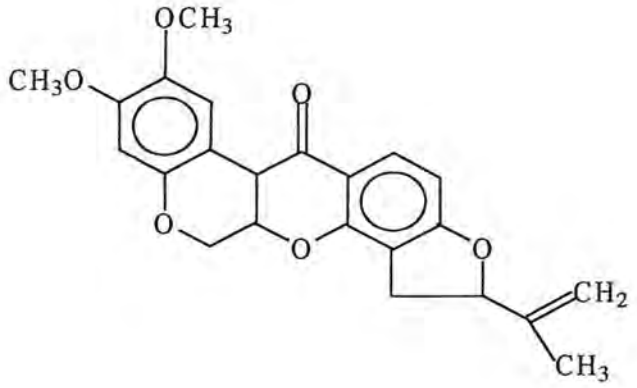
These compounds are highly unstable to light, air, moisture and alkali. Knowledge of their structure and their high cost stimulated the structural optimization of synthetic derivatives, which has resulted in reduced costs, wide affordability and acceptability of analogues (Duke, 1990; Kirk and Orthmer, 1992; Jacobson *et al.*, 1971; Jacobson, 1975; Bushell *et al.*, 1998). Examples of these synthetic pyrethroids include; phenoxybenzyl-*DL-cis,trans*-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylate (permethrin) (**31**) and α-cyano-3-phenoxybenzyl-*DL-cis*-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane carboxylate (decamethrin) (**32**). Although synthetic prethroids offer improved selectivity (over the other synthetic insecticides) and lower mammalian toxicity, incidences of mosquito resistance to these insecticides have however been recently reported (WHO, 1996; Kristinsson, 1998).



		X	Y
31	Permethrin	Cl	Cl
32	Decamethrin	Br	Br

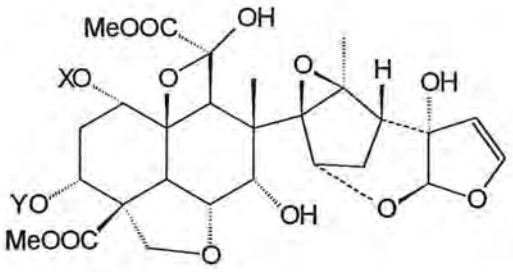


The genera *Derris, Lonchocarpus* and *Tephrosia* are known to be rich in rotenone (**33**) from their roots. Root extracts of plants in these genera were commercially used as insecticides in the 1930's. The use of rotenone-yielding roots as insecticides in the USA was developed as a result of federal laws against high residues of lead, arsenic and fluorine in edible products (Jacobson, 1975; Jacobson and Crosby 1971). Rotenone is harmless to plants, highly toxic to insects, relatively toxic to fish, pigs and honey bees. Twenty-one (21) species of *Tephrosia*, 12 of *Derris*, 12 of *Lonchocarpus*, 10 of *Milletia* and several of *Mundulea*, have been reported to contain rotenoids (Kirk and Orthmer, 1992).



33

Neem is a fast growing tree originally from the Indian sub-continent but now found in all parts of Africa and Asia. It contains several insecticidal compounds. The main one is azadirachtin (**34**), which deters and kills many serious insect pests and vectors. The seeds of neem retain their killing power for a year if kept in darkness (Rappaport, 1992). The main disadvantage is the instability of neem extracts to light.



X = $CH_3CH{=}C(CH_3)CO$

Y = Ac

34

Other insecticidal plants, include, *Hyptis spicigera* a herb that grows wild throughout Africa and belongs to the Labiatae family; *Guiera senegalensis*, a small tree that grows in arid Africa and *Annona* species (Rappaport, 1992).

2.2 The status of vector resistance to insecticides

Insecticide resistance has appeared in almost every major group of arthropod vectors of diseases. By 1991, resistance to all major classes of insecticides in public health use was reported in more than 150 species of vectors and nuisance pests (WHO, 1996). Today, the number of the resistant vectors is much greater than it was in 1991. This number has been increasing steadily. Furthermore, cross-resistance has occurred between older insecticides such as DDT and synthetic pyrethroids in different species of mosquitoes and among different pyrethroids themselves.

For *Anopheles gambiae* it has been reported in several west African countries that this vector is resistant to permethrin with decreased susceptibility to deltamethrin and lambdacyhalothrin (WHO, 1996).

2.3 Repellents

Repellents are chemical substances that protect animals, plants or materials such as fabrics, grain and timber from insect attack by rendering them unattractive, unpalatable or offensive (Metcalf and Flint, 1962). The substances, which may not be poisonous or mildly toxic, are rarely effective against all kinds of insects.

The practical problem of repellency is essentially a behavioural one (to alter and intercept a normal response operating through chemosensory pathways) (Mafong and Kaplan, 1997).
To be effective, a repellent compound must first be capable of stimulating some sensory system other than that which mediates attraction. Secondly, the repellent must also act upon a system which has some influence on locomotion or feeding since the response of the organism depends upon which sensory system has been stimulated, and which reflex arcs are placed in operation (Dethier, 1956). Vapour repellents act in the gaseous phase and are most often stimulants of the olfactory receptors. Contact repellents (deterrents) are those compounds, which the insect must come into direct contact with and act on receptors not normally sensitive to vapours (Dethier *et al.*, 1960).

The search for new repellents during the 2nd world war led to establishment of criteria for a good repellent against blood-sucking insects. These are: effective protection of the treated area for several hours, on all types of subjects and 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, touch and harmlessness to clothing; protection against a wide variety of biting insects; low cost and availability. No compound has ever been found which meets all these requirements satisfactorily (Metcalf *et al.*, 1962; Kirk and Orthmer, 1992).

During world war II there was need to search for new repellents that could be used by the military. Almost 7000 synthetic organic compounds were screened for repellency against mosquitoes on human skin and clothing in USA (Knippling, 1949). Dimethyl phthalate (DMP), 2-ethyl-1,3-hexanediol (Rutgers 6/2) and *n*-butylmesityloxide oxalate (indalene) are some of the earliest repellents synthesized in the laboratory. Each showed differences in repellency, which was found to be specific for various mosquito species. Differences disappeared and effectiveness was enhanced when they were mixed together. The mixtures are effective against a wide range of mosquito species.

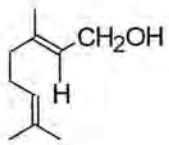
The search for new and longer acting repellents resulted in the discovery of diethyl-*m*-toluamide (DEET) and cyclohexamethylene carbamide (CHMC), which are the most potent of the modern synthetic repellents. DEET was introduced in 1950's and shown to be a more effective repellent than DMP and 2-ethyl-1,3-hexanediol (Kirk and Orthmer, 1992), though these two are still available in some insect repellent preparations. Important disease vector species such as *Anopheles pulcherrimus* (Zhogolev, 1968), *A. albimanus* (Curtis *et al.*, 1987; Schreck, 1977; 1985; Yang and Zhuang, 1974), *A. gambiae* (Curtis *et al.*, 1987) and *Glossina morsitans* (Schmidt, 1977) are less susceptible to DEET than *Aedes aegypti*.

N,N-diethyl-*m*-toluamide (DEET), dimethylphthalate (DMP), 2-ethyl-1,3-hexanediol and 2-phenylcyclohexanol have been used as general-purpose repellents, whereas *n*-butyl-6,6-dimethyl-5,6-dihydro-1,4-pyran-2-carboxylate, *cis*-dimethylbicyclo[2.2.1]-5-heptane-2,3-dicarboxylate, 2-ethyl-2-butyl-1,3-propanediol and *n*-propyl-N,N-diethyl succinate have been used as mosquito repellents.

Most of the synthetic organic repellents act as solvents for lacquers and should not be applied to watch crystals, spectacle frames, synthetic fabrics, paints and varnishes. This is a major undoing for synthetic repellent formulations. Disadvantages of synthetic repellents include, resistance developed by insects, toxicity to other animals, high cost and environmental pollution (Stinecipher *et al.*, 1997).

Pyrethrin is an excito-repellent/insecticide (WHO, 1984) with moderate repellency of up to 7 hrs (Johnson, 1947). Mosquitoes not repelled by pyrethrin die after biting (Curtis *et al.*, 1991). Mosquito coils have been produced from pyrethrum flowers for protection against indoor mosquito bites. Coils burn for 6 to 8 hours; therefore, one can be left dangerously unprotected. Studies on the repellency of mosquito coils in Tanzania and Papua New Guinea revealed protection of 40 to 80% (Hudson *et al.*, 1971; Charlwood *et al.*, 1984).
A coil containing 0.5% natural pyrethrins, reduced the landing rate of *A. gambiae* by 40% while knockdown began after 2 minutes, but a few took some blood and others recovered (Curtis and Hill, 1988). Whereas no resistance has been observed with natural pyrethroids, synthetic analogues have induced resistance in mosquitoes (WHO, 1996).

Essential oils from some plants have mosquito repellent properties. This has been observed in the oils of Cassia, Camphor, Citronella, Lemon grass, Clove, Thyme, Geranium, Bergamot, Baylaurel, Pine, Wintergreen, Penyroyal and Eucalyptus (Knippling, 1949). These oils have been the basis of most commercial natural repellents and many different varieties were produced and tested, until the advent of synthetic compounds such as diethyltoluamide (DEET). Completely unrelated plants in most cases share some of the repellent constituents. Some examples of natural repellents, which have been isolated from a wide range of plant species, include geraniol (**35**), citronellal (**36**), linalool (**37**), camphor (**38**), δ-pinene (**39**), *p*-menthane-1,8-diol (**40**), 1,8-cineole (**41**), and eugenol (**42**). Such substances are active as repellents or attractants for other non-biting insects (Dethier, 1947) and are insecticides at high concentrations.



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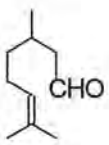

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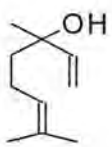

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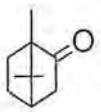

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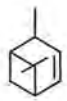

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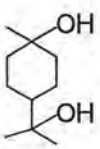

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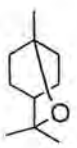

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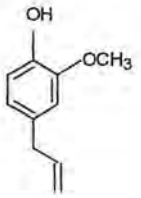

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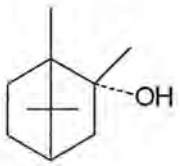
Citronella oil is a popular repellent in India and is still used as a repellent in Europe and North America. The main consituent of citronella oil is citronellal, which was found to be as effective as dimethylphthalate (DMP) when freshly applied. A synthetic derivative of citronellal has been used as an ingredient of commercial repellents. A fresh application of this derivative was found to be as effective as DEET against sand flies and mosquitoes (Buescher *et al.*, 1952; Rutledge *et al.*, 1982; 1983; 1985).

Burning of certain herbs such as *Artemesia* and *Calamus* species is still practised in remote villages in China to repel mosquitoes (Curtis, 1991). Essential oil of *Artemesia vulgaris* has linalool (**37**), camphor (**38**), borneol (**43**) and terpinen-4-ol (**44**) as the main repellent constituents. Terpinen-4-o1 was found to be the most active and as effective as DMP (Curtis *et al.*, 1991).



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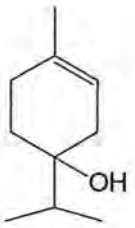

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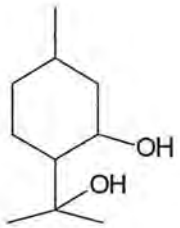
Oil of the lemon eucalyptus plant, *Eucalyptus maculata* Citriodon has some repellent effect on mosquitoes. Its main ingredients are citronellal, citronellol, geraniol, isopulegol, δ-pinene and sesquiterpenes. However, laboratory assays of these ingredients for repellency against *Aedes aegypti* was disappointing (Zhuang *et al.*, 1974). This showed that the active principle(s) was not in the plant oil. It was finally discovered in the waste distillate of the lemon eucalyptus oil, and named quwenling, meaning "effective mosquito repeller".



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The main ingredient of this material was found to be *p*-menthane-3,8-diol (**45**) which is a white waxy material now sold in 30 or 40% alcoholic solution (Trigg, 1996). Cotton nets impregnated with quwenling are effective for 8.5 days on average (4 - 6 hrs daily usage with storage in plastic bags between tests).

This is slightly less than that of DEET impregnation, which lasted 9.7 days on average. This repellent is now commercially available as Mosiguard® and is rapidly replacing DEET and other synthetic repellents used in malaria control.

An extract labeled 1247 has also been made in China from wild mint (*Mentha haplocalyx*) and consists of *D*-8-aceto-oxycarbotanacetone. Laboratory tests of its alcoholic solution were reported to have had a protection time of 6-12 hours against *Ae. albopictus*. Another derivative designated 9525 has been isolated from the leaves and stems of *Clausena kwangsiensis*. Cage studies of a 50% solution of this material in alcohol, rubbed on the back of the hand at a dosage of 0.001 mg/cm^2 showed a protection time of 10 hrs against *Ae. albopictus* (Curtis et al., 1991). The effective ingredient of 9525 is *p*-menthane-3,8-diol (**45**).

Before the advent of synthetic chemicals, people have used plants and their derived substances to repel or kill mosquitoes and other insect pests. Ancient races used smoke from burning cattle or goat dung to drive out mosquitoes from their caves or huts before sleeping. Later on, certain herbs or barks of some trees were added to the smoldering fire to enhance the repellent action of smoke. The Ainu people of Hakkaido, Japan and Micmac Indians (Curtis *et al.*, 1991) of New Foundland wore leggings of sedge, bark or cloth to reduce insect biting nuisance, which is concentrated around the lower legs. Ancient Chinese had many prescriptions of repellents against mosquitoes among other blood-sucking flies. During World War II, the military used aromatic oils like citronella, bergamot, eucalyptus, peppermint, turpentine and spirit of camphor in various formulations.

Herbs of the basil family (Labiatae) have many traditional medicinal uses in Africa and Asia. In East and West Africa they are also used as mosquito repellents (Dalziel, 1937; Kokwaro, 1993). In Northern Tanzania (Fivawo, 1985) basil-like herbs (*Ocimum* spp. and *Hyptis soaveolens*) and neem (*Azadirachta indica*) leaves were used for repelling mosquitoes. White (1973) showed that, when smeared on the legs, the juice of *Ocimum* spp reduced biting by caged *A. gambiae*. Later the repellency was attributed to eugenols (Chogo and Crank, 1981; Hassanali, 1996), which are main components of clove and other essential oils with repellent properties.

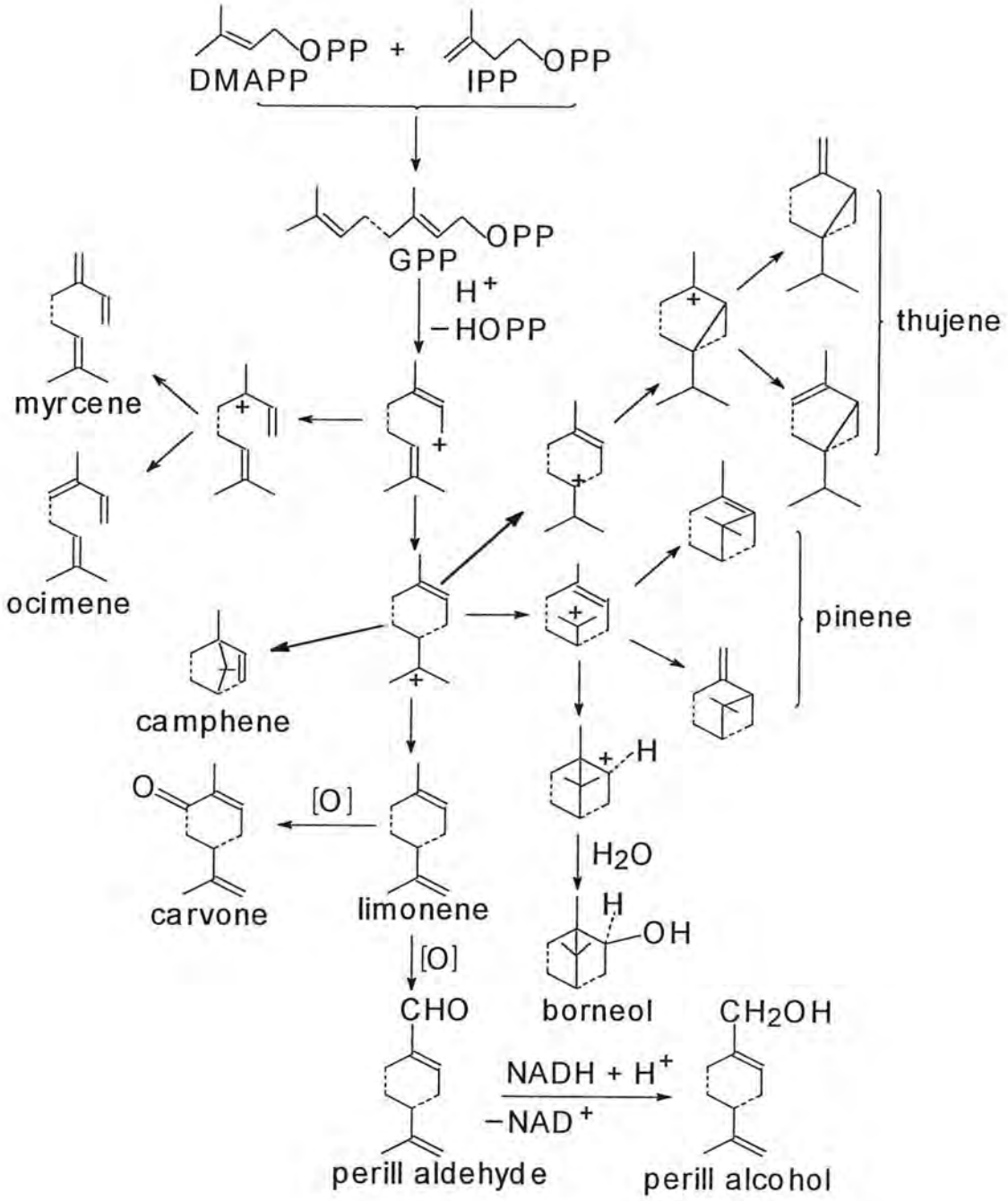
The woods and resins of aromatic trees are widely sold in the markets, under the collective term 'Churai' in Gambia to be burned as mosquito repellent. However, children in households using Churai are shown to suffer from as much malaria as those in neighbouring households, which do not use it (Srou *et al.*, 1987; Greenwood *et al.*, 1987).

Dry smouldering sticks of thyme (*Thymus serphyllum*) were reported to give 85 to 90% protection for 60 to 90 minutes in the open air in U.S.S.R. (Rubtzov, 1946; Philip *et al,* 1945). In India, women smearing their bodies with turmeric and gingili or mustard oil before bathing with soap had much reduction in bites of *A. fluviatilis* (Philip *et al,* 1945). Men, who do not, had more bites. A hand anointed this way was not bitten by caged mosquitoes, which preferred untreated hand.

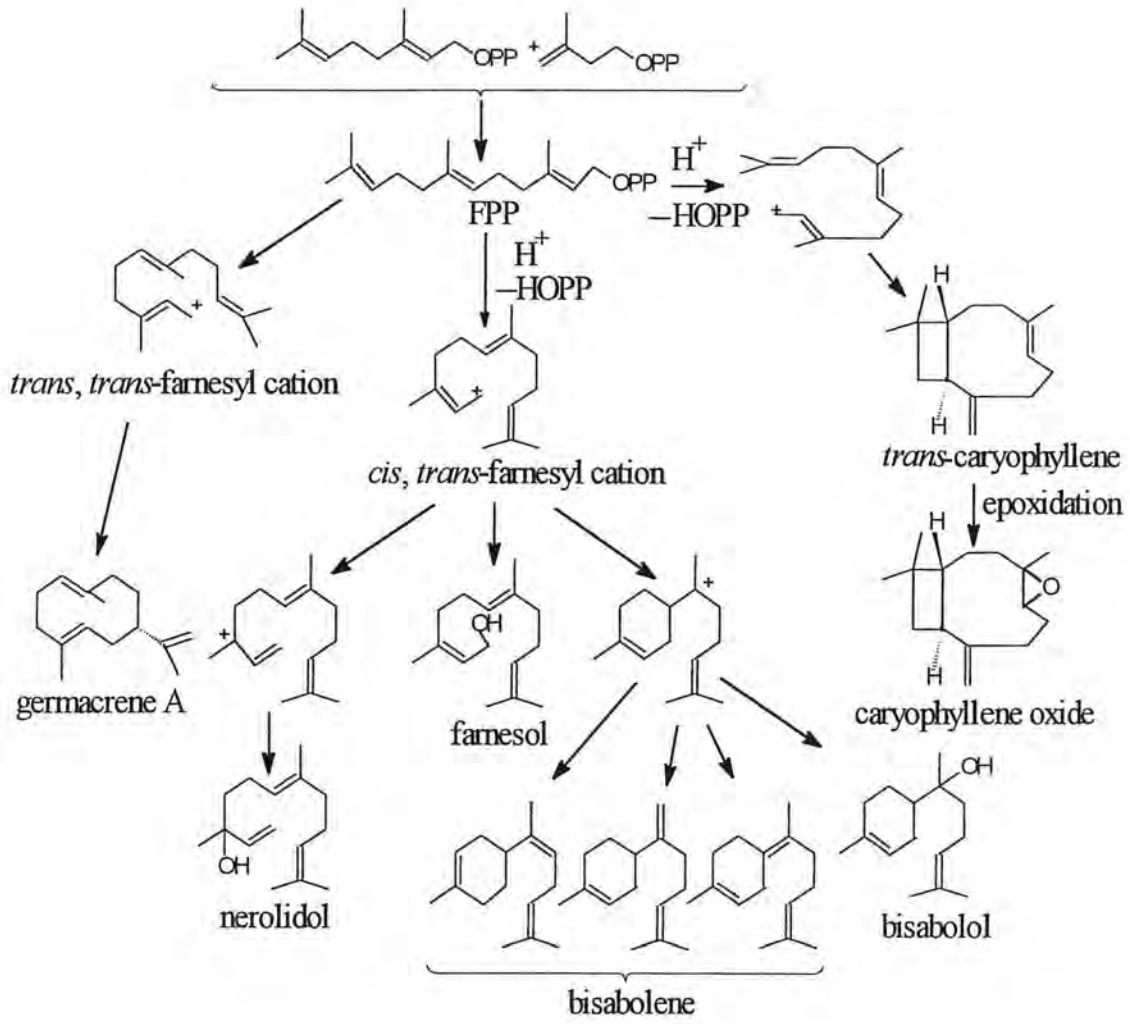
Components of the plant-derived essential oils are mainly mono and sesquiterpenoid compounds. Their biosynthesis has been subject to extensive research in the last century.

2.4 Biosynthetic pathway to monoterpenes and sesquiterpenes

Biosynthesis of terpenes is thought to begin from isopentyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP) units. The source of IPP is glucose, one of the products



Scheme 1. Biosynthesis of monoterpenes



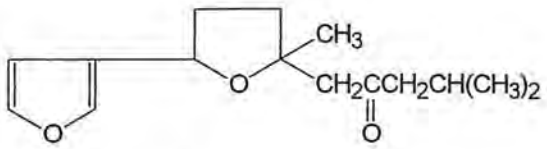
Scheme 2. Biosynthesis of sesquiterpenes

The role played by monoterpenes and sesquiterpenes in plant and insect defense is well understood and need no over-emphasis. Monoterpenes are plant products and well-known constituents of many essential oils. However, it is interesting to note that monoterpenes have also been found in animals. A good example of this is the isolation of citronellol from the scent glands of alligators. It has also been shown that, certain insects produce monoterpenes as components of their defensive secretions (Pridham, 1967).

The function of monoterpenoids and sesquiterpenoids in plants range from repellents to attractants for pollinators. They also act as allelopathic agents or defensive agents against competitors predators and pathogens. For instance, geese in a field containing peppermint will never feed on this plant (Thomson, 1994).

Lemon grass is one of the least attractive grasses to livestock in tropical Africa, and terpenes are clearly repellent to ruminants in south Australia. It has been suggested that trees under attack by insects or other leaf predators may produce certain monoterpenoids to stimulate other trees to resist the attack (Thomson, 1994). The best example is the production of a high concentration of 1,8-cineole by eucalyptus trees in Australian forests. High concentration of this monoterpene is said to stimulate other trees to resist attack by insects or other leaf predators. Plants may also produce chemicals that attract predators of the attacking pest (synomones).

Like monoterpenes, sesquiterpenes are also secondary metabolites produced by plants mainly for the purpose of defense. One of the best examples is that of the sweet potato (*Ipomea batatus*), whose roots respond to infection with black rot fungus, *Ceratocystis fimbriata*, by synthesizing comparatively large amounts of the sesquiterpene ipomeamarone (**46**) (Pridham, 1967).



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This sesquiterpene exhibits a potent anti-fungal action against the pathogen (Pridham, 1967; Uritani and Akazawa, 1959) and the amounts synthesized by various strains of the sweet potato reflect the degree of resistance of the strains.

The discovery of useful compounds such as mosquito repellent/insecticidal molecules from plants is coincidental since the mosquito may not be a pest of the concerned plant. However, the same compound(s) may have some similar role(s) against another target pest/vector.

2.5 Strategies in the search for anti-arthropod botanicals

It is generally recognized that there are 5 systematic approaches to choosing plants that may contain new biological agents:

- In *local random approach*, all available species are collected from a particular site regardless of any previous knowledge. This approach is however, not practical (Soejarto, 1993).
- *Ethnobotanical route*, gives credence to oral or written information on the indigenous medicinal or pesticidal use of the plant, and based on this information, the plant is collected for validation. This is a popular basis for selecting plants especially, in societies where traditional medicine practice is a major form of health care, like in the Indian, Chinese or African systems.
- In *taxonomic approach*, plants of pre-determined family or genera considered to be of interest are sought, from diverse locations.
- In *chemotaxonomical* (phytochemical) *approach*, a particular class of compounds like isoquinoline alkaloids is chosen for investigation, and different species of plants anticipated to produce related alkaloids are collected (Waterman, 1993).
- Lastly is *information managed approach*, where ethnobotanical, biological, and chemical information is collected on the basis of computerized data bases such as NAPRALERT (Natural Products Alert), Chemical Abstracts (CA) or Biological Abstracts (BA). The data is then prioritized to afford a list of plants for specific collection (Cordell *et al.*, 1993).

2.6 Plants

About 1200 plants have been listed in the literature as being of potential insecticidal value (Roark, 1947). Most remain uninvestigated either chemically or biologically. Many more plants used in traditional practices for preventing mosquito bites and hence malaria control remain uninvestigated and the bio-active compounds therein are therefore unknown. In the current research project, 33 plants have been investigated. Out of these, 6 very active new mosquito repellent plants, which also exhibited mosquitocidal effect, have been discovered. These plants belong to Compositae, Verbeneceae, and Labiatae families. They are described below.

2.6.1 The family compositae

It comprises of shrubs, herbs, trees and climbers. The active plants from this family were *Conyza newnii* Benth, and *Tarchonanthus camphoratus* Benth; both of which were collected from West Pokot and Naivasha, respectively.

2.6.1.1 *Conyza newii* (Plate II)

It is also known as Kisegeyo by the Shambaa (Kokwaro, 1993). The plant is a herb with thin leaves, dented on their margins and pointed apex. The leaf veins are branched, and the flowers yellow. The seeds are very light, and so get easily dispersed by wind. The leaves produce a pungent aroma and are chewed for chest troubles. The roots are used as an emetic by, boiling them and drinking the warm decoction (Kokwaro, 1993). This plant was collected in Kacheliba, West Pokot, Rift Valley province.

Plate II. *Conyza newii* **(with yellow flowers)**

The essential oil chemistry and mosquito repellency/insecticidal activity of this plant has not been reported in the literature. Neither has any compound with these properties been isolated from the plant.

2.6.1.2 *Tarchonanthus camphoratus* (Plate **III**)

It is known as Muririchwa (Kikuyu), and Oleleshwa (Maasai). Its common name in Eritrea is Sarakan. *T. camphoratus* is a bushy tree that is widespread in Africa. It is found in a range of altitudes; from coastal dunes, semi-desert to the edges of mountain forests.

In Eritrea, it is common in evergreen or semi-deciduous bush land and bushed grassland, especially on stoney soils. It occurs widely over the highlands, such as around Wogret, Rora-habab, Halhal, Seharti, Adi-keih, and Quatit, 1800-3000 m above sea level (Bein *et al.*, 1996). In Kenya it is widely distributed in Rift Valley area.

Plate III. flowering *Tarchonanthus camphoratus*

T. camphoratus is an aromatic shrub or small tree 1-9 m tall, the whole tree is silver-grey in appearance and all parts smelling strongly of camphor. The bark is brown-grey, longitudinally fissured, and peeling in long strips. The leaves are alternate, leathery, grey-green, above, felty pale grey-white-silver below, and strongly scented when crushed. The leaf has narrow oblong, usually 5-10 cm long. The leaf base is narrowed to a short stalk and the edge is sometimes toothed when young. The flowers have tiny florets, are tubular, cream-white or pale yellow, 4-5 mm, and are grouped into 3-5 flower heads, 1 cm across.

They are massed in branched pyramidal clusters 5-20 cm, all covered with white wholly hairs. The fruit has tiny nutlets covered with white woolly hairs; heads resemble balls of cotton wool, and are about 12 x 9 cm. (Bein *et al.*, 1996).

This plant is propagated by seedlings, cuttings, and wildings. It grows fairly fast and coppices well. It tends to be invasive in overgrazed areas. The wood will burn even when it is green. The heavy, hard timber has been used elsewhere to make furniture. The plant is used as firewood, fodder (leaves), hut construction (stems and branches). It is also used for soil conservation and as a windbreak. The Maasai use this plant for repelling mosquitoes.

The essential oil chemistry of this plant was reported by Mwangi *et al.* (1994). However, its mosquito repellent/insecticidal activity is not mentioned any where in the literature. No compound with mosquitocidal activity has been previously isolated from *T. camphoratus*.

2.6.2 The family verbenaceae

This family constitutes trees, shrubs, climbers, or herbs. The leaves of plants in this family are opposite or verticillate, exstipulate, entire or compound. Flowers are bisexual, zygomorphic, with a corolla tube and 4 or 5 lobes and 4 stamens. Plants produce a drupe type of fruit. The active plants under this family were from the genus *Lippia*.

The genus *Lippia* comprises of shrubs or woody herbs, with leaves that are opposite or verticillate and glandular. Flowers are in pedunculate, with crowded spikes. Corolla is obscurely 2-lipped, with 3 lobes. The fruit is of 2 dry mericarps; each 1-seeded and very small. *L. ukambensis* Vatke and *L. javanica* Vatke were collected from Naivasha and Nairobi respectively.

2.6.2.1 *Lippia javanica* (Plate **IV**)

Its local names are Ang'we rao, Mweny (Luo), Sulasula (Luhya), Muthiriti (Kikuyu), Kyulu or Mutithi (Kamba), Ol-sinoi (Maasai), Mwokyot (Kipsigis), Onyinkwa (Kisii), Mwokio (Marakwet), Chepngosoriet (Nandi), Sunoni (Samburu), and Orwo (Acholi).

This plant is a shrub, 0.5-3 m tall, with leaves that are opposite (rarely in threes), aromatic, ovate or elliptic, base cuneate, apex acute, and margin crenate.

The leaf is 2-8 by 0.6-3 cm, sand papery above and bubscent beneath. The flowers are white or cream with yellow throat, in short - pedunculed (rarely long-stalked), with crowded spikes 0.5-1 cm long and corolla tube of about 2 mm long.

The plant is locally abundant in secondary bush land or grassland, but less often in wooden grassland. It is a problem in range land pastures, and widely distributed in Loita highlands, Aberdare highlands, eastern highlands, Kitui, Kisii, Rift Valley, Machakos, Kajiado, Nairobi, (Beentje, 1994).

Plate IV. flowering *Lippia javanica*

An infusion of the leaves of this plant is given to patients with fever. Leaves and flowers are sniffed to clear a stuffy nose. The leaves and flowers are first rubbed between the hands to get the maximum scent emitted, and when sniffed, the subject is set sneezing; which then clears the nose. For the treatment of malaria, a decoction of boiled leaves is taken and the whole body bathed in the same fluid. Pounded leaves can also be applied on cut wounds, or soaked in water, and the juice drunk for the treatment of tapeworms and for indigestion (Kokwaro, 1993).

The essential oil chemistry of this plant has been reported (Guenther, 1949; Mwangi *et al.*, 1991b; Chagonda *et al.*, 2000). Repellent activity of the ethanol extract of *L. javanica* against *Anopheles arabiensis* was reported during the course of this project by Govere *et al.* (2000). However, there is no report on the activity of the plant oil against *Anopheles gambiae*.

2.6.2.2 *Lippia ukambensis* (Plate V)

Its locally known by the following names: Muthiethi, Muthirithi (Kamba), Muthiriti (Kikuyu, Meru), Mwokiot (Kipsigis), Mosonyon (Pokot), and Sinoni (Samburu). It is a shrub 0.5-3.6 m tall.

Plate V. *Lippia ukambensis* **(held)**

The leaves are opposite (rarely in threes), aromatic, ovate or elliptic, base cuneate, apex acute or obtuse, margin crenate, 2-12 by 0.8-4.5 cm, and sand papery on both sides. The flowers are white, with yellow throat, in long pedunculate crowded spikes 0.5-1.5 cm long: the corolla tube is 2-4 mm long, and the fruit red. Like *L. javanica*, *L. ukambensis* is also a problem in rangeland.

The leaves of *L. ukambensis* are sometimes used for tea. Its essential oil is a component of the Naturub®, an ointment used for relief from congestion, aches and insect bites. The chemical composition of the leaf oil of this plant has been reported (Mwangi *et al.*, 1991a). No work has however been reported on repellent/mosquitocidal activity of the leaf oil of the plant. Neither has any compound with either of these activities been reported in literature.

2.6.3 The family labiatae/lamiaceae

This family has herbs or shrubs that usually, but not always have rectangular stems. These herbs/shrubs have opposite leaves that are often aromatic. Their flowers are bisexual, with zygomorphic corolla that is 2-lipped. They have 2-4 stamens with the style emerging from the base of the ovary. The fruit produced has 4 small nut-lets, mostly hidden in the persistent calyx. The active plants from this family were *Plectranthus marrubioides* Benth, and *Tetradenia riparia* (*Iboza multiflora*) Benth. They were collected near the Baboon Cliff in Naivasha.

2.6.3. 1 *Plectranthus marrubioides* (Plate **VI**)

Its local names are: Barbarisa (Borana), Sali (Samburu), Dalol (Somali), Akurau, Nakhwara (Turkana). The plant is a creeper shrub, 0.3-3 m in length. Its slightly fleshy, succulent and often with arching branches which may clamber through other shrubs. It often flowers when leafless. The leaves are broadly elliptical to almost orbicular, succulent, base crenate, apex rounded or acute and the margin entire to crenate. The pubescent is about 1-3 by 1-2.5 cm long. Flowers are blue or violet, in racemes 4-22 cm long and the corolla is 10-19 mm long.
Its usually found in dry bush land or bushed grassland on rocky sites. The plant is propagated by stem cuttings. This plant is found in the Rift Valley, around Naivasha, Turkana, Marsabit and Samburu (Kokwaro, 1993). Mwangi *et al.* (1986) reported the essential oil chemistry of this plant. The mosquito repellency and adulticidal activities have not been mentioned any where in the literature. The compounds responsible for the activities have not been isolated before.

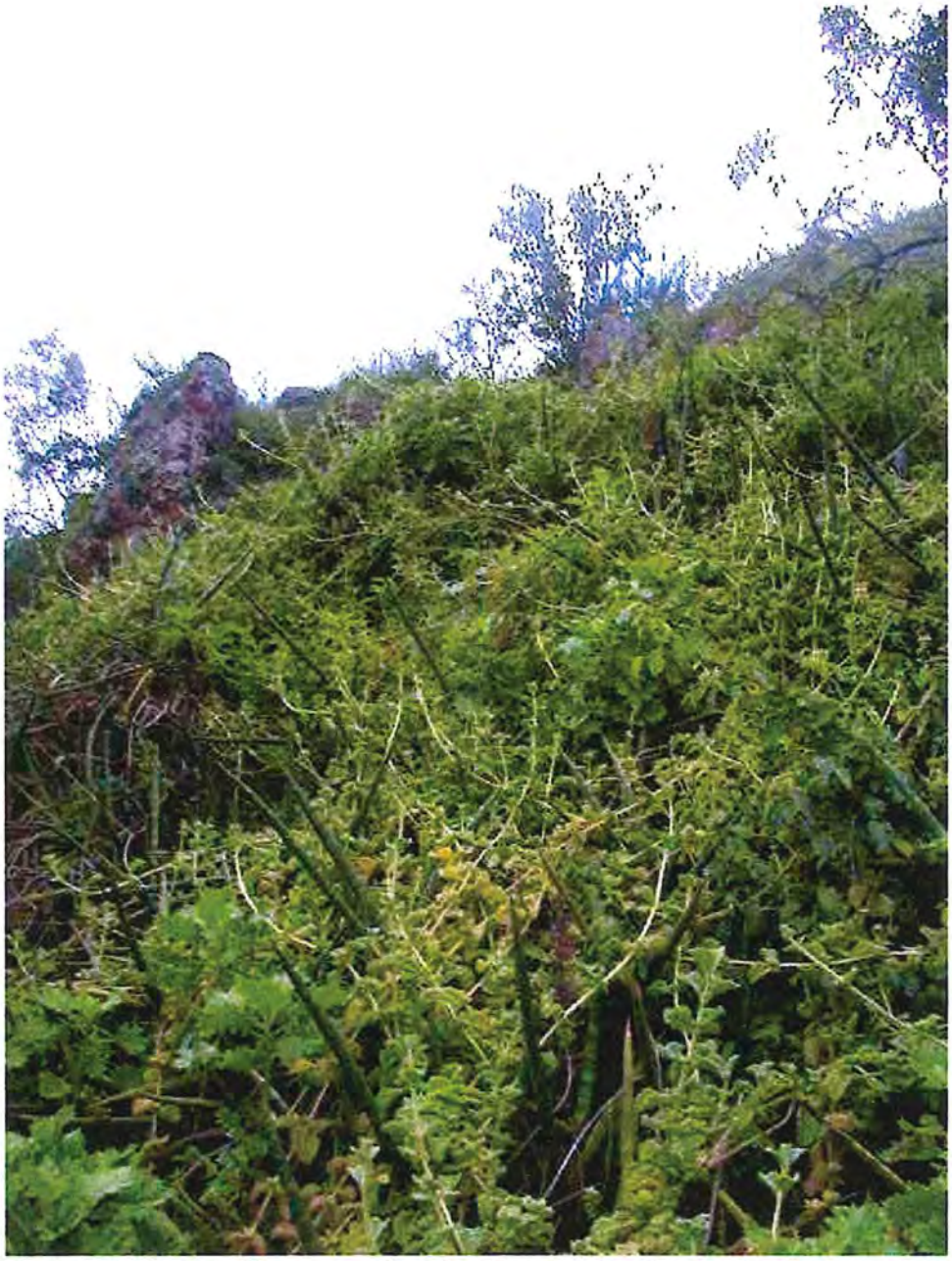


Plate VI. *Plectranthus marrubioides* **(clambering through other shrubs)**

2.6.3.2 *Tetradenia riparia* (*Iboza multiflora*) (Plate **VII**)

In Tanzania, the plant is locally known as Fukufuku (Nyika), Lilaaku (Lunyore), Mshunshu (district de Baroka) and Mwache (Pare), while in Kenya, Honwa (Marakwet), Maraka (Meru) and Okita Dala (Luo). This plant is usually common in rocky areas, and specifically on the cliffs. It has weak stem and grows up to about 3-5 m in height. The leaves are broad and elliptical, non-succulent, aromatic with rough margin and branched veins. The plant flowers when leafy.

Plate VII. flowering *Tetradenia riparia*

The leaves are used to treat stomach problems and are known to attract banana weevil. The leaves, together with the bark of *Parkia filicoidea* or fruit of *Capsicum annuur* are used to treat diseases of chicken. The Chaggas around Mt. Kilimanjaro use it as live fence, and its leaves are fed to cattle as an anti-helminthic. The roots are believed to have anti-bilharzia activity. The chewed decoction of roots is also used to manage rhumatism and pneumonia (http://pc4.sisc.ucl.ac.be; Kokwaro, 1993). The essential oil has anti-malarial activity (Campbell *et al.*, 1997).

Essential oil chemistry of *T. riparia* has been reported (Campbell *et al.*, 1997). However, there is no work done on the mosquito repellent and adulticidal activities.

CHAPTER 3: BIO-PROSPECTING

3.1 Plants collected

During the bio-prospecting exercise, a total of 33 plants were collected from different parts of Nyanza, Western, Rift Valley, and Central provinces. Essential oils from 16 plants were bio-assayed, while 17 plants did not have enough oil. The residue from steam distillation of the plants was extracted with chloroform to check if there were any polar residual insecticidal principles that could not be extracted by steam distillation. All these were bio-assayed for insecticidal and repellent activity. The water extracts were also bio-assayed.

3.2 Preliminary repellency assays

Whereas no water extract had high activity, only three of the 33 chloroform extracts had some repellency at 0.05 g/ml (*Tetradenia riparia*, 85%, *Conyza newii*, 83% and *Hyptis pectinata*, 81%), while the essential oils of 8 out of 16 plants had a protective efficacy of 90% and above at a concentration of 0.1 g/ml (10% solution). Although *Schinus molle* had the highest yield of essential oils, its repellency at 10 and 0.1% concentration (58 and 27%, respectively) showed that it was the poorest of all the 16 plants. *Conyza newii* oil was the best repellent with 100% and 46% repellency at 10 and 0.1% concentration respectively. The repellency for the rest of the plants ranged from 65 - 95% at 10% and 32 - 64% at 0.1%.

The oils and chloroform extracts of the 16 plants were also tested for their insecticidal activity by fumigation and tarsal contact assays.

3.3 Preliminary insecticidal assays

The essential oils of seven plants exhibited insecticidal activity by fumigation. The insecticidal times (T_{i50} and T_{i100}) ranged from 1.33 – 24 and 1.67 - 24 hrs, respectively. The knock down time (T_{KD100}) ranged from 10 mins to 1.5 hrs. Among the seven plants, *Conyza newii* and *Plectranthus marrubioides* had the highest insecticidal activity, with T_{i100} values of 1.67 hrs for both. *Ocimum lamiifolia* was the slowest in insecticidal action with T_{i100} of 24 hrs. *Lippia javanica* had the shortest knock down time (T_{KD100} 10 mins), whereas *Lippia ukambensis* had the longest knock down time (T_{KD100} 1.5 hrs). The results are summarised in table 1.

Table 1. Mosquitocidal activity data of 0.1g/ml solution of plant essential oils.

Plant Oil	T_{i50}	T_{i100}	T_{KD100}
Tarchonanthus camphoratus.	80 min	140 min	80 min
Lippia javanica	90 min	140 min	10 min
Plectranthus marrubioides	80 min	100 min	30 min
Tetradenia riparia	90 min	130 min	80 min
Lippia ukambensis	40 min	160 min	90 min
Conyza newii	80 min	100 min	60 min
Ocimum lamiifolia	90 min	24 hours	-
Croton dichogamus (M)	24 hours	-	-
Croton dichogamus (R)	24 hours	-	-
Bidens pilosa	-	-	-
Schinus molle	-	-	-
Lantana camara	-	-	-
Teclea simplisifolia	-	-	-
Helicrysum spp	-	-	-
Hyptis pectinata	-	-	-
Psidia punctulata	-	-	-

The extracts were also evaluated using tarsal contact bio-assay. However, none of the extracts from the 33 plants showed mosquitocidal activity.

The repellency and insecticidal bio-assay results revealed six plants (*Conyza newii*, *Lippia javanica*, *L. ukambensis*, *Plectranthus marrubioides*, *Iboza multiflora*, and *Tarchonanthus camphoratus*) whose essential oils had both mosquito repellent and insecticidal activities. These plants were, therefore, subjected to further biological assay and chemical investigation.

3.4 Detailed mosquito repellency assay of active plants oils

The detailed repellency bio-assay results of the essential oils from the six plants confirmed the superiority of *C. newii* with a PE (protective efficacy) range of 45 - 100% between a concentration range of 10^{-5} and 10^{-2} g/ml (Fig. 2). This was followed by *P. marrubioides* with PE of 41 - 100% (Fig. 3), *Tetradenia riparia* 35 - 88% (Fig.4), *L. ukambensis* 34 - 84% (Fig. 5), *L. javanica* 29 - 97% (Fig. 6) and *T. camphoratus* 25 - 100% (Fig. 7). *C. newii, L. javanica* and *P. marrubioides* had PE of 100, 89 and 88% PE at 10^{-2} g/ml whereas *T. camphoratus*, *L. ukambensis* and *Tetradenia riparia* had 64, 62 and 60%, respectively, at the same concentration.

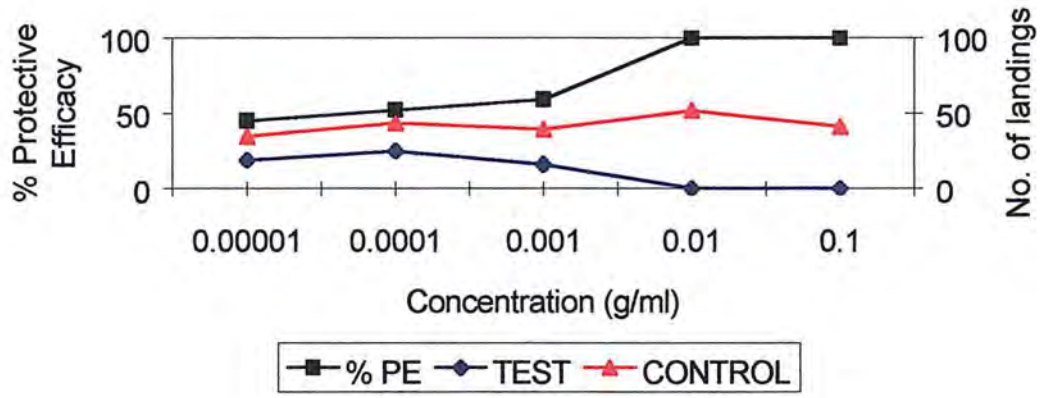


Fig. 2. Repellency assay of *Conyza newii* oil

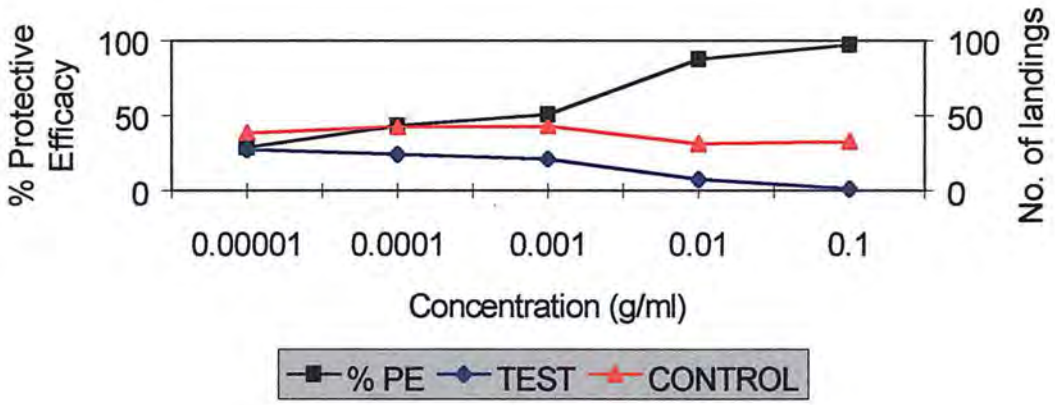


Fig. 3. Repellency assay of *Lippia javanica* oil

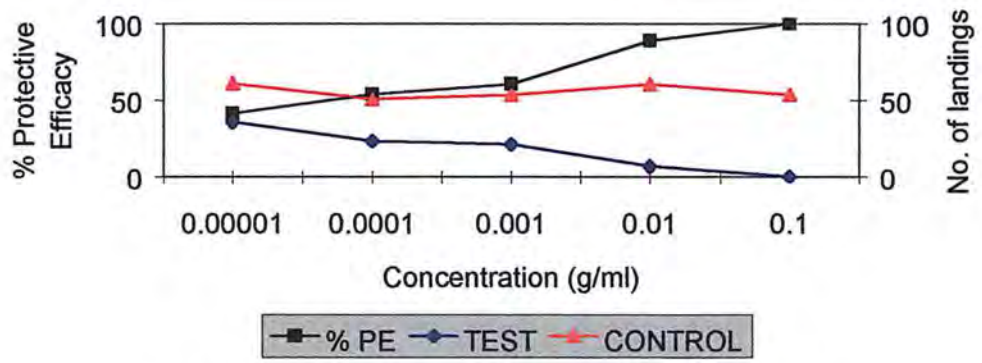


Fig. 4. Repellency assay of *Plectranthus marrubioides* oil

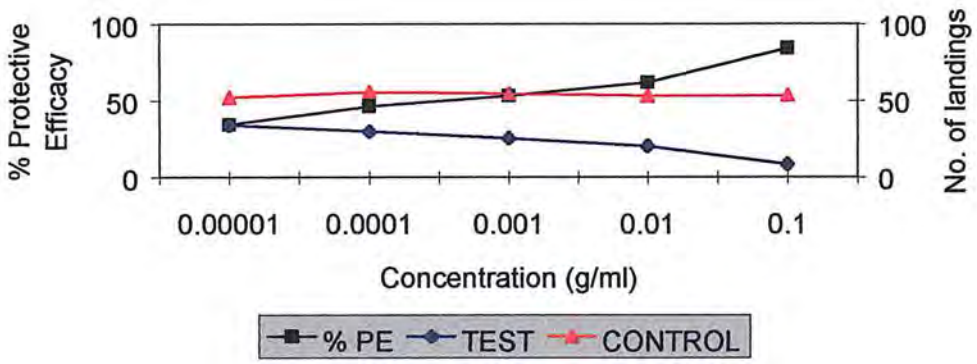


Fig. 5. Repellency assay of *Lippia ukambensis* oil

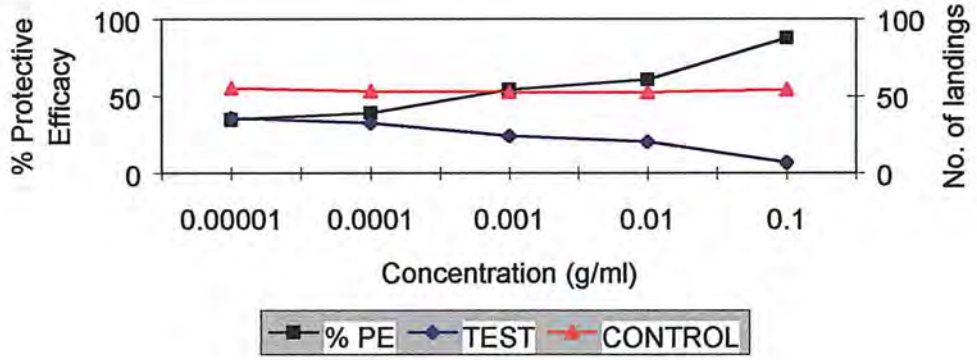


Fig. 6. Repellency assay of *Tetradenia riparia* oil

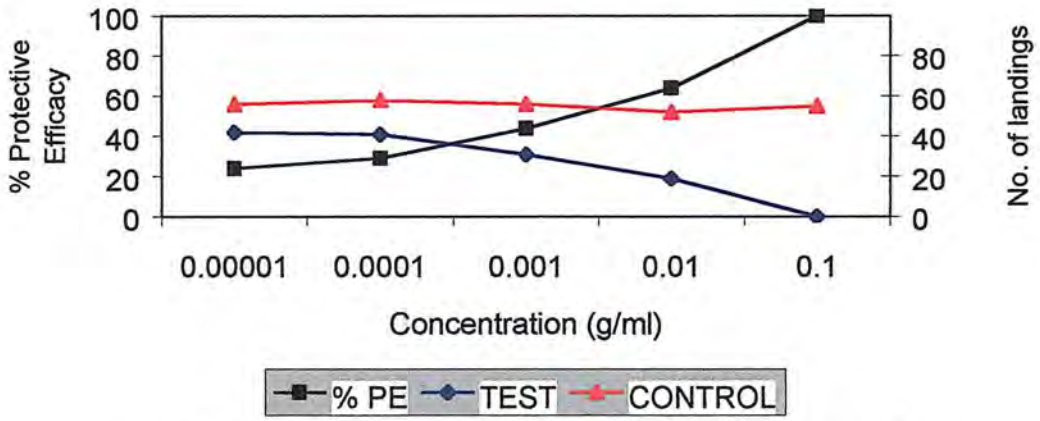


Fig. 7. Repellency assay of *Tarchonanthus camphoratus* oil

From the bio-assay data obtained, probit analyses (Busvine, 1971) were done to calculate the RD_{25}, RD_{50} values, and the variances, v of m, the estimated log. RD_{50}. The RD_{50} values obtained for the essential oils of the six repellent/insecticidal plants were 9×10^{-5}, 9×10^{-5}, 3×10^{-4}, 4×10^{-4}, 5×10^{-4} and 2×10^{-3} mg/cm^2 for *C. newii*, *P. marrubioides*, *L. javanica*, *L. ukambensis*, *T. riparia* and *T. camphoratus*, respectively (Table 2).

Table 2. RD values for essential oils of the six plants

Plant Oil	**RD_{25} (mg/cm^2)**	**RD_{50} (mg/cm^2)**
Conyza newii	2.6367×10^{-8}	8.86×10^{-5}
Plectranthus marrubioides	2.9835×10^{-6}	8.85×10^{-5}
Lippia javanica	2.192×10^{-5}	2.618×10^{-4}
Lippia ukambensis	3.5342×10^{-6}	4.32×10^{-4}
Tetradenia riparia	7.7964×10^{-6}	5.04×10^{-4}
Tarchonanthus camphoratus	2.9712×10^{-5}	2.408×10^{-3}

The results confirmed the order of repellency as *C. newii* > *P. marrubioides* > *L. javanica* > *L. ukambensis* > *T. riparia* > *T. camphoratus*.

3.5 Detailed mosquitocidal assays

The detailed fumigation mosquitocidal bio-assays were done for all the six plant oils and the results examined by probit analysis. The LD_{25}, LD_{50}, and LD_{75} values, and the variances, v of m, the estimated log. LD_{50} were obtained. Again, the order of insecticidal activity (LD_{50}) was found to be *C. newii* >, *P. marrubioides* > *T. camphoratus* > *L. javanica* > *T. riparia* > *L. ukambensis* (Table 3).

Table 3. LD values for the essential oils of the six plants

Plant Oil	**LD_{25} (mg/cm^3)**	**LD_{50} (mg/cm^3)**	**LD_{75} (mg/cm^3)**
Conyza newii	1.042×10^{-3}	1.965×10^{-3}	3.711×10^{-3}
Lippia javanica	2.429×10^{-3}	4.338×10^{-3}	7.746×10^{-3}
Plectranthus marrubioides	1.204×10^{-3}	2.809×10^{-3}	6.542×10^{-3}
Lippia ukambensis	2.690×10^{-3}	4.655×10^{-3}	8.063×10^{-3}
Tetradenia riparia	2.563×10^{-3}	4.429×10^{-3}	7.655×10^{-3}
Tarchonanthus camphoratus	2.218×10^{-3}	3.788×10^{-3}	6.465×10^{-3}

From the detailed bio-assays results, further work was deemed necessary on the chemical composition and the bio-active constituents of the essential oils.

CHAPTER 4: ESSENTIAL OIL COMPOSITION

4.1 Chemical composition of the repellent plants

The essential oil chemistry of *T. riparia, P. marrubioides, Lippia javanica, Lippia ukambensis*, and *Tarchonanthus camphoratus* has been done, (Campbell *et al.*, 1997; Mwangi *et al.*, 1994; 1991a; 1991b; 1986). During the course of our investigations, mosquito repellency of ethanol extract of *L. javanica* was reported against *An. arabiensis* (Govere *et al.*, 2000). However, no work has been done on the mosquito repellency and mosquitocidal activity of the oils of these plants. To our knowledge, the essential oil chemistry of *Conyza newii* has not been reported anywhere in the chemical literature. The bio-active compounds in the oils of these six plants remain uninvestigated as far as mosquito repellency/mosquitocidal activity is concerned.

A total of 62 compounds were identified in the essential oils from the six plants. In all the GC profiles, the numbered peaks represent the following compounds:

1. α-thujene
2. α-phellandrene
3. *trans*-β-ocimene
4. α-pinene
5. camphene
6. β-phellandrene
7. sabinene
8. β-pinene
9. α-fenchyl alcohol
10. β-myrcene
11. δ-2-carene
12. α-terpinene
13. terpinolene
14. *p*-cymene
15. limonene
16. 1,8-cineole
17. γ-terpinene
18. fenchone
19. myrcenol
20. *trans*-sabinene hydrate
21. linalool
22. thujone
23. camphor
24. terpen-4-ol
25. borneol
26. limonene oxide
27. *cis-p*-menth-3-en-1-ol
28. α-terpineol
29. neral
30. fenchyl acetate
31. verbenone
32. verbenol
33. cuminal
34. carvacrol
35. thymol
36. limonene dioxide
37. myrtenyl acetate
38. peril aldehyde
39. peril alcohol
40. carvone
41. eugenol
42. solalone
43. ylangene
44. germacrene B
45. α-cubebene
46. methyl eugenol
47. α-copaene
48. β-bourbonene
49. α-gurjunene
50. β-caryophyllene
51. α-caryophyllene
52. alloaromadendrene
53. aromadendrene
54. germacrene D
55. β-selinene
56. α-farnesene
57. γ-cadinene
58. δ-cadinene
59. α-amorphene
60. caryophyllene oxide
61. spathulenol
62. α-bisabolol

There are some many other small peaks, which were identified but can not be seen clearly on these GC profiles.

4.1.1 *Conyza newii*

Fifty (50) compounds were identified in the leaf oil of *C. newii* by GC, GC-MS and GC co-injection (CO) with authentic standards. The major components of the oil were perillaldehyde (29.28%), limonene (10.06%), 2-methyl-5-(methylethyl)-2-cyclohexen-1-ol (7.34%), 1,8-cineole (6.84%), perill alcohol (4.27%), germacrene B (1.45%), *trans*-β-ocimene (1.35%), geraniol (1.17%), β-myrcene (1.16%) and α-amorphene (1.11%). Several other monoterpenoid and sesquiterpenoid compounds were identified (Table 4) but these were present in amounts < 1%. Twenty-three compounds were present in trace (t) amounts (< 0.1%), six compounds in 0.1 - 0.2%, four compounds in 0.2 - 0.4%, two compounds in 0.4 - 0.6%, three compounds in 0.6 - 0.8% and two in 0.8 - 1% (Table 4). Out of the identified compounds, 56% were oxygenated terpenes, which constituted most of the major components of the leaf oil.

Table 4. Chemical composition of the essential oil of *C. newii* leaves

Compound	% Area	Identification	Compound	% Area	Identification
α-pinene	0.35	GC-MS, CO	*trans*-β-ocimene	1.35	GC-MS, CO
camphene	0.13	GC-MS, CO	1,3,8-p-menthatriene	t	GC-MS
β-eudesmol	t	GC-MS, CO	*trans*-sabinene hydrate	t	GC-MS, CO
β-pinene	0.18	GC-MS, CO	perillaldehyde	29.28	GC-MS, CO
β-myrcene	1.16	GC-MS, CO	peill alcohol	4.27	GC-MS, CO
neral	t	GC-MS, CO	myrtenyl acetate	0.12	GC-MS, CO
δ-2-carene	t	GC-MS, CO	geranyl acetate	0.69	GC-MS, CO
myrtenol	t	GC-MS, CO	α-fenchyl alcohol	0.21	GC-MS, CO
δ-4-carene	t	GC-MS, CO	*cis*-2-pinanol	t	GC-MS, CO
limonene	10.06	GC-MS, CO	α-terpinolene	t	GC-MS, CO
1,8-cineole	6.84	GC-MS, CO	α-caryophyllene	0.56	GC-MS, CO
α-terpinene	t	GC-MS, CO	germacrene B	1.45	GC-MS, CO
carvone	t	GC-MS, CO	germacrene D	0.65	GC-MS
linalool	0.11	GC-MS, CO	isocaryophyllene	0.58	GC-MS, CO
p-cymene	t	GC-MS, CO	α-amorphene	1.11	GC-MS, CO
γ-terpinene	t	GC-MS, CO	*cis*-sabinene hydrate	t	GC-MS, CO
α-fenchene	t	GC-MS, CO	methyleugenol	0.37	GC-MS, CO
geraniol	1.17	GC-MS, CO	phenylethyl alcohol	t	GC-MS, CO
α-copaene	0.27	GC-MS, CO	monoterpene alcohol	0.88	GC-MS, CO
α-cadinol	t	GC-MS, CO	limonenyl-10-acetate	0.89	GC-MS
γ-curcumene	t	GC-MS	β-phellandrene	t	GC-MS, CO
camphor	0.17	GC-MS, CO	artemisia ketone	0.12	GC-MS
α-terpineol	t	GC-MS, CO	spathulenol	0.19	GC-MS, CO
borneol	t	GC-MS, CO	4-isopropylbenzaldehyde	0.78	GC-MS, CO
ylangene	t	GC-MS	2-methyl-5-(methylethyl)-2-cyclohexen-1-ol.	7.34	GC-MS

The GC profile of the plant's leaf oil is shown in figure 8a.

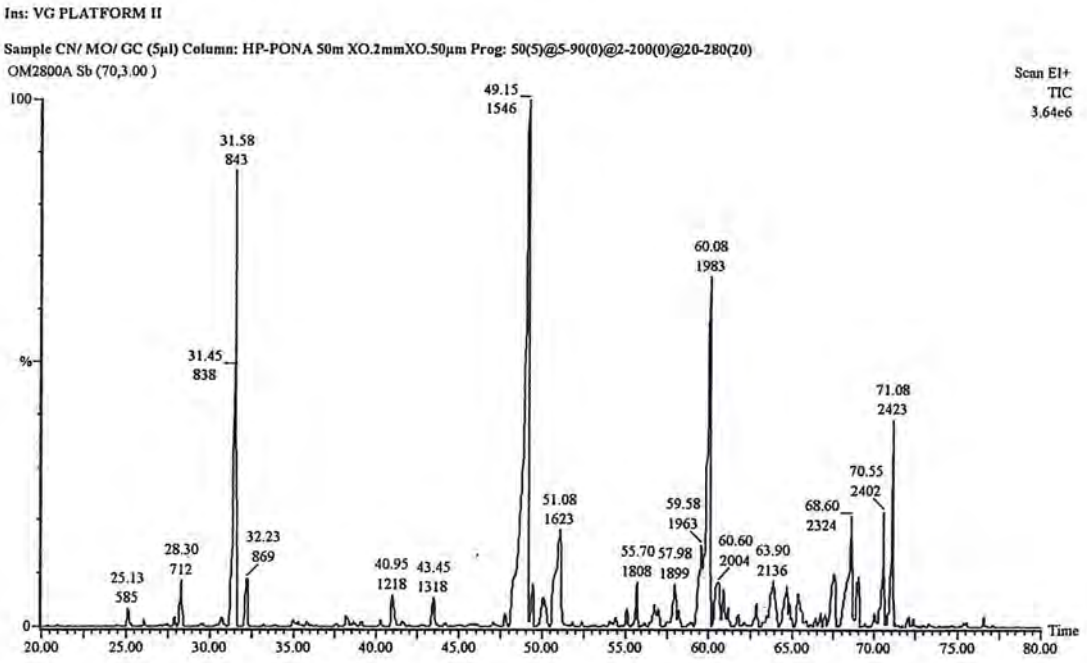


Fig. 8a The GC profile of *Conyza newii* oil

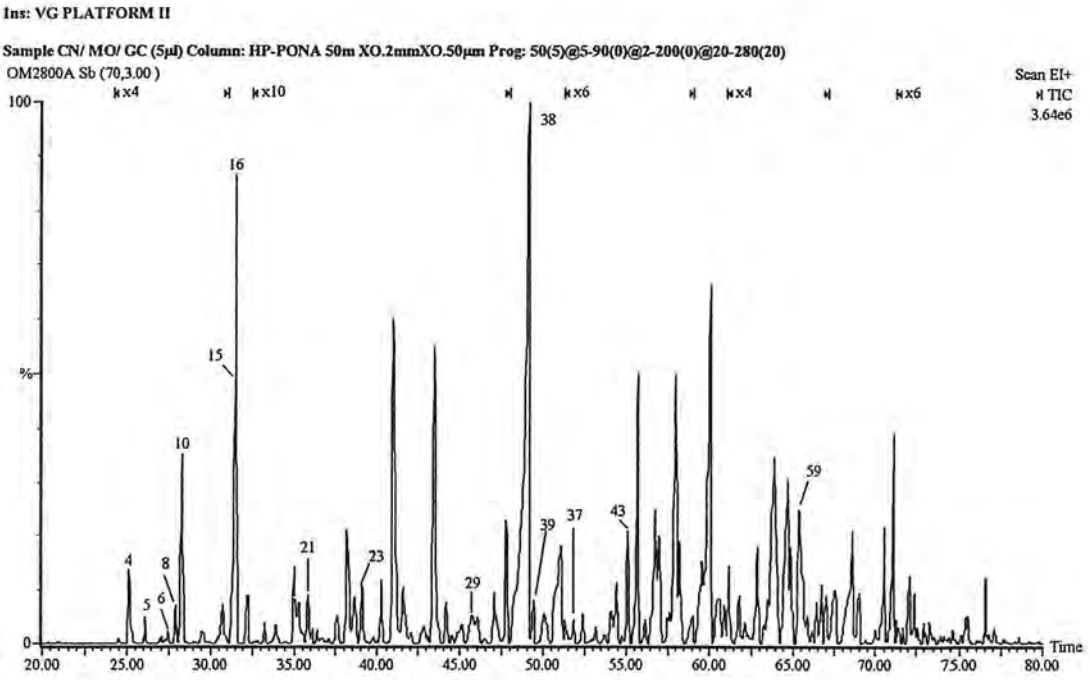


Fig. 8b The magnified GC profile of *Conyza newii* oil

4.1.2 *Tetradenia riparia*

A total of 38 compounds were identified from the leaf oil of this plant, by the same methods used for *C. newnii*. Fenchone (64.82%), limonene (2.02%), 1,8-cineole (1.50%) and *trans*-β-ocimene (1%) were the main constituents of the leaf oil of this plant. Other monoterpenes and sesquiterpenes identified (Table 5) were present in amounts less < 1%. Eighteen compounds were present in trace amounts (< 0.1%), four compounds in the range 0.1 - 0.2%, three compounds in 0.2 - 0.4%, six compounds in 0.4 - 0.6%, two compounds in 0.6 - 0.8% and another two in 0.8 - 1% (Table 5). Campbell *et al.*, (1997) reported slightly different results in which, apart from fenchone (13.6%), other main constituents were α-terpineol (22.6%), β-fenchyl alcohol (10.7%), β-caryophyllene (7.9%) and perill alcohol (6.0%).

Table 5. Chemical composition of the essential oil of *T. riparia* (*I. maltiflora*) leaves

Compound	% Area	Identification	Compound	% Area	Identification
α-pinene	t	GC-MS, CO	β-phellandrene	0.49	GC-MS, CO
camphene	t	GC-MS, CO	α- phellandrene	0.49	GC-MS, CO
β-pinene	0.78	GC-MS, CO	*trans*-β-ocimene	1.00	GC-MS, CO
β-myrcene	0.80	GC-MS, CO	*trans*-sabinene hydrate	t	GC-MS, CO
δ-2-carene	t	GC-MS, CO	terpen-4-ol	t	GC-MS, CO
limonene	2.02	GC-MS, CO	pinocarvone	t	GC-MS
δ-3-carene	t	GC-MS, CO	α-fenchyl alcohol	0.73	GC-MS, CO
1,8-cineole	1.50	GC-MS, CO	*cis*-verbenol	t	GC-MS, CO
δ-4-carene	t	GC-MS, CO	fenchone	64.82	GC-MS, CO
α-terpinene	t	GC-MS, CO	α-pyronene	0.42	GC-MS
p-cymene	0.55	GC-MS, CO	thujone	t	GC-MS, CO
γ-terpinene	0.96	GC-MS, CO	*p*-menth-3-en-1-ol	0.37	GC-MS
camphor	0.13	GC-MS, CO	α-fenchyl acetate	0.39	GC-MS, CO
α-terpineol	t	GC-MS, CO	p-menth-2-en-1-ol	t	GC-MS
α-copaene	0.13	GC-MS, CO	germacrene B	0.13	GC-MS, CO
α-cubebene	t	GC-MS, CO	germacrene D	0.59	GC-MS
α-fenchene	t	GC-MS, CO	isocaryophyllene	0.1	GC-MS, CO
α-cadinol	t	GC-MS, CO	β-bourbonene	0.43	GC-MS, CO
γ-curcumene	t	GC-MS	borneol	t	GC-MS, CO

Figure 9a shows a typical GC profile for the leaf oil of *T. riparia* (*I. Multiflora*).

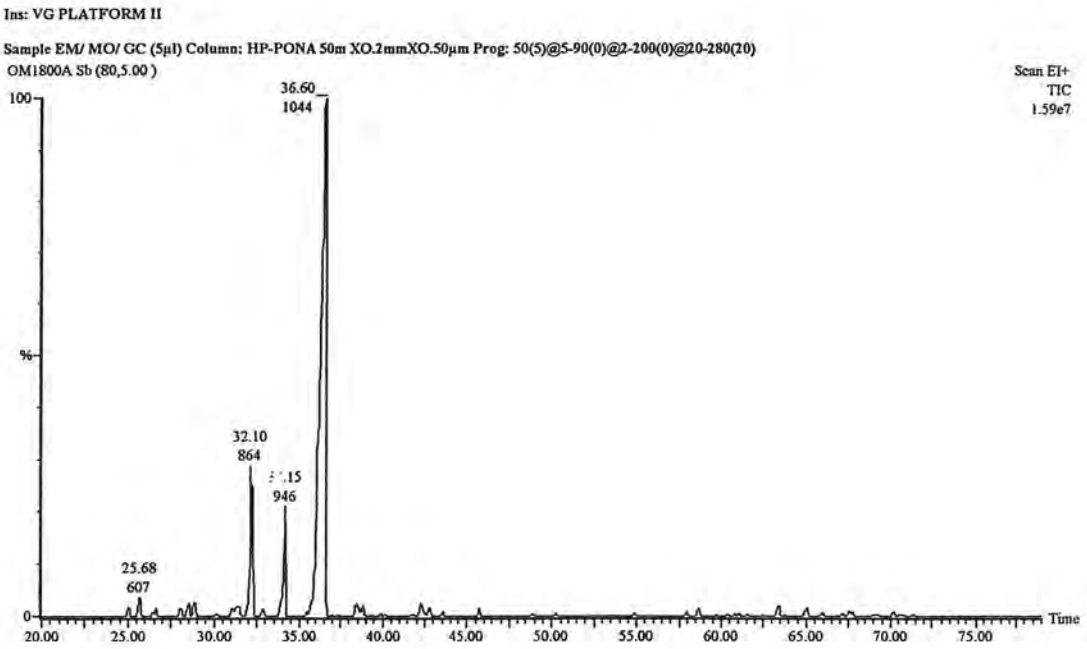


Fig. 9a The GC profile of *Iboza multiflora* oil

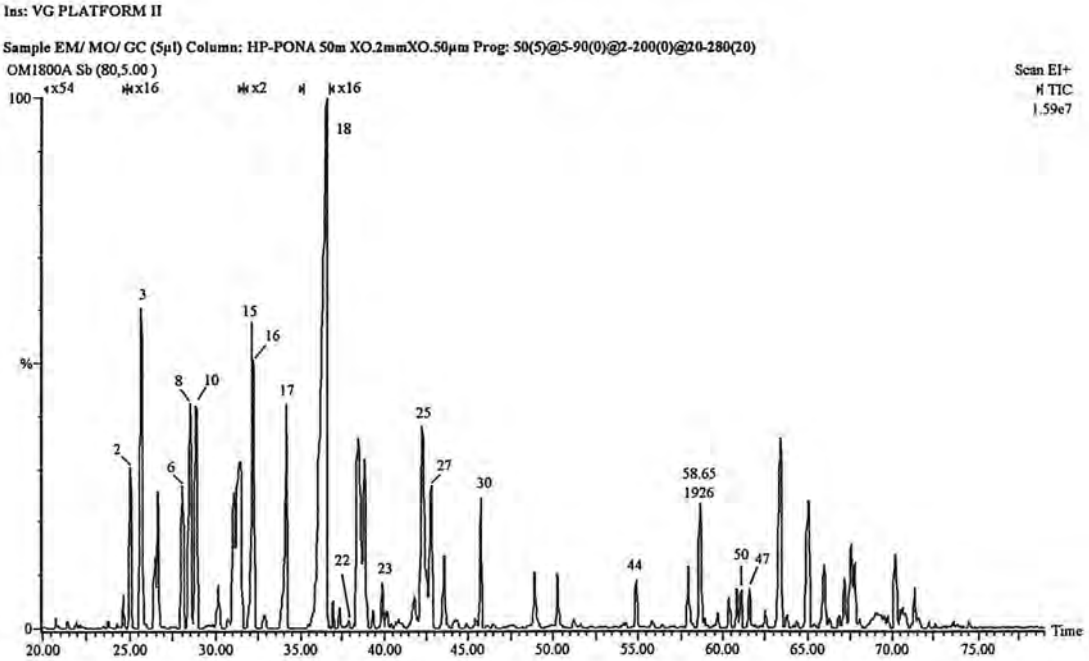


Fig. 9b The magnified GC profile of *Iboza multiflora* oil

4.1.3 *Plectranthus marrubioides*

The essential oil from *P. marrubioides* was analysed by GC, GC-MS and GC co-injection with the known standards. A total of 70 compounds accounting for 87.17% of the oil were identified. Camphor (48.80%), 1,8-cineole (9.0%), *p*-cymene (3.08%), α-terpinene (2.58%), limonene dioxide (2.5%), fenchone (1.75%), isocaryophyllene (1.67%), viridiflorol (1.61%), camphene (1.58%), β-selinene (1.50%), *trans*-sabinene hydrate (1.19%), caryophyllene oxide (1.13%) and terpen-4-ol (1.08%) were the main components. Minor constituents included 27 compounds in trace amounts (< 0.1%), 14 compounds in the range 0.1 - 0.2%, 10 compounds in 0.2 - 0.4%, 4 compounds in 0.4 - 0.6% and 3 compounds in 0.6 - 1% (Table 6). Camphor had already been reported as the major constituent of the leaf oil of *P. marrubioides* by Mwangi *et al.* (1986).

Table 6. Chemical composition of the essential oil of *P. marrubioides* leaves

Compound	% Area	Identification	Compound	% Area	Identification
α-pinene	0.17	GC-MS, CO	β-phellandrene	0.15	GC-MS, CO
camphene	1.58	GC-MS, CO	α- caryophyllene	0.30	GC-MS, CO
β-pinene	0.81	GC-MS, CO	*trans*-β-ocimene	0.14	GC-MS, CO
β-myrcene	0.16	GC-MS, CO	*trans*-sabinene hydrate	1.19	GC-MS, CO
δ-2-carene	t	GC-MS, CO	terpen-4-ol	1.08	GC-MS, CO
limonene	t	GC-MS, CO	pinocarvone	t	GC-MS
carvone	t	GC-MS, CO	myrtenol	t	GC-MS, CO
1,8-cineole	9.00	GC-MS, CO	piperitone oxide	t	GC-MS, CO
δ-4-carene	t	GC-MS, CO	fenchone	1.75	GC-MS, CO
α-terpinene	2.58	GC-MS, CO	α-campholene aldehyde	t	GC-MS
p-cymene	3.08	GC-MS, CO	*cis*-carveol	0.20	GC-MS, CO
γ-terpinene	0.96	GC-MS, CO	*p*-menth-2-en-1-ol	t	GC-MS
camphor	48.80	GC-MS, CO	*cis*-sabinene hydrate	t	GC-MS, CO
α-terpineol	0.38	GC-MS, CO	linalyl propanoate	0.55	GC-MS
α-copaene	0.12	GC-MS, CO	limonene dioxide	2.50	GC-MS, CO
α-cubebene	t	GC-MS, CO	germacrene D	0.15	GC-MS
α-fenchene	0.10	GC-MS, CO	isocaryophyllene	1.67	GC-MS, CO
α-selinene	0.21	GC-MS, CO	α-terpinolene	0.27	GC-MS, CO
γ-curcumene	t	GC-MS	borneol	0.36	GC-MS, CO
linalool	t	GC-MS, CO	1,3,8-p-menthatriene	t	GC-MS
thujol	t	GC-MS, CO	linalool oxide	t	GC-MS
carvacrol	0.10	GC-MS, CO	phellandral	t	GC-MS
sabinol	t	GC-MS	camphene hydrate	t	GC-MS
solalone	t	GC-MS, CO	*cis*-2-pinanol	t	GC-MS, CO
α-amorphene	t	GC-MS, CO	cycloisosativene	t	GC-MS
β-eudesmol	t	GC-MS, CO	spathulenol	t	GC-MS, CO
eugenol	t	GC-MS, CO	isospathulenol	0.50	GC-MS
β-selinene	1.50	GC-MS, CO	*trans*-chrysanthemal	t	GC-MS
β-elemene	0.44	GC-MS	α-gurjunene	0.17	GC-MS, CO
viridiflorol	1.61	GC-MS	4-isopropylbenzaldehyde	t	GC-MS, CO
δ-cadinene	0.20	GC-MS, CO	ascaridol	0.3	GC-MS, CO
thymol	0.12	GC-MS, CO	alloaromadendrene	0.11	GC-MS, CO
ledol	0.20	GC-MS	caryophyllene oxide	1.13	GC-MS, CO
γ-cadinene	0.33	GC-MS, CO	isoascaridol	t	GC-MS
p-cymen-8-ol	0.36	GC-MS, CO	*p*-mentha-1,8-dien-2-ol	t	GC-MS

The GC profile of the leaf oil is shown in figure 10a.

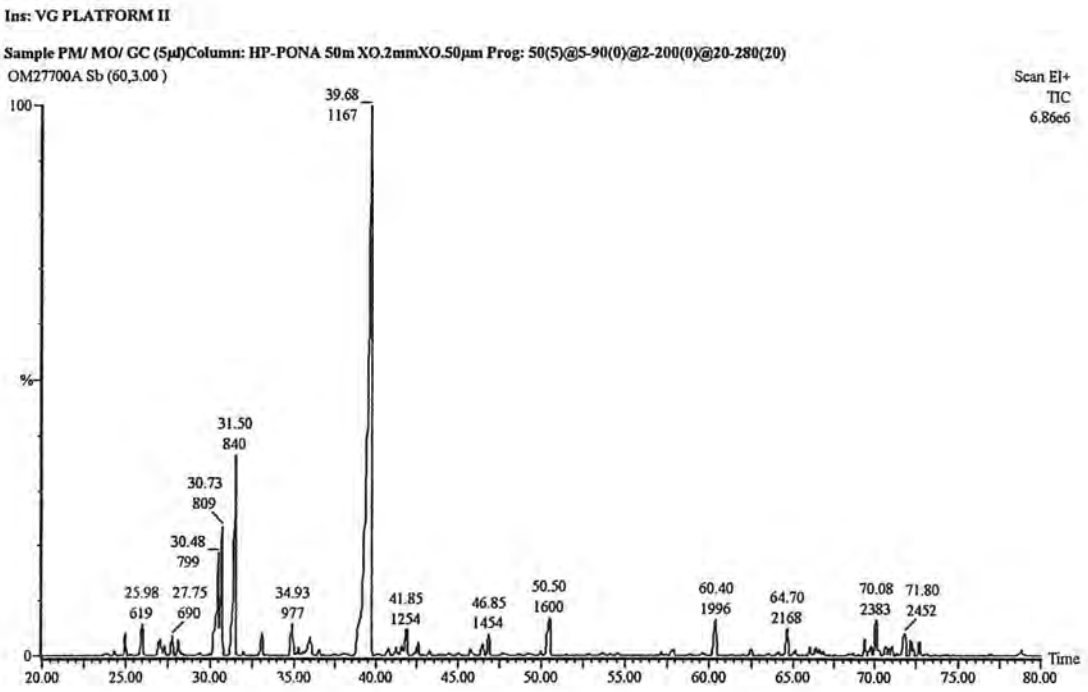


Fig. 10a The GC profile of *Plectranthus marrubioides* oil

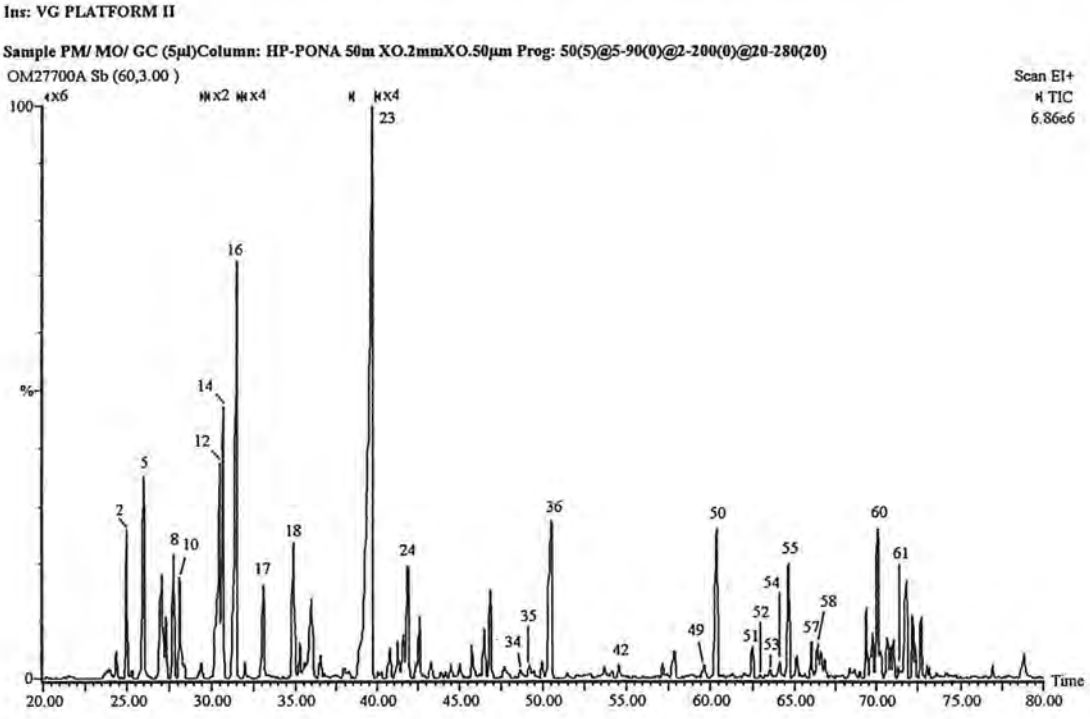


Fig. 10b The magnified GC profile of *Plectranthus marrubioides* oil

4.1.4 *Lippia ukambensis*

From the leaf oil of this plant, a total of 37 compounds that constituted 84% of the oil were identified. The main constituents of the leaf oil were camphor (39.84%), *trans*-sabinene hydrate (14.21%), camphene (8.63%), 1,8-cineole (2.42%), β-myrcene (1.69%), germacrene D (1.45%), γ-terpinene (1.42%), *p*-menth-3-en-1-ol (1.39%), α-phellandrene (1.28%), β-phellandrene (1.21%), borneol (1.14%) and α-pinene (1.13%). Minor components included 11 compounds present in trace amounts (< 0.1%), 2 compounds in the range 0.1 - 0.2%, 4 compounds in 0.2 - 0.4%, 3 compounds in 0.4 - 0.6%, another 3 in 0.6 - 0.8% and 4 compounds in 0.8 - 1.0% range (Table 7). The results reported in this thesis are similar to what was found by Mwangi *et al.* (1991a) on the chemical composition of leaf oil of this plant. However, they identified fewer compounds than these.

Table 7. Chemical composition of the essential oil of *L. ukambensis* leaves

Compound	% Area	Identification	Compound	% Area	Identification
α-pinene	1.13	GC-MS, CO	β-phellandrene	1.21	GC-MS, CO
camphene	8.63	GC-MS, CO	α- phellandrene	1.28	GC-MS, CO
β-pinene	1.00	GC-MS, CO	*trans*-β-ocimene	0.59	GC-MS, CO
β-myrcene	1.69	GC-MS, CO	*trans*-sabinene hydrate	14.21	GC-MS, CO
δ-2-carene	t	GC-MS, CO	pinocarvone	0.76	GC-MS
limonene	0.29	GC-MS, CO	verbenone	0.78	GC-MS, CO
linalool	0.40	GC-MS, CO	*p*-menth-2-en-1-one	t	GC-MS
1,8-cineole	2.42	GC-MS, CO	fenchone	0.17	GC-MS, CO
α-terpinolene	0.86	GC-MS, CO	α-thujene	0.82	GC-MS, CO
α-terpinene	t	GC-MS, CO	methyleugenol	0.90	GC-MS, CO
p-cymene	0.67	GC-MS, CO	*p*-menth-3-en-1-ol	1.39	GC-MS
γ-terpinene	1.42	GC-MS, CO	α-caryophyllene	0.12	GC-MS, CO
camphor	39.84	GC-MS, CO	*p*-mentha-1,4,8-triene	t	GC-MS
α-terpineol	t	GC-MS, CO	*p*-menth-2-en-1-ol	t	GC-MS
α-copaene	0.38	GC-MS, CO	germacrene B	t	GC-MS, CO
α-cubebene	t	GC-MS, CO	germacrene D	1.45	GC-MS
α-farnesene	t	GC-MS, CO	isocaryophyllene	0.43	GC-MS, CO
borneol	1.14	GC-MS, CO	β-bourbonene	0.49	GC-MS, CO
			caryophyllene oxide	t	GC-MS, CO

The GC profile of the leaf oil is as shown in figure 11a.

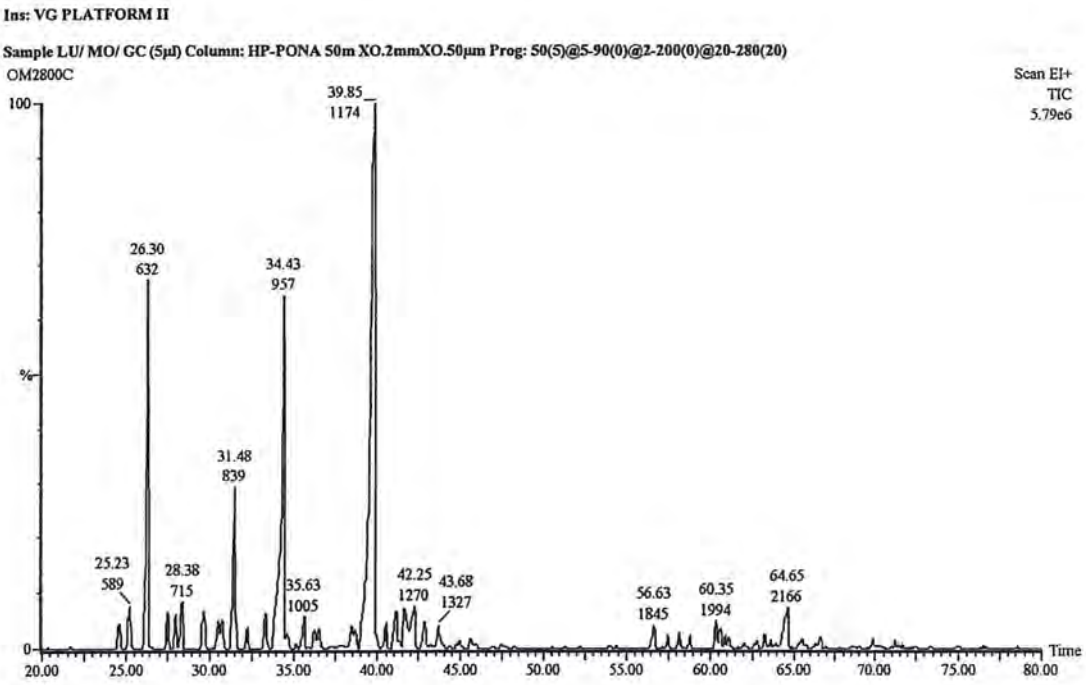


Fig. 11a The GC profile of *Lippia ukambensis* oil

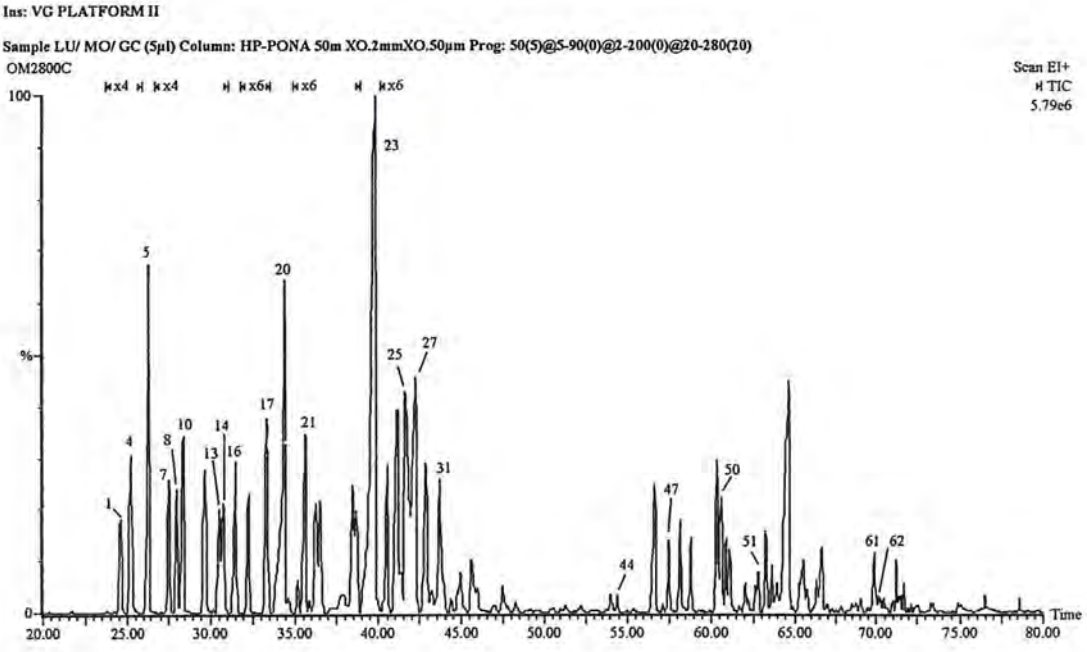


Fig. 11b The magnified GC profile of *Lippia ukambensis* oil

4.1.5 *Tarchonanthus camphoratus*

A total of 46 compounds accounting for 79.3% of the essential oils of the plant's leaf were identified. The major constituents were camphene (16.82), α-pinene (16.62%), α-fenchyl alcohol (14.76%), 1,8-cineole (6.51%), α-terpineol (3.78%), terpen-4-ol (3.28%), *p*-cymene (2.89%), isocaryophyllene (1.36%), alloaromadendrene (1.19%), and caryophyllene oxide (1.06%). Minor ones included 10 compounds present in trace amounts (< 0.1%), 3 compounds in the range of 0.1 - 0.2%, 10 compounds in 0.2 - 0.4%, 6 compounds in 0.4 - 0.6%, 2 compound in 0.6 - 0.8% and finally 6 compounds in 0.8 - 1.0% (Table 8). The GC profile of the leaf oil of the plant is as indicated in figure 12a. These results are comparable with what was reported by Mwangi *et al.* (1994) on the composition of the essential oil of *T. camphoratus*. However, the compounds reported here are fewer than those identified earlier.

Table 8. Chemical composition of the essential oil of *T. camphoratus* leaves

Compound	% Area	Identification	Compound	% Area	Identification
α-pinene	16.62	GC-MS, CO	*trans*-β-ocimene	t	GC-MS, CO
camphene	16.82	GC-MS, CO	*trans*-sabinene hydrate	0.21	GC-MS, CO
β-pinene	0.78	GC-MS, CO	terpen-4-ol	3.28	GC-MS, CO
β-myrcene	t	GC-MS, CO	myrtenol	t	GC-MS, CO
δ-4-carene	0.91	GC-MS, CO	β-bourbonene	t	GC-MS, CO
limonene	0.69	GC-MS, CO	α-fenchyl alcohol	14.76	GC-MS, CO
1,8-cineole	14.38	GC-MS, CO	*cis*-carveol	0.34	GC-MS, CO
α-terpinene	0.27	GC-MS, CO	p-menth-2-en-1-ol	0.54	GC-MS
p-cymene	2.89	GC-MS, CO	fenchone	0.43	GC-MS, CO
γ-terpinene	0.56	GC-MS, CO	α-fenchyl acetate	0.83	GC-MS, CO
camphor	0.38	GC-MS, CO	isocaryophyllene	1.36	GC-MS, CO
α-terpineol	3.78	GC-MS, CO	α-terpinolene	0.38	GC-MS, CO
α-copaene	t	GC-MS, CO	borneol	t	GC-MS, CO
α-cubebene	t	GC-MS, CO	linalool oxide	t	GC-MS
α-fenchene	0.12	GC-MS, CO	*cis*-verbenol	0.30	GC-MS, CO
γ-curcumene	0.15	GC-MS	*trans*-2-pinanol	t	GC-MS, CO
linalool	0.88	GC-MS, CO	monoterpene alcohol	0.24	GC-MS
α-cadinol	0.84	GC-MS, CO	spathulenol	0.48	GC-MS, CO
α-thujene	0.29	GC-MS, CO	myrtenal	0.38	GC-MS, CO
β-eudesmol	3.12	GC-MS, CO	α-ar-curcumene	0.98	GC-MS, CO
α-bisabolol	0.42	GC-MS, CO	alloaromadendrene	1.19	GC-MS, CO
p-cymen-8-ol	0.96	GC-MS, CO	caryophyllene oxide	1.06	GC-MS, CO
δ-cadinene	0.41	GC-MS, CO	viridiflorol	0.34	GC-MS

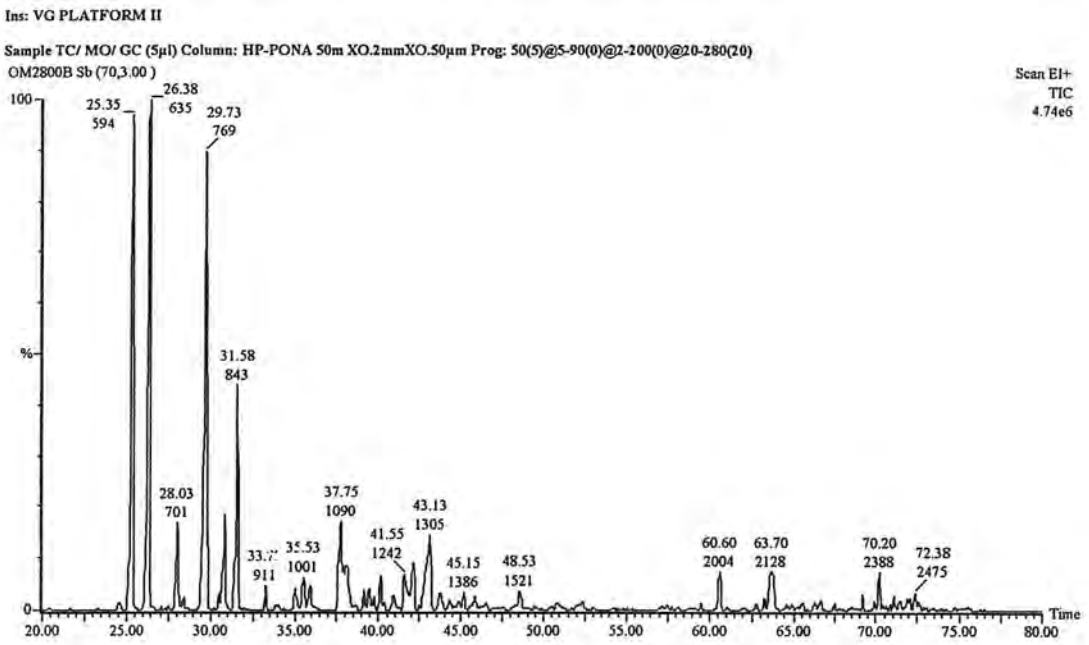


Fig. 12a The GC profile of *Tarchonanthus camphoratus* oil

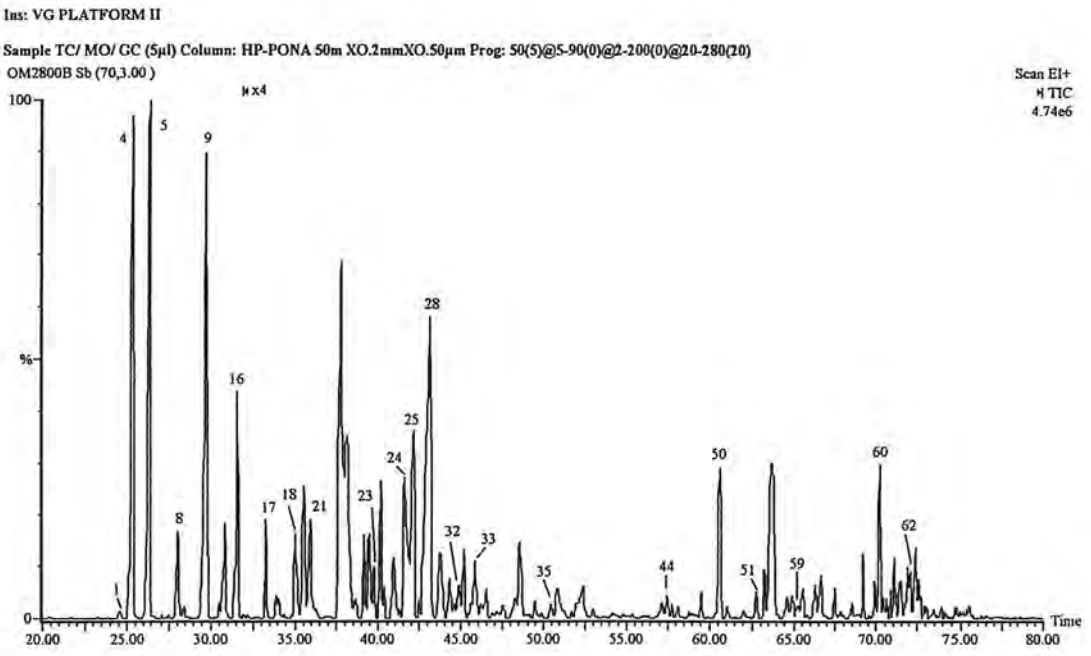


Fig. 12b The magnified GC profile of *Tarchonanthus camphoratus* oil

4.1.6 *Lippia javanica*

Forty six (46) compounds were identified from the leaf oil of *L. javanica* by the same analytical techniques afore-stated earlier on. The major constituents of the leaf oil were limonene oxide (38.99%), *cis*-verbenol (11.33%), verbenone (6.06%), β-myrcene (3.16%), artemisia ketone (2.78%), linalool (2.69%), limonene (2.58%), α-terpineol (2.04%), isocaryophyllene (1.38%), piperitenone (1.07%) and *trans*-β-ocimene (1.06%). Other constituents included 16 compounds present in trace amounts (< 0.1%), 7 compounds in the range of 0.1 - 0.2%, another 7 in 0.2 - 0.4%, 3 compounds in 0.4 - 0.6% and another 3 compounds in 0.6 - 0.8% (Table 9). The GC profile of the leaf oil constituents is shown in figure 13a.

Table 9. Chemical composition of the essential oil of *L. javanica* leaves

Compound	% Area	Identification	Compound	% Area	Identification
α-pinene	t	GC-MS, CO	β-phellandrene	0.23	GC-MS, CO
camphene	0.31	GC-MS, CO	α- phellandrene	t	GC-MS, CO
β-pinene	t	GC-MS, CO	*trans*-β-ocimene	1.06	GC-MS, CO
β-myrcene	3.16	GC-MS, CO	*trans*-sabinene hydrate	t	GC-MS, CO
δ-3-carene	0.76	GC-MS, CO	limonene oxide	38.99	GC-MS, CO
limonene	2.58	GC-MS, CO	alloaromadendrene	0.13	GC-MS, CO
linalool	2.69	GC-MS, CO	β-bisabolene	t	GC-MS
1,8-cineole	t	GC-MS, CO	eugenol	0.46	GC-MS, CO
α-terpinene	t	GC-MS, CO	α-pyronene	0.12	GC-MS
carvone	0.40	GC-MS, CO	methyleugenol	0.27	GC-MS, CO
camphor	0.75	GC-MS, CO	aromadendrene	0.31	GC-MS, CO
α-terpineol	2.04	GC-MS, CO	α-caryophyllene	0.14	GC-MS, CO
α-copaene	t	GC-MS, CO	*p*-mentha-1,3,8-triene	t	GC-MS
α-farnesene	0.18	GC-MS, CO	citronellal	t	GC-MS, CO
borneol	t	GC-MS, CO	artemisia ketone	2.78	GC-MS
γ-cadinene	0.17	GC-MS, CO	germacrene D	0.24	GC-MS
eucarvone	0.10	GC-MS	isocaryophyllene	1.38	GC-MS, CO
terpen-4-ol	t	GC-MS, CO	β-bourbonene	0.40	GC-MS, CO
myrcenol	0.73	GC-MS, CO	caryophyllene oxide	0.20	GC-MS, CO
verbenone	6.06	GC-MS, CO	α-ar-curcumene	t	GC-MS, CO
carvacrol	0.46	GC-MS, CO	nerolidol	t	GC-MS, CO
cis-verbenol	11.33	GC-MS, CO	chrysanthenone	0.55	GC-MS
carvacrol	0.46	GC-MS, CO	piperitenone	1.07	GC-MS

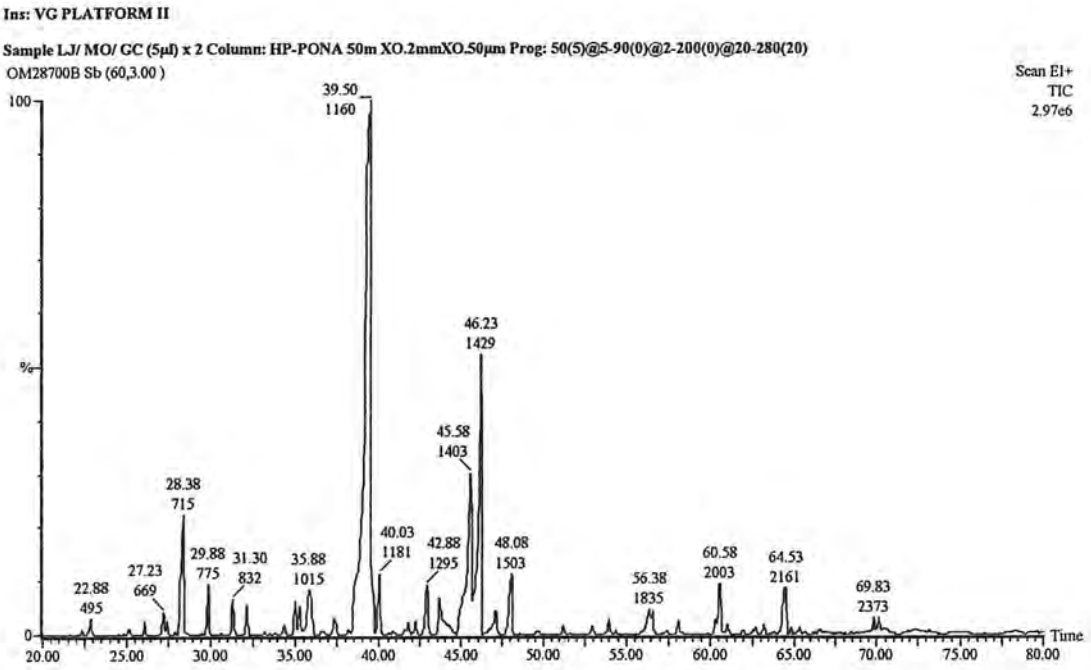


Fig. 13a The GC profile of *Lippia javanica* oil

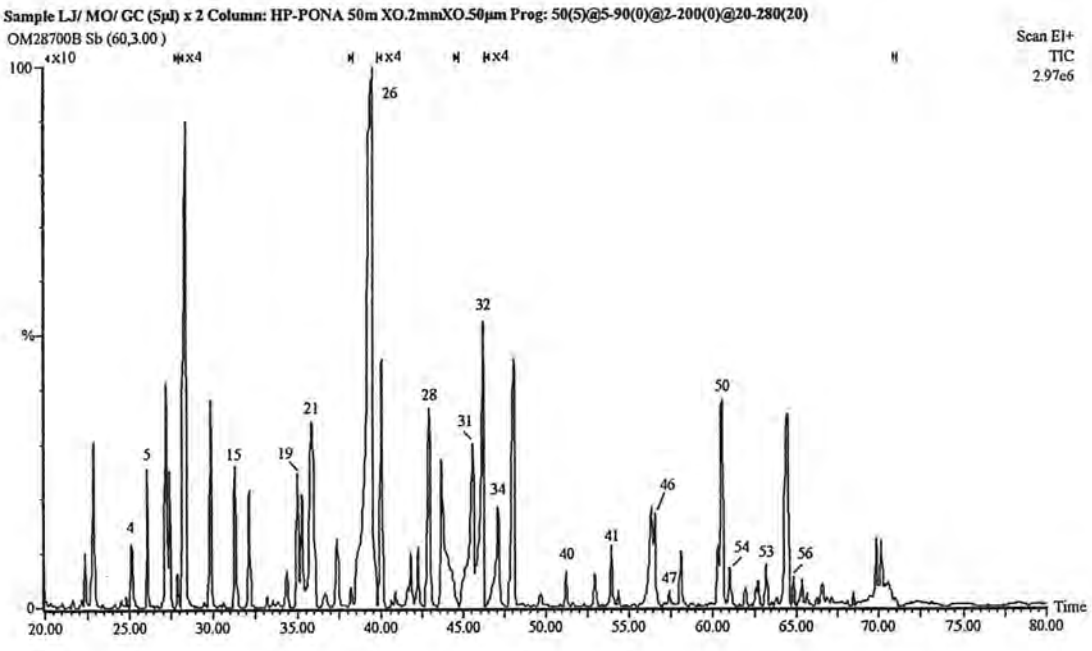


Fig. 13b The magnified GC profile of *Lippia javanica* oil

It is quite interesting to note that, even though the chemical composition of the leaf oil of *Lippia javanica* has been reported by Mwangi *et al.* (1991b), Chagonda *et al.* (2000) and Guenther (1949), the major constituents reported by these three different teams were very different from one another and also from what is being reported in this thesis. Whereas myrcene (20.9 - 49.7%), *cis*-ocimenone (24.9 - 39.9%), *trans*-ocimenone (11.4 - 20.6%) and *trans*-targetone (0 - 4.6%) were the major components found by Mwangi *et al.*, (1991b), Chagonda *et al.*, (2000) had linalool (1.8 - 68.8%), myrcene (0.5 - 54%), limonene (0.4 - 39.9%), 2,6-dimethylstyrene (trace - 26.9%), while Guenther (1949) reported p-cymene, linalool and β-caryophyllene as the principal constituents. These variations could be attributed to the different geographical factors and/or poor plant identification.

With the 62 compounds identified from the repellent/insecticidal essential oils from 6 plants, it became necessary to evaluate them individually or in a blend for their insecticidal/repellent properties to understand their contribution to the overall activity of the essential oils of these plants.

CHAPTER 5: BIO-ASSAY OF PURE COMPOUNDS

The authentic standards of the identified compounds from the six repellent plants were bio-assayed for their repellent and insecticidal activities according to WHO (1996) protocol.

5.1 Tarsal contact mosquitocidal assays

The insecticidal activity of 37 compounds was investigated by tarsal contact bio-assay (WHO, 1996). Out of these, only two compounds, perillaldehyde and perill alcohol were found to be active, with LD_{50} of 4.8675 x 10^{-3} mg/cm^2 and 5.2753 x 10^{-3} mg/cm^2, respectively. Any compound with a tarsal contact activity lower than LD_{50} value of 0.16 mg/cm^2 (WHO, 1996) is considered as a good potential insecticide. The LD_{50} values of perillaldehyde and perill alcohol being < 0.16 mg/cm^2, indicate that they meet the WHO requirements as compounds with tarsal contact insecticidal activity. The insecticidal activity of the two compounds is being reported for the first time in this thesis.

5.2 Fumigation mosquitocidal assays

All the 37 compounds were evaluated in fumigation mosquitocidal assay (WHO, 1996). Again the active compounds were perillaldehyde and perill alcohol with LD_{50} of 1.0451 x 10^{-4} mg/cm^3 and 2.5183 x 10^{-4} mg/cm^3 in that order. The rest of the compounds had no activity even at the highest concentration (0.01 g/ml) bio-assayed.

5.3 Repellency assays

All the 37 out of 62 compounds identified through GC co-injection in all the six repellent plants were assayed for their repellency against the female *Anopheles gambiae* [ex-Ifakara (Tanzania) strain] mosquitoes.

5.3.1 *Conyza newii*

Twenty-three (23) compounds were assayed from the list of 50 identified compounds in the leaf oil of this plant. Out of these, 3 compounds (perillaldehyde, perill alcohol and geraniol) had PE of 100% at a concentration of 0.01 g/ml. Carvone had a PE within the range of 90 - 99%, α-terpineol in 80 - 89%, 8 compounds (α-terpinene, γ-terpinene, terpinolene, α-fenchyl alcohol, linalool, citral, camphor and 1,8-cineole) within 70 - 79%,

4 compounds (camphene, borneol, myrtenol and geranyl acetate) in 60 - 69% and 6 compounds (limonene, β-pinene, *p*-cymene, α-pinene, isocaryophyllene and isopropylbenzaldehyde) had PE < 60% at the same concentration. It was generally noted that there was a dose to response relationship from the lowest to the highest dosage.

The compounds responsible for the repellent activity of the leaf oil of this plant might be perillaldehyde, perill alcohol, 1,8-cineole, carvone, α-terpineol, α-terpinene, γ-terpinene, terpinolene, α-fenchyl alcohol, linalool, citral, camphor and geraniol. The repellency of the 4 major components of the essential oil (perillaldehyde, perill alcohol, limonene and 1,8-cineole) was done as a blend. The results clearly indicated that these four compounds have major contribution to the repellent activity of *C. newii* leaf oil. A blend of all the four compounds had a higher activity (75% PE) at 0.001 g/ml than that of the plant leaf oil (59.15% PE) at the same concentration. Limonene lowered the activity of perillaldehyde and peril alcohol mixture to 63.94%, while 1,8-cineole showed some synergistic effect to the mixture of the two compounds (78.93%) at the same concentration.

5.3.2 *Tetradenia riparia* (*I. Multiflora*)

From the 38 identified compounds in the leaf oil of *T. riparia*, 17 compounds were assayed. Verbenol had a PE of 100% at 0.01 g/ml concentration, two compounds (α-terpineol and terpen-4-ol) had PE within the range of 80 - 89, 5 compounds (α-terpinene, γ-terpinene, α-fenchyl alcohol, camphor and 1,8-cineole) had their PE in 70 - 79% range, 3 compounds (camphene, borneol and fenchone) in 60 - 69% and 6 (limonene, β-pinene, *p*-cymene, α-pinene, isocaryophyllene and thujone) had PE < 60% at the same concentration. Dose-response relationship was clearly observed.

The repellent activity of the leaf oil of *T. riparia* might probably be due to the major components (fenchone, limonene and 1,8-cineole) in a blend. In fact, the % PE of this blend at 0.1 g/ml (91.42%), 0.01 g/ml (69.03%) and 0.001 g/ml (55.73%) were higher than those of the plant oil (87.68, 60.82 and 54.09%) at the same concentrations.

There might also be some contribution from γ-terpinene and synergistic action between fenchone (the major component) and minor constituents like verbenol, α-terpeneol, terpen-4-ol, α-fenchyl alcohol, α-terpinene, 1,8 -cineole and camphor.

5.3.3 *Plectranthus marrubioides*

A total of 22 out of the 70 compounds identified were assayed from the leaf oil of this plant. Two compounds (carveol and caryophyllene oxide) had PE of 100% at 0.01 g/ml, carvone had a PE of 90 - 99%, 3 compounds (eugenol, α-terpineol and terpen-4-ol) in 80 - 89%, 6 compounds (α-terpinene, γ-terpinene, linalool, terpinolene, camphor and 1,8-cineole) within 70 - 79%, 4 compounds (camphene, myrtenol, borneol and fenchone) in 60 - 69% and 6 (4-isopropylbenzaldehyde, limonene, α-pinene, isocaryophyllene, *p*-cymene and β-pinene) had PE less than 60% at that concentration.

The repellency of the leaf oil of *P. marrubioides* might most likely be due to the major components (camphor, 1,8-cineole and α-terpinene) as a blend giving 90.77% PE at 0.1 g/ml concentration. Minor contributions from carveol, caryophyllene oxide, α-terpineol, terpen-4-ol, eugenol, terpinolene, linalool and γ-terpinene cannot be ruled out since the leaf oil gave 100% PE at the same concentration.

5.3.4 *Lippia ukambensis*

Seventeen (17) out of 37 identified compounds were assayed from the leaf oil of this plant. One compound, caryophyllene oxide had a PE of 100% at 0.01 g/ml, α-terpineol had a PE within the range 80 - 89%, 7 compounds (1,8-cineole, verbenone, camphor, linalool, terpinolene, α- terpinene and γ-terpinene) in 70 - 79%, 3 compounds (camphene, fenchone and borneol) in 60 - 69%, and 5 (isocaryophyllene, α-pinene, *p*-cymene, limonene and β-pinene) had PE < 60%.

The repellent property of the leaf oil of *L. ukambensis* might most likely be due to synergistic interaction between camphor and other compounds like γ-terpinene, caryophyllene oxide, α-terpineol, verbenone, 1,8-cineole, linalool and terpinolene.

The bio-assay results of the major compounds (camphor, camphene and 1,8-cineole) in a blend indicated that, other than these three compounds, there might be some other(s) that could be contributing to the repellent activity of the leaf oil of this plant. This was from the fact that at 0.1 g/ml this blend had a PE (74.08%) lower than that of the plant leaf oil (84.11%) at the same concentration. The compounds most likely to be synergizing these three compounds may be caryophyllene oxide and α-terpeneol, which are present in the essential oil in low amounts but show high activity individually.

5.3.5 *Tarchonanthus camphoratus*

Twenty-two (22) out of the 46 identified compounds from the leaf oil of this plant were assayed for their mosquito repellency. Three of them (verbenol, carveol and caryophyllene oxide) had PE of 100% at 0.01 g/ml, 2 compounds (terpen-4-ol and α-terpineol) had PE falling within the range of 80 - 89%, 7 compounds (camphor, linalool, α-fenchyl alcohol, terpinolene, α-terpinene, 1,8-cineole and γ-terpinene) in 70 - 79%, 5 compounds (camphene, borneol, myrtenol, myrtenal and fenchone) in 60 - 69% and another 5 (α-pinene, limonene, *p*-cymene, β-pinene and isocaryophyllene) had PE < 60% at the same concentration. There was a clear dose to response relationship for all the compounds bioassayed.

Repellency of the leaf oil of *T. camphoratus* might likely be due to the synergistic action of the major compounds (camphene, α-pinene, 1,8-cineole, α-fenchyl alcohol, terpen-4-ol and α-terpineol) together with the contribution of caryophyllene oxide, verbenol and carveol. This was confirmed by the bio-assay of the blend, which gave a PE of 96.62% compared to 100 % for the leaf oil at 0.1 g/ml. The synergistic effect of camphor, linalool, terpinolene, α-fenchyl alcohol, α-terpinene and γ-terpinene towards the overall activity of the leaf oil of this plant cannot be neglected.

5.3.6 *Lippia javanica*

Twenty-one (21) out of the 46 identified compounds were assayed for their mosquito repellency from the leaf oil of this plant. Two compounds (verbenol and caryophyllene oxide) had PE of 100% at 0.01 g/ml, 3 compounds (carvone, citronellal, and nerolidol) had their PE falling within the range of 90 - 99%, another 3 (eugenol, α-terpinene and terpen-4-ol) within

80 - 89%, 5 compounds (1,8-cineole, verbenone, camphor, linalool and α-terpinene) within 70 - 79%, 4 (camphene, aromadendrene, borneol and limonene oxide) within 60 - 69% and lastly another 4 compounds (limonene, α-pinene, isocaryophyllene and β-pinene) had PE of < 60% at that concentration.

The repellent activity of the leaf oil of *L. javanica* might be mainly due to the major compounds (limonene oxide, *cis*-verbenol and verbenone) in a blend. This was confirmed by the bio-assay of the major compounds as a blend giving 95.24% PE as compared to 100% for the leaf oil at 0.1 g/ml. There might however, be synergistic action of limonene oxide with *cis*-verbenol, verbenone, α-terpineol, linalool, camphor, 1,8-cineole and α-terpinene.

A total of 37 compounds were assayed for their repellent activities against *A. gambiae*. Nine of them showed a PE > 90% at 0.01 g/ml (1%) concentration. Perillaldehyde, perill alcohol, caryophyllene oxide, verbenol, geraniol and carveol gave 100% protection at this concentration. Nerolidol, carvone and citronellal gave 94 - 95% protective efficacy. Four compounds (eugenol, α-terpineol, terpen-4-ol and linalyl acetate) had their PE falling between 80 - 89%. Nine compounds had a PE of 70 - 79% while 8 compounds had a PE of 60 - 69%. The rest of the compounds had a PE of less than 60% (Table 10).

Table 10. The repellency assay data of identified compounds

Compound	% Protective Efficacy of the various solutions ± 0.05			
	0.00001g/ml	**0.0001g/ml**	**0.001g/ml**	**0.01g/ml**
Camphene	39.24	42.06	59.60	65.42
Limonene	60.00	49.18	45.01	23.24
β-Pinene	40.78	46.15	50.00	57.69
p-Cymene	43.33	36.23	16.05	-15.38
αTerpinene	28.38	35.26	51.36	78.28
γ-Terpinene	28.23	36.05	44.80	76.28
Terpinolene	26.16	38.16	47.18	74.28
α-Pinene	20.38	31.24	43.38	51.06
Aromadendrene	36.92	39.01	57.14	68.05
Isocaryophyllene	13.04	-5.50	-22.25	-18.76
Nerolidol	43.90	57.33	83.05	93.95
α-Fenchyl alcohol	33.34	43.21	45.12	75.77
Perill alcohol	37.76	61.18	71.75	100
Verbenol	40.92	49.73	76.88	100
Carveol	34.93	46.29	80.74	100
Geraniol	29.19	51.19	80.77	100
α-Terpineol	31.28	42.36	53.48	89.48
Eugenol	28.02	39.89	54.56	88.48
Terpen-4-ol	24.50	34.01	42.20	85.13
Myrtenol	16.94	32.3	38.67	63.64
Borneol	36.01	46.16	60.00	68.42
Linalool	32.00	37.87	47.50	71.42
Citronellal	27.78	43.18	53.76	95.19
Perillaldehyde	33.13	42.46	61.29	100
Citral	29.28	38.23	54.30	78.36
Myrtenal	26.70	29.20	49.30	60.82
Isopropylbenzaldehyde	0	22.25	34.06	41.75
Camphor	37.24	48.62	62.06	78.02
Verbenone	28.28	31.18	62.07	71.23
Fenchone	26.24	38.06	44.32	68.23
Carvone	27.91	43.89	45.76	94.34
Thujone	25.65	30.65	44.70	58.26
Caryophyllene oxide	33.13	41.47	50.58	100
Limonene oxide	35.02	41.60	48.06	68.24
1,8-Cineole	36.56	43.87	50.62	78.01
Geranyl acetate	21.15	27.58	48.00	63.23
Linalyl acetate	22.36	37.28	52.35	80.38

From the repellency data, the RD_{50} (dose required to repel 50% of the insect population) values were calculated by probit analysis for eight compounds that showed the highest repellency towards *A. gambiae* (Table 11).

The RD_{50} values revealed the order of repellency as nerolidol > perill alcohol > *cis*-verbenol > *cis*-carveol > geraniol > citronellal > perillaldehyde > caryophyllene oxide.

Table 11. RD_{50} of eight most repellent compounds

Compound	RD_{50} (mg/cm^2)
Nerolidol	4.2×10^{-5}
Perill alcohol	6.3×10^{-5}
cis-Verbenol	7.5×10^{-5}
cis-Carveol	1.0×10^{-4}
Geraniol	1.05×10^{-4}
Citronellal	2.21×10^{-4}
Perillaldehyde	3.2×10^{-4}
Caryophyllene oxide	1.2×10^{-3}
DEET	5.0×10^{-2}

It therefore appears that the sesquiterpene alcohols > cyclic monoterpene alcohols > acyclic monoterpene alcohols > monoterpene aliphatic aldehydes > cyclic monoterpene aldehydes > cyclic sesquiterpene epoxides in their repellency of *A. gambie*. Explanation to this may be found in the relative volatilities of these compounds with the exception of caryophyllene oxide. This observation is further supported by the fact that all sesquiterpene and monoterpene alcohols assayed for repellency had protective efficacies of 75 - 100% and 63 - 100%, respectively at 0.01 g/ml concentration, the aldehydes 60 - 100%, esters 63 - 80%, ketones 54 - 94% and hydrocarbons 23 - 78% (Table 10). Among the cyclic monoterpene alcohols, it appears that monocyclic alcohols are generally much better repellents than the bicyclic ones. Bicyclic monoterpene alcohols had repellency in the range of 60 - 69% as compared to the monocyclic ones, which were within the range of 85 - 100%. The presence of hydroxyl group implies better repellency. This has been clearly demonstrated by *p*-menthane-3,8-diol (Schreck and Leonard, 1991) and 2-ethyl-1,3-hexanediol (Kirk and Orthmer, 1992) both of which have 2 hydroxyl groups.

Out of the eight compounds, two (geraniol and citronellal) have been reported (Curtis *et al.*, 1991; Dethier, 1947) as mosquito repellents. The remaining six compounds (caryophyllene oxide, nerolidol, verbenol, perill alcohol, carveol and perillaldehyde) are being reported for the first time as repellents of *A. gambiae* mosquitoes in this thesis.

5.3.7 Repellency bio-assay of formulated compounds

The 8 compounds showing good repellency against *A. gambiae*, when dissolved in acetone and applied to the skin were formulated in various carrier media and bio-assayed once again to determine the longevity of the protection. Several bases, oils and gels were investigated. These included aqueous base, Tween 80, Tween 60, and emulsion gel. This was necessary since a carrier medium that gives a homogeneous mixture is required for such formulations. The compounds were therefore mixed with various media and left to stand. If the two separated after some time then the medium was considered unsuitable. Formulation of pure compounds controls the release rate of repellents and, therefore, is a precondition for good longevity of such products. Formulated compounds (10%) in aqueous base were assayed for their repellent activity against *A. gambiae* after 0, 2, 4, 6 and 8 hrs after application and their protective efficacies (PE) calculated (Table 12 and Figure 14). A standard commercial repellent with a 10% commercial DEET formulation (Urtan®) was similarly bio-assayed for comparison.

Table 12. Durational % PE of formulated compounds

	% PE ± 0.005								
Time (hrs)	Perill aldehyde	Perill alcohol	Caryophyllene oxide	Verbenol	Nerolidol	Carveol	Geraniol	Citronellal	DEET
0	100	100	100	100	100	100	100	100	100
2	88	100	100	89	98	93	97	72	100
4	82	76	100	66	87	77	62	65	100
6	71	62	83	58	75	54	55	45	100
8	49	57	77	48	54	30	45	36	95

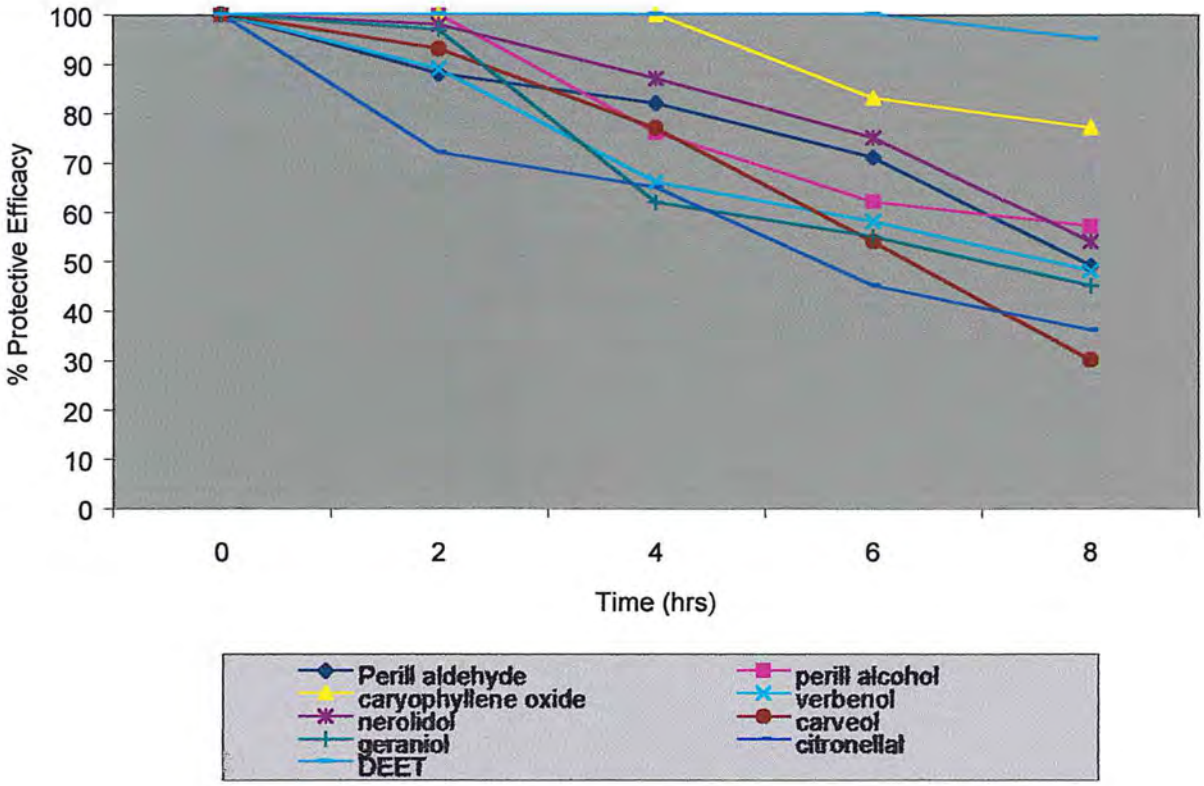


Fig. 14 Longevity of protection of the eight formulated compounds

Protective efficacy > 70% was considered effective. The best compound was found to be caryophyllene oxide with 8 hrs protection followed by nerolidol (6 hrs), perillaldehyde (6 hrs), carveol (4 hrs), perill alcohol (4 hrs), geraniol (2 hrs), verbenol (2 hrs) and citronellal (2 hrs). DEET on the other hand gave 100% protection for 6 hours. This dropped to 95% after 8 hours. It is therefore evident that the PE of caryophyllene oxide compares favourably with that of DEET. It may therefore be necessary to carry out further formulation studies of this compound singly or as a blend with the aim of enhancing PE and longevity of protection.

CHAPTER 6: CONCLUSION AND FUTURE DIRECTION

6.1 Conclusions

The family Compositae, which was represented by *Tarconanthus camphoratus* and *Conyza newii*, was found to be richer in oxygenated monoterpenes than the hydrocarbon monoterpenes. There was an almost equal distribution of both oxygenated and non-oxygenated terpenes in both *C. newii* and *T. camphoratus*, with 62% and 65% composition of monoterpenes, respectively, compared to the sesquiterpenes. The chemical constitution of the major constituents in the leaf oils of the two plants was found to be quantitatively and qualitatively different. Whereas *C. newii* was rich in perillaldehyde (29.28%), limonene (10.06%), 2-methyl-5-(methylethyl)-2-cyclohexen-1-ol (7.34%), 1,8-cineole (6.84%) and perill alcohol (4.27%), *T. camphoratus* on the other hand had camphene (16.82%), α-pinene (16.62%), α-fenchyl alcohol (14.76%), 1,8-cineole (6.51%), α-terpineol (3.78%) and terpen-4-ol (3.28%) as the major components. However, the two plants also showed some qualitative similarities in their minor components like, α-terpinene, *trans*-sabinene hydrate, *p*-cymene, γ-terpinene, β-eudesmol and α-cadinol, which were quantitatively, present in *T. camphoratus* than in *C. newii*. The results obtained for *T. camphoratus* were qualitatively similar to but quantitatively different from what was reported by Mwangi *et al.* (1994). Perhaps this is due to the difference in the ecotypes, ages or geographical location of the plant used in this case.

The two *Lippia* species, *Lippia javanica* and *Lippia ukambensis* which represented the family verbeneceae, were found to possess a lot of qualitative difference in the chemical constitution of their leaf oils. *L. ukambensis* was found to be rich in camphor (39.84%), *trans*-sabinene hydrate (14.21%), camphene (8.63%) and 1,8-cineole (2.42%), while *L. javanica* had limonene oxide (38.99%), *cis*-verbenol (11.33%), verbenone (6.06%), β-myrcene (3.16%) and artemisia ketone (2.78%). Whereas *L. javanica* had moderate amounts of β-myrcene and verbenone, *L. ukambensis* had much lower amounts of the two compounds but much higher content of 1,8-cineole. A similar observation was made for the leaf oil of *L. ukambensis*, which had quantitatively high levels of camphor and *trans*-sabinene hydrate but these, were present in trace amounts in leaf oil of *L. javanica*.

The results obtained for the chemical constitution of leaf oil of *L. javanica* are different from those reported by Guenther (1949), Mwangi *et al.*, (1991b) and Chagonda *et al.*, (2000) who reported *p*-cymene, linalool and β-caryophyllene; myrcenone, *cis*-ocimenone, *trans*-ocimenone and myrcene; and linalool, myrcene, limonene and 2,6-dimethylstyrene respectively, as the major constituents of *L. javanica* leaf oil. This reflects large quantitative differences in the essential oil composition obtained from different sources. On the other hand, the results of the chemical composition of *L. ukambensis* leaf oil were similar to that reported by Mwangi *et al.* (1991a).

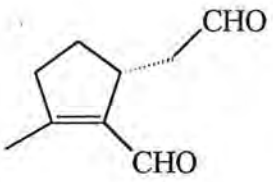
Tetradenia riparia and *Plectranthus marrubioides,* which represented the family labiatae showed some qualitative similarities in their essential oils. The principal compounds in the leaf oils of both plants were ketones. *T. riparia* was found to be rich in fenchone (64.82%), 1,3,8-trimethylbicyclo[2.2.1]heptan-2-one (11.68%), limonene (2.02%) and 1,8-cineole (1.50%), while *P. marrubioides* had camphor (48.80%), 1,8-cineole (9.0%) and α-terpinene (3.08%). Only small amounts of fenchone (1.75%) and camphor (0.13%) were present in *P. marrubioides* and *T. riparia*, respectively; while 1,8-cineole, α-terpinene, *p*-cymene, *trans*-sabinene hydrate, terpen-4-ol, α-terpineol and borneol were comparatively higher in *P. marrubioides* than in *T. riparia*. The converse was however, observed for γ-terpinene, limonene, β-myrcene and *trans*-β-ocimene in *T. riparia* and *P. marrubioides*.

The most effective mosquito repellent plants were *Conyza newii* and *Plectranthus marrubioides* with RD_{50} of 8.86×10^{-5} mg/cm^2 and 8.85×10^{-5} mg/cm^2, respectively. *Tetradenia riparia* was found to be the least effective repellent (RD_{50} 5.04×10^{-4} mg/cm^2) of the six plants tested. The high mosquito repellency of *Conyza newii* leaf oil could be due to mainly, perillaldehyde and perill alcohol, which happened to be the principal compounds of the leaf oil of this plant. Nevertheless, it might be difficult to ignore the contributions of geraniol, geranyl acetate, carvone, α-terpineol and γ-terpinene among other many minor components, which could be acting synergistically with the two major constituents of the plant leaf oil. In fact, α-terpineol, geraniol and γ-terpinene are known mosquito repellents (Dethier, 1947; Curtis, 1990).

Although camphor, the major constituent of the leaf oil of *Plectranthus marrubioides* and *Lippia ukambensis* is a reported mosquito repellent (Curtis, 1990), it exhibited low repellent activity as compared to the oils of the two plants. A similar observation was made with fenchone and α-fenchyl alcohol, the major constituents of *Tetradenia riparia* and *Tarchonanthus camphoratus*, respectively. Consequently, the repellency of the leaf oils of these four plants could most likely be arising from the synergistic action between the minor components like (linalool, terpen-4-ol, α-terpineol, γ-terpinene, borneol, caryophyllene oxide *cis*-carveol, among many others) and the major constituents named earlier. In fact, caryophyllene oxide, *cis*-carveol, terpen-4-ol, α-terpineol, linalool and borneol exhibited good repellent properties singly.

Even though the leaf oil of *Lippia javanica* showed good repellent activity, limonene oxide, the major component of the leaf oil of *L. javanica* had moderate repellency. The mosquito repellency of the leaf oil of this plant could mainly be attributed to *cis*-verbenol and synergistic action of caryophyllene oxide, carvone, citronellal, nerolidol, eugenol, verbenone, α-terpinene and terpen-4-ol with the major component (limonene oxide).

The best plant derived repellent compounds were found to be nerolidol, perill alcohol, *cis*-verbenol, *cis*-carveol, geraniol, citronellal, perillaldehyde and caryophyllene oxide. The RD_{50} values of nerolidol (4.2×10^{-5} mg/cm^2), perill alcohol (6.3×10^{-5} mg/cm^2), *cis*-verbenol (7.5×10^{-5} mg/cm^2), cis-carveol (1×10^{-5} mg/cm^2), suggested that these compounds could be much better repellents than geraniol (1.05×10^{-4} mg/cm^2) and citronellal (2.21×10^{-4} mg/cm^2), which happen to be among some well known natural mosquito repellents (Curtis, 1990).
Interestingly, only terpenoid alcohols and aldehydes seem to give good mosquito repellency. The alcohols seem to be better than the aldehydes. In addition to structural effects, this may be partly attributed to the lower volatility of alcohols due to hydrogen bonding and their fairly high polarity, which increases with the number of the hydroxyl groups as in 2-ethyl-2-butyl-1,3-propanediol, 2-ethyl-1,3-hexanediol (Kirk and Orthmer, 1992) and *p*-menthane-3,8-diol, which has been reported as a mosquito repellent (Trigg, 1996), whose efficacy compares favourably to DEET. Rotundial (**47**) is a good example of a mosquito repellent aldehyde isolated from *Vitex rotundifolia* (Grayson, 2000).



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Although caryophyllene oxide was the least active of the eight compounds, durational repellency bio-assay of formulated compounds revealed that caryophyllene oxide was the best as a topical repellent. A 10% formulation of this compound in aqueous base gave the longest protection of 4 hours at 100% repellency, dropping to 77% after 8 hours. This observation confirms the importance of formulation in the efficacy of topical repellents. Controlled release may be invoked in explaining this observation. The remaining 7 compounds did not protect for long. This could be attributed to their high volatility, which made them evaporate from the surface of the skin at a fast rate shortly after their application. It would, therefore, be interesting to investigate similar compounds, incorporating the three functional groups (alcohol, aldehyde and epoxide) in one molecule, for mosquito repellency.

Conyza newii oil (LD_{50} 1.965 x 10^{-3} mg/cm^3) and *Plectranthus marrubioides* oil (LD_{50} 2.809 x 10^{-3} mg/cm^3) were the most effective mosquitocidal oils, when evaluated by fumigation method. The order of activity of the oils of the other four plants was *Tarchonanthus camphoratus* (LD_{50} 3.788 x 10^{-3} mg/cm^3) > *Lippia javanica* (LD_{50} 4.338 x 10^{-3} mg/cm^3) > *Tetradenia riparia* (LD_{50} 4.429 x 10^{-3} mg/cm^3) > *Lippia ukambensis* (LD_{50} 4.655 x 10^{-3} mg/cm^3). None of the six plants showed tarsal contact activity. This could be due to their high volatility, which made them evaporate from the surface of the filter paper prior to the introduction of the insects.

Of all the tested compounds identified from the leaf oils of the six plants, only two compounds (perillaldehyde and perill alcohol) from *C. newii* oil were found to be exhibiting mosquitocidal activity by both tarsal contact and fumigation methods, although, *C. newii* oil itself did not show tarsal contact activity. Of the two compounds, perillaldehyde (LD_{50} 4.8675 x 10^{-3} mg/cm^2 in tarsal contact and 1.0451 x 10^{-4} mg/cm^3 in fumigation) was found to be more active than perill alcohol (LD_{50} 5.2753 x 10^{-3} mg/cm^2 in tarsal contact and 2.5183 x 10^{-4} mg/cm^3 in fumigation). The insecticidal activity of these two compounds has not been reported before.

6.2 Future directions

- Bio-assays of the other identified compounds in the leaf oils of the six plants should be done. This might give some more potent repellent or mosquitocidal compounds.
- It would be interesting to investigate the activity of the solvent extracts of these plants as repellents and insecticides.
- Optimisation of formulations of the compounds that showed good repellent activity needs to be done. This might probably lead to good topical repellents of plant origin.
- Although perillaldehyde, is a good insecticide and moderate repellent, it is a volatile compound that may require structural modifications to reduce volatility and to optimise the insecticidal activity. This may lead to compound(s) with good insecticidal/repellent activity, which may be useful in the treatment of mosquito bed nets.
- Further bio-prospecting activities should be carried out in the regions covered, as well as in the other parts of the country not covered by this research project. There is no doubt that there could still be many more unknown mosquito repellent/mosquitocidal plants in Kenya.

CHAPTER 7: EXPERIMENTAL

7.1 Glassware

The general-purpose glassware were well cleaned with 6 M HNO_3 acid, rinsed with water and acetone before drying them in an oven. The glassware for collection and storage of the sample extracts were chemically cleaned by soaking them in freshly prepared chromic acid overnight and cleaned with distilled water. They were then rinsed with the appropriate solvents and dried in the oven before use.

7.1.1 Chemicals and solvents

The solvents (acetone, chloroform and dichloromethane) used were pure analytical HPLC grades, purchased from Sigma-Aldrich Chemical Company. All the essential oil standards used for the GC co-injection and bio-assay were bought from Sigma-Aldrich Chemical Company and Fluka Chemika Company.

7.2 Plant collection, identification, and preparation

Collection of the plants was based on chemo-taxonomy, phytochemical, and ethno-botanical information and random selection of aromatic plants. *Conyza newii* (aerial parts) and *Tarchonanthus camphoratus* (leaves) were collected from West Pokot and Naivasha, respectively. Similarly, the leaves of *Lippia javanica* and *Lippia ukambensis* were collected from Oyugis and Naivasha (slightly past the Baboon Cliff), respectively. *Plectranthus marrubioides* and *Tetradenia riparia* (*Iboza multiflora*) leaves were collected from Naivasha (near the Baboon Cliff). The collected plants were identified by a plant taxonomist from the University of Nairobi (UoN), Botany Department and the National Museums of Kenya. Sample specimens were then deposited at the E.A and UoN Herbaria.

The samples (leaves, flowers or whole aerial parts) were dried under shade for seven days before extraction.

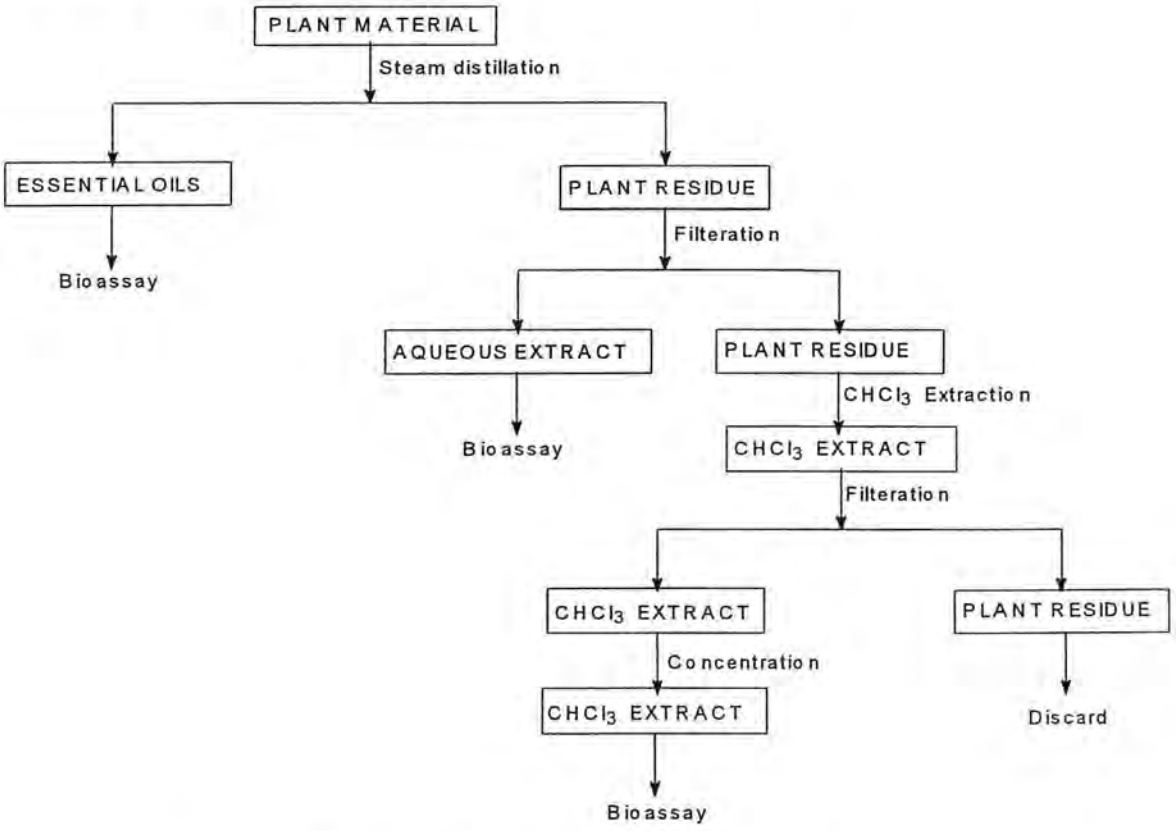
7.3 Extraction

The essential oils from the plant samples (leaves, flowers or whole aerial parts) were extracted by steam distillation using modified Clevenger or the Dean-Stark apparatus.

Various quantities (700 – 1300 g) of each of the plant material were put into a 5 litre round-bottom flask and 1500 ml of tap water added.

The flask was then fitted with the Clavenger/Dean-Stark apparatus and a double pocket condenser. The plant material was steam distilled for 8 hours. The essential oil produced was collected on water layer in the Clavenger/Dean-stark apparatus. It was separated, dried with anhydrous sodium sulphate and stored in amber-coloured vials at 0°C until use. The amount of essential oil from each plant is summarised in table 13.

The water extract left in the flask was carefully decanted into a clean glass bottle (250 ml) and stored in the fridge for bio-assay. Non-volatile organic compounds left in the plant residue after steam distillation were extracted with chloroform (2.5 - 3 litres) for 24 hours. The chloroform extract of each of the plant samples was decanted, dried, filtered and concentrated using the rotor vapour. The samples were kept in clean vials for bio-assay. The amounts of chloroform extracts are summarised in table 13. The whole process of extraction is also summarised in scheme 3.



Scheme 3. The flow chart of the extraction process

Table 13. The amount of essential oil and chloroform extract from each plant

Plant name	% Yield of oil	% Yield of $CHCl_3$ extract
*Tarchonanthus camphoratus**	0.580	2.495
*Lippia javanica**	1.040	2.352
*Plectranthus marrubioides**	1.850	2.835
*Tetradenia riparia**	0.350	2.865
*Lippia ukambensis**	1.760	2.468
*Conyza newii**	4.360	2.935
Croton dichogamus (M) *	0.580	2.395
Croton dichogamus (R) *	0.580	2.388
*Ocimum lamiifolia**	0.360	2.698
*Bidens pilosa**	0.040	1.064
*Schinus molle**	4.760	3.005
*Lantana camara**	0.400	2.824
*Teclea simplisifolia**	0.340	2.893
Helicrysum spp*	0.050	1.288
*Hyptis pectinata**	0.340	2.729
*Psidia punctulata**	0.070	1. 159
Ajuga remota¶	0.004	0.965
Teclea nobilis¶	0.013	2.348
Teclea trichocarpa¶	0.010	2.258
Leonotis molisina¶	0.012	2.338
Clerodendrum rotundifolia¶	0.010	2.266
Salvia cocainea¶	0.014	2. 195
Hoslundia opposita¶	0.013	2.832
Lippia grandifolia¶	0.013	2.545
Plectranthus A¶	0.013	3.361
Plectranthus B¶	0.012	2.806
Plectranthus barbetus¶	0.010	1.290
Fuestas africana¶	0.010	2.972
Leonotis nepetifolia¶	0.010	2.423
Acalyfa fruticosa¶	0.010	1.214
Neoboutania macrocalyx¶	0.010	1.247
Tithonia diversifolia¶	0.004	0.896
B. bagshawei¶	0.006	1.556

Key: * Repellency bioassay of oil done, ¶ Repellency bioassay of leaf oil not done

7.4 Purification and chemical identification

Characterization, identification and determination of the components of the essential oils from the repellent/insecticidal plants was done by gas chromatography (GC), gas chromatography - mass spectrometry (GC-MS), and GC co-injection of the essential oils with authentic standards.

7.4.1 Gas chromatography (GC)

Gas chromatographic separation was performed on a capillary gas chromatograph, Hewlett Packard (HP) model 5890 Series II equipped with a splitless capillary injector system, a flame ionization detector (FID) coupled to an integrator (HP 3393A Series II). The separation was done in a cross-linked methylsilicone capillary column, 50 m x 0.2 mm (i.d) x 0.33μm (film thickness) supplied by Hewlett Packard. Carrier gas was white spot nitrogen at a flow rate of 0.7 ml/min. The fuel used was hydrogen (analytical grade) together with medical air (pure oxygen). The temperature programme was 50°C (5 min.) to 280°C @ 5° C/min (10 min.).

For GC analysis 10% solutions of the essential oils in CH_2Cl_2 were used, and 1-2 μl quantities were injected with a 10 μl syringe. For standards, 2% solutions in CH_2Cl_2 were similarly analysed and their retention times compared to those of the components in the essential oils of the plants. The GC profiles for the essential oils of the six plants are given in figures 7-11.

7.4.2 Gas chromatography - mass spectrometry (GC-MS)

GC-MS analyses were carried out on a HP 8060 Series II Gas Chromatograph coupled to a VG Platform II Mass Spectrometer. The mass spectrometer was operated in the electron ionization (EI) mode at 70 eV and an emission current of 200 μA. The temperature of the source was held at 180°C and the multiplier voltage was 300 V. The pressure of the ion source was held at 9.4×10^{-6} mBar, while that of the analyser (MS detector) was 1.4×10^{-5} mBar. The spectrometer had a scan cycle of 1.5 seconds (scan duration of 1 second and interscan delay of 0.5 second). The mass range was set at m/z 1-1400. The scan range for the samples was however from m/z 38-650. The instrument was calibrated using heptacosafluorotributylamine [$CF_3(CF_2)_3$]$_3$N,

(Apollo Scientific Ltd. UK). The GC column used was the same as the one described for the GC analysis except for the film thickness of 0.5 μm. The temperature programme is illustrated in figure 15.

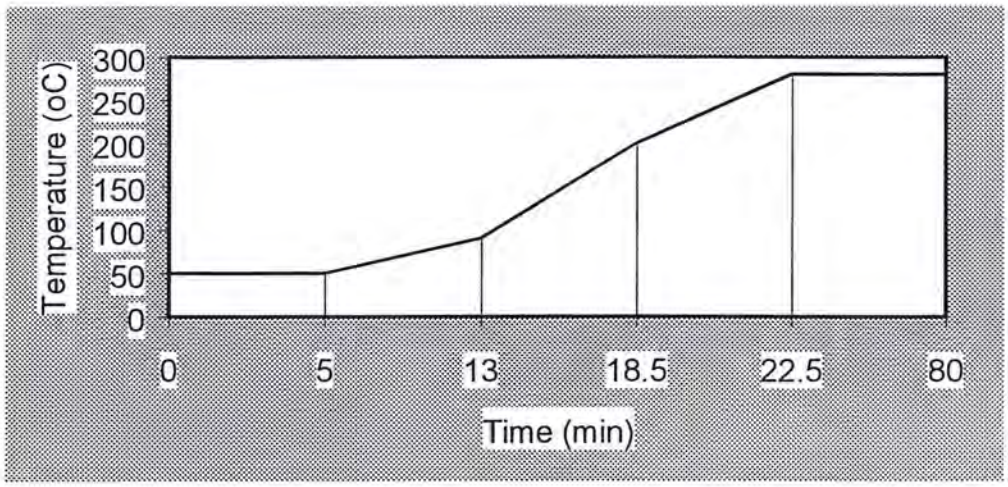


Figure 15. The temperature programme for the GC-MS.

The GC-MS was linked to a computer with MS data library (NIST and WILEY). The compounds were identified by comparing their MS with those of authentic samples or with MS library data. This was further confirmed by GC co-injection.

7.4.3 GC co-injection

Identity of the components of the essential oils was confirmed by peak enhancement upon GC co-injection of the crude essential oils with authentic standards.

A list of all the essential oils identified in the six repellent/ insecticidal plants is given together with their % peak areas in table 14. In the table, t = traces (< 0.001%) while *Conyza newii*, *Lippia javanica*, *Plectranthus marrubioides*, *Lippia ukambensis*, *Tetradenia riparia*, and *Tarchonanthus camphoratus* are represented by the initials: CN, LJ, PM, LU, TR and TC, respectively.

Table 14. The chemical composition of essential oil of the six plants

Compound	CN	LJ	PM	LU	TR	TC
α-Pinene	0.35	t	0.17	1.13	t	16.62
Camphene	0.13	0.31	1.58	8.63	t	16.82
α-Phellandrene		t		1.28	0.49	
β-Phellandrene	t	0.23	0.15	1.21	0.49	
β-Pinene	0.18	0.08	0.81	1.00	0.78	0.78
β-Myrcene	1.16	3.16	0.16	1.69	0.8	t
1,3,8-*p*-Menthatriene	t	t	t			
δ-2-Carene	t		t	t	t	
trans-β-Ocimene	1.35	1.06	0.14	0.59	1.0	t
δ-3-Carene		0.76			t	
δ-4-Carene	t		t		t	0.91
Limonene	10.06	2.58	t	0.29	2.02	0.69
1,8-Cineole	6.84	t	9.00	2.42	1.50	6.51
Limonene oxide		38.99				
α-Terpinene	t	t	2.58	t	t	0.27
trans-Sabinene hydrate	t	t	1.19	14.21	t	0.21
Linalool	0.11	2.69	t	0.40		0.88
p-Cymene	t		3.08	0.67	0.55	2.89
o-Cymene						t
Citronellal		t				
Thujol			t			
γ-Terpinene	t		0.96	1.42	0.96	0.56
Linalool oxide			t			t
cis-Sabinene hydrate	t		t			
Geraniol	1.17					
Limonene dioxide			2.50			
Phenylethyl alcohol	t					
Artemisia ketone	0.12	2.78				
α-Terpinolene	t		0.27	0.86		0.38
cis-Carveol			0.20			0.34
Camphor	0.17	0.75	48.80	39.84	0.13	0.38
α-Terpineol	t	2.04	0.38	t	t	3.78
Borneol	t	t	0.36	1.14	t	t
α-Campholene aldehyde			0.06			
Limonenyl-10-acetate	0.89					
Myrtenol	t		t			t
Neral	t					
Carvone	t	0.4	t			
Perillaldehyde	29.28					

Table 14. Continued

Compound	CN	LJ	PM	LU	TR	TC
Perill alcohol	4.27					
Phellandral			t			
Myrtenyl acetate	0.12					
Geranyl acetate	0.69					
Camphene hydrate			t			
p-Cymen-8-ol			0.36			0.96
Terpen-4-ol		t	1.08		t	3.28
cis-p-Mentha-1,8-dien-2-ol			t			
Pinocarvone			t	0.76	t	
Chrysanthenone		0.55				
Myrcenol		0.73				
α-Fenchyl alcohol	0.21				0.73	14.76
Piperitenone		1.07				
Piperitone oxide			0.54			
Verbenone		6.06		0.78		
cis-Verbenol		11.33			t	0.30
Carvacrol		0.46	0.1			
Linalyl propanoate			0.55			
Sabinol			t			
Fenchone			1.75	0.17	64.82	0.43
cis-2-Pinanol	t		t			
α-Pyronene		0.12			0.42	
Thujone					t	
Solalone			0.13			
p-Menth-3-en-1-ol				1.39	0.37	
α-Fenchyl acetate					0.39	0.83
α-Thujene				0.82		0.29
p-Menth-2-en-1-ol			0.06	0.07	t	0.54
Ylangene	t					
α-Caryophyllene	0.56	0.14	0.30	0.12		
Germacrene B	1.45			t	0.13	
Germacrene D	0.69	0.24	0.15	1.45	0.59	
Cycloisosativene			t			
Isocaryophyllene	0.58	1.38	1.67	0.43	0.10	1.36
α-Amorphene	1.11		t			
Spathulenol	0.19		t			0.48
β-Bisabolene		t				

Table 14. Continued

Compound	CN	LJ	PM	LU	TR	TC
Isospathulenol			0.50			
p-Menth-2-en-1-one				t		
Ascaridole			0.30			
Eugenol		0.46	t			
Methyleugenol	0.37	0.27		0.9		
α-Copaene	0.27	t	0.12	0.38	0.13	0.04
β-Bourbonene		0.40		0.49	0.43	t
Alloaromadendrene		0.13	0.11			1.19
Aromadendrene		0.31				
α-Ar-curcumene		t				0.98
α-Farnesene		0.18		t		
Nerolidol		t				
Isoascaridole			t			
Caryophyllene oxide		0.20	1.13	t		1.06
γ-Cadinene		0.17	0.33			
Eucarvone		0.10				
δ-Cadinene			0.20			0.41
Monoterpene alcohol	0.88					0.24
Thymol			0.12			
α-Cubebene			t	t	t	t
β-Bisabolol						0.42
β-Elemene			0.44			
α-Gurjunene			0.17			
β-Selinene			1.50			
α-Fenchene	0.08		0.10		t	0.12
α-Selinene			0.21			
Ledol			0.20			
β-Eudesmol	t		0.40			3.12
Viridiflorol			1.61			0.34
α-Cadinol	t				t	0.84
γ-Curcumene	t		t		t	0.15
trans-Pinan-2-ol						t
Myrtenal						0.38
cis-p-Menth-2-en-1-ol						0.13
4-Isopropylbenzaldehyde	0.78		t			
1,3,8-Trimethylbicyclo[2.2.1]heptan-2-one					11.68	
2-Methyl-5-(Methylethyl)-2-cyclohexen-1-ol	7.34					

7.5 Bio-assays

The essential oils, chloroform, and water extracts were bio-assayed for their repellency and mosquitocidal activity against the female *Anopheles gambiae* mosquitoes [ex-Ifakara (Tanzania) strain] that were reared under standard conditions at the ICIPE Duduville mosquito insectary. Both repellency and mosquitocidal assays were based on the WHO (1996) protocols for the laboratory and field evaluation of insecticides and repellents.

7.5.1 Mosquito repellency assays (WHO, 1996)

The bio-assays were carried out in a dark room with red light as the only source of illumination. The room temperature and humidity were artificially set using a heater and humidifier to mimic the host feeding conditions for the female *A. gambiae* (Temp. 27-35°C and Relative Humidity > 65%). All the repellency tests were carried out on 5-7 days old female *A. gambiae* mosquitoes that had been starved over night, but previously fed on 6% glucose solution.

Six (6) human volunteers were used in repellency assays. They were not allowed to use lotions, perfumes, oils and perfumed soaps on the day of the bio-assay.

7.5.1.1 Preliminary screening

The preliminary screening of the essential oils and chloroform extracts were done with (0.001, 0.1 and 10%) and (0.05, 0.5 and 5%) solutions of the essential oils and chloroform extracts in acetone, respectively. Each of these solutions was screened using six different human volunteers (3 females and 3 males). A total of 18 cages each measuring 50 x 50 x 50 cm were used, with 25 starved female *Anopheles gambiae* mosquitoes in each cage. Test solution (0.5 ml) was dispensed on the right forearm of a volunteer from the wrist to the elbow. The rest of the hand was covered with a glove to make it unattractive to the mosquitoes. Acetone (0.5 ml) was dispensed on the left forearm, to act as control. The arms were swapped regularly to eliminate any bias. The control arm was introduced into the cage immediately after releasing the 25 insects and kept there for 3 minutes. The mosquitoes that landed on that arm during the test duration were recorded. The treated arm was then introduced into the cage and kept there for 3 minutes. The number of mosquitoes that landed on the treated arm was recorded.

The screening was done sequentially starting with the lowest dose (0.001%) and ending with the highest one (10%). Each concentration was screened with fresh batch of mosquitoes. After the bio-assay of each concentration, the arms were washed with bar soap, rinsed well with tap water and then allowed to dry for 15-20 minutes, before application of the next dose of the test sample. The % protective efficacy (PE) was calculated as follows:

$$PE = (\frac{PCM - PTM}{PCM}) \text{ X } 100\%$$

Where PCM is the percent control mean and PTM is the percent test mean of mosquitoes landing on the control and treated arms respectively (Mehr *et al.*, 1985). The results of the preliminary repellency assay of essential oils and chloroform extracts are summarised in tables 15 to 16.

Table 15. Preliminary repellency assay data of the essential oils

	% PE ± 0.05		
Plant oil	**10^{-1} g/ml (10%)**	**10^{-3} g/ml (0.1%)**	**10^{-5} g/ml (0.001%)**
T. camphoratus.	98.5	34.2	22.3
L. javanica	90.3	57.9	48.7
P. marrubioides	81.8	58.3	33.2
T. riparia	79.6	42.7	37.7
L. ukambensis	83.9	52.2	32.4
C. newii	100	45.5	27.9
C. dichogamus (M)	73.0	45.8	35.2
C. dichogamus (R)	81.6	32.5	21.8
O. lamiifolia	81.4	60.6	27.5
B. pilosa	90.5	64.3	13.9
S. molle	57.8	26.5	25.5
L. camara	91.6	49.4	43.2
T. simplisifolia	99.5	44.9	32.8
Helicrysum spp	65.4	32.4	41.3
H. pectinata	90.2	46.6	44.4
P. punctulata	93.8	43.7	32.5

Table 16. Preliminary repellency assay data of chloroform extracts

	% PE ± 0.05		
Plant	**0.0005 g/ml**	**0.005 g/ml**	**0.05 g/ml**
T. camphoratus	18.4	25.9	45.4
L. javanica	25.2	32.4	48.6
P. marrubioides	22.8	37.9	50.9
T. riparia	38.5	48.4	85.3
L. ukambensis	23.7	35.8	52.5
C. newii	22.3	28.3	83.2
C. dichogamus (M)	46.9	43.5	31.8
C. dichogamus (R)	44.5	38.4	30.7
O. lamiifolia	30.3	34.6	56.3
B. pilosa	18.9	24.8	38.5
S. molle	12.2	21.6	28.7
L. camara	21.8	31.5	38.3
T. simplisifolia	13.6	22.6	36.6
Helicrysum spp	23.3	50.4	48.7
H. pectinata	36.7	39.8	80.9
P. punctulata	24.4	36.5	60.1
A. remota	18.9	26.7	53.6
T. nobilis	20.6	28.5	39.4
T. trichocarpa	22.4	30.3	36.5
L. molisina	14.5	21.9	28.6
C. rotundifolia	10.1	16.5	26.7
S. coccinae	13.7	19.3	25.4
H. opposita	18.3	22.5	36.3
L. grandifolia	21.5	27.9	38.6
Plectranthus A	5.8	16.7	24.4
Plectranthus B	8.6	14.2	23.5
P. barbetus	10.5	16.7	28.8
F. africana	6.8	18.3	31.3
L. nepetifolia	13.4	19.5	25.2
A. fruticosa	5.8	13.3	27.5
N. macrocalyx	6.4	17.9	24.5
T. diversifolia	22.6	38.5	63.9
B. bagshawei	20.5	28.6	42.3

The aqueous extract (0.5 ml) was similarly dispensed on the right forearm of a volunteer and the same quantity of distilled water on the left arm to act as control. Repellency tests were done and % protective efficacy calculated as previously described. The repellency results are summarised in table 17.

Table 17. Preliminary repellency assay data of water extracts

Plant	Mean No. of insects on Test arm ± 0.05	Mean No. of insects on Control arm ± 0.05	% Protective Efficacy ± 0.005
T. camphoratus	18.2	17.5	-5.88
L. javanica	14.6	16.4	12.50
P. marrubioides	17.3	19.8	10.52
T. riparia	10.8	14.4	28.57
L. ukambensis	20.6	18.6	-11.11
C. newii	16.9	18.5	11.11
C. dichogamus (M)	14.5	17.3	17.65
C. dichogamus (R)	13.4	15.4	13.33
O. lamiifolia	19.3	22.8	13.64
B. pilosa	15.4	18.7	16.67
S. molle	20.8	20.4	0
L. camara	17.5	20.8	15.00
T. simplisifolia	16.3	18.6	11.11
Helicrysum spp	12.5	14.4	14.28
H. pectinata	14.7	16.9	12.50
P. punctulata	17.3	19.5	10.53
A. remota	11.4	13.2	15.85
T. nobilis	14.9	14.8	0
T. trichocarpa	16.4	17.1	5.88
L. molisina	18.7	19.0	5.26
C. rotundifolia	12.4	14.3	14.28
S. coccinae	10.9	11.7	9.09
H. opposita	13.3	13.4	0
L. grandifolia	14.8	17.5	17.65
Plectranthus A	10.7	10.2	0
Plectranthus B	12.5	13.0	7.69
P. barbetus	18.3	18.8	0
F. africana	15.9	18.6	16.67
L. nepetifolia	13.4	14.3	7.14
A. fruticosa	20.9	21.1	4.76
N. macrocalyx	19.6	18.9	-5.56
T. diversifolia	17.3	18.6	5.56
B. bagshawei	19.5	20.8	5.00

7.5.1.2 Detailed mosquito repellency bio-assay of essential oils

Detailed bio-assay of the plant extracts with good preliminary mosquito repellency was done. In this bio-assay, 1 g of the neat oil from each plant sample was dissolved in 10 ml of acetone to give 10^{-1} g/ml (10%) solution. By serial dilution with acetone, 1% (10^{-2} g/ml), 0.1% (10^{-3} g/ml), 0.01% (10^{-4} g/ml), and 0.001% (10^{-5} g/ml) solutions were prepared. All the solutions were assayed, with each being subjected to a fresh batch of 100 female *A. gambiae* mosquitoes in each cage.

For each dosage, the volume of repellent solution on the test arm ranged from 0.6 to 1.0 cm^3 (depending on the surface area of the arm). The surface area of the arms of volunteers were estimated using a paper and were found to be 426.50 cm^2, 428.50 cm^2, 484.25 cm^2, 528.75 cm^2, 530.50 cm^2, and 602.75 cm^2 respectively. The average surface area of a volunteer was approximated at 500 cm^2. The volume of the repellent dispensed on each volunteer arm was 0.6, 0.6, 0.7, 0.8, 0.8, and 1.0 ml respectively. This gave the average volume of repellent dispensed on the volunteers' arms to be 0.75 ml. The highest dose of repellent was 100 mg/ml (10%) for essential oil. For 10% solution, 0.75 ml dispensed, corresponds to 75 mg of the essential oil mixture applied on a 500 cm^2 (0.15 mg/cm^2). For 1% solution, dispensing 0.75 ml corresponds to 7.5 mg of the essential oil applied on a 500 cm^2 (0.015 mg/cm^2). Hence for 0.1% solution, the dose is 1.5×10^{-3} mg/cm^2. The lowest concentration (0.001%) therefore corresponds to 1.5×10^{-5} mg/cm^2. The % protective efficacy was calculated as detailed above. The results of the detailed repellency assay of essential oils from the 6 plants are summarised in tables 18 to 23. In these tables, DW, MA, JA, JT, FM, and DK are the initials for the names of the volunteers used to produce the desired number of replicates; while C and T represent control and treated arms, respectively. The numbers for C and T represent mosquitoes landing on control and treated hands, respectively. P.E is the protective efficacy calculated as described earlier.

Table 18. Detailed repellency assay data of *Conyza newii* oil

Conc. (g/ml)	% PE ± 0.005	Assay	DK	FM	JT	MA	DW	JA	Mean ± 0.005
10^{-5}	44.93	C	25	33	18	36	70	25	34.5
		T	21	21	15	27	21	9	19
10^{-4}	52.11	C	30	38	39	50	58	46	43.5
		T	16	16	26	27	20	20	24.83
10^{-3}	59.15	C	33	25	40	49	56	32	39.17
		T	11	13	15	23	18	16	16
10^{-2}	100	C	39	33	49	54	74	61	51.67
		T	0	0	0	0	0	0	0
10^{-1}	100	C	36	20	40	54	48	48	41
		T	0	0	0	0	0	0	0

Initial: Temp. 26^{0}C % RH. 80 Time. 8.00 a.m Final: Temp. 28^{0}C % RH. 78 Time. 12.30 p.m

Table 19. Detailed repellency assay data of *Lippia javanica* oil

Conc. (g/ml)	% PE ± 0.005	Assay	DW	MA	JA	JT	FM	DK	Mean ± 0.005
10^{-5}	28.67	C	38	42	43	33	30	44	38.33
		T	23	41	25	25	20	30	27.33
10^{-4}	43.36	C	56	40	30	75	10	45	42.67
		T	13	28	18	47	2	37	24.17
10^{-3}	50.76	C	85	20	80	26	17	30	43
		T	34	10	51	5	7	20	21.17
10^{-2}	87.58	C	45	40	47	15	17	22	31
		T	26	4	4	2	4	5	7.5
10^{-1}	96.94	C	40	30	36	24	28	38	32.67
		T	6	0	0	0	0	0	1

Initial: Temp. 27^{0}C % RH. 84 Time. 8.30 a.m Final: Temp. 29^{0}C % RH. 80 Time. 1.00 p.m

Table 20. Detailed repellency assay data of *Plectranthus marrubioides* oil

Conc. (g/ml)	% PE ± 0.005	Assay	JA	MA	DW	DK	JT	FM	Mean ± 0.005
10^{-5}	41.36	C	56	48	62	68	63	68	60.83
		T	38	30	34	39	38	35	35.67
10^{-4}	53.8	C	42	50	58	45	55	53	50.5
		T	16	23	34	15	35	17	23.33
10^{-3}	60.43	C	55	50	58	56	48	54	53.5
		T	21	20	24	25	21	16	21.17
10^{-2}	88.68	C	68	56	68	95	35	40	60.33
		T	10	2	8	15	0	2	6.83
10^{-1}	100	C	52	58	45	60	58	48	53.5
		T	0	0	0	0	0	0	0

Initial: Temp. 28^{0}C % RH. 78 Time. 8.00 a.m Final: Temp. 32^{0}C % RH. 75 Time. 12.35 p.m

Table 21. Detailed repellency assay data of *Lippia ukambensis* oil

Conc. (g/ml)	% PE ± 0.005	Assay	JA	DK	JT	DW	FM	MA	Mean ± 0.005
10^{-5}	34.28	C	40	50	56	60	58	48	52
		T	28	30	33	38	48	28	34.17
10^{-4}	46.25	C	50	56	55	62	60	50	55.5
		T	28	34	30	33	29	25	29.83
10^{-3}	53.21	C	52	50	51	64	58	52	54.5
		T	28	21	20	34	26	24	25.5
10^{-2}	61.76	C	45	52	56	60	55	51	53.17
		T	18	20	21	25	19	19	20.33
10^{-1}	84.11	C	53	55	40	58	60	55	53.5
		T	8	9	10	10	9	5	8.5

Initial: Temp. 28^{0}C % RH. 78 Time. 8.15 a.m Final: Temp. 30^{0}C % RH. 78 Time. 12.50 p.m

Table 22. Detailed repellency assay data of *Tetradenia riparia* oil

Conc. (g/ml)	% PE ± 0.005	Assay	FM	MA	JA	DK	DW	JT	Mean ± 0.005
10^{-5}	34.75	C	55	50	62	56	60	48	55.17
		T	38	34	38	39	40	27	36
10^{-4}	38.88	C	50	48	60	55	56	50	53.17
		T	32	30	31	35	33	34	32.5
10^{-3}	54.09	C	52	54	58	52	46	56	53
		T	26	27	29	20	20	24	24.33
10^{-2}	60.82	C	46	58	50	48	54	58	52.33
		T	20	24	20	18	21	20	20.5
10^{-1}	87.68	C	50	57	48	46	60	64	54.17
		T	8	9	5	5	7	6	6.67

Initial: Temp. 26^{0}C % RH. 84 Time. 8.05 a.m Final: Temp. 29^{0}C % RH. 81 Time. 12.55 p.m

Table 23. Detailed repellency assay data of *Tarchonanthus camphoratus* oil

Conc. (g/ml)	% PE ± 0.005	Assay	DK	JA	MA	DW	JT	FM	Mean ± 0.005
10^{-5}	24.55	C	50	45	60	55	68	60	56.33
		T	40	32	45	40	50	48	42.5
10^{-4}	29.52	C	62	49	56	48	72	63	58.33
		T	40	31	40	36	52	48	41.17
10^{-3}	44.83	C	59	46	58	58	60	58	56.5
		T	32	28	32	30	34	31	31.17
10^{-2}	63.69	C	45	48	55	50	62	54	52.33
		T	20	18	20	19	20	17	19
10^{-1}	100	C	55	49	60	51	64	56	55.83
		T	0	0	0	0	0	0	0

Initial: Temp. 26^{0}C % RH. 82 Time. 8.15 a.m Final: Temp. 29^{0}C % RH. 78 Time.12.5 0 p.m

The RD_{50} values for the essential oils were calculated by probit analysis (Busvine, 1971). The results are summarised in tables 24 to 29.

Table 24. Probit analysis of repellency assay data of *Conyza newii* leaf oil

% Repellency	Dose (mg/cm^2)	Log dose +5	Emperical Probit	Regression Equation	Expected Probit	Working Probit	Weighting Coefficient	Weight	
		x			y			w	wx
100	1.5×10^{-1}	4.176	_	$y = 4.82 + 0.19x$	5.61	6.42	0.558	55.8	233.0208
100	1.5×10^{-2}	3.176	_		5.42	6.34	0.601	60.1	190.8776
59.15	1.5×10^{-3}	2.176	5.23		5.23	5.23	0.627	62.7	136.4352
52.11	1.5×10^{-4}	1.179	5.05		5.04	5.06	0.637	63.7	74.9112
44.93	1.5×10^{-5}	0.176	4.85		4.85	4.87	0.634	63.4	11.1584

$Sw = 305.7$; $Swx = 646.4032$; $Swx^2 = 115085.4221$; $\bar{X} = \frac{Swx}{Sw} = 2.1145$; When $y = 5$, $x = m = 0.9474$;

$RD_{50} = 0.0000886 mg/cm^2$; $V = \frac{1}{b^2}\left\{\frac{1}{Sw} + \frac{(m-\bar{x})^2}{Swx^2 - \frac{(Swx)^2}{Sw}}\right\} = 0.0909$

Table 25. Probit analysis of repellency assay data of *Lippia javanica* leaf oil

% Repellency	Dose (mg/cm^2)	Log dose +5	Emperical Probit	Regression Equation	Expected Probit	Working Probit	Weighting Coefficient	Weight	
		x			y			w	wx
96.94	1.5×10^{-1}	4.176	6.88	$y = 4.118 + 0.622x$	6.72	6.84	0.208	20.8	86.8608
87.58	1.5×10^{-2}	3.176	6.18		6.09	6.14	0.405	40.5	28.6280
50.76	1.5×10^{-3}	2.176	5.03		5.47	4.98	0.581	58.1	26.4256
43.36	1.5×10^{-4}	1.179	4.82		4.85	4.83	0.634	63.4	74.5584
28.67	1.5×10^{-5}	0.176	4.45		4.23	4.46	0.503	50.3	8.8528

$Sw = 233.1$; $Swx = 425.3256$; $Swx^2 = 45710.7204$; $\bar{X} = \frac{Swx}{Sw} = 1.8246$; When $y = 5$, $x = m = 1.4180$;

$RD_{50} = 0.0002618 mg/cm^2$; $V = \frac{1}{b^2}\left\{\frac{1}{Sw} + \frac{(m-\bar{x})^2}{Swx^2 - \frac{(Swx)^2}{Sw}}\right\} = 0.0111$

Table 26. Probit analysis of repellency assay data of *Plectranthus marruboides* leaf oil

% Repellency	Dose (mg/cm^2)	Log dose +5	Emperical Probit	Regression Equation	Expected Probit	Working Probit	Weighting Coefficient	Weight	
		x			y			w	wx
100	1.5×10^{-1}	4.176	_	$y = 4.569 + 0.455x$	6.46	6.94	0.302	30.2	126.1152
88.68	1.5×10^{-2}	3.176	6.23		6.01	6.18	0.439	43.9	139.4264
60.43	1.5×10^{-3}	2.176	5.25		5.56	5.25	0.581	58.1	126.4256
53.8	1.5×10^{-4}	1.179	5.08		5.10	5.09	0.634	63.4	74.5584
41.36	1.5×10^{-5}	0.176	4.77		4.65	4.78	0.601	60.1	10.5776

$Sw = 255.7$; $Swx = 477.1032$; $Swx^2 = 56999.0377$; $\bar{X} = \frac{Swx}{Sw} = 1.8659$; When $y = 5$, $x = m = 0.9473$;

$RD_{50} = 0.0000885\ mg/cm^2$; $V = \frac{1}{b^2}\left\{\frac{1}{Sw} + \frac{(m-\bar{x})^2}{Swx^2 - \frac{(Swx)^2}{Sw}}\right\} = 0.0190$

Table 27. Probit analysis of repellency assay data of *Lippia ukambensis* leaf oil

% Repellency	Dose (mg/cm^2)	Log dose +5	Emperical Probit	Regression Equation	Expected Probit	Working Probit	Weighting Coefficient	Weight	
		x			y			w	wx
84.11	1.5×10^{-1}	4.176	5.99	$y = 4.475 + 0.321x$	5.82	5.98	0.503	50.3	210.0528
61.76	1.5×10^{-2}	3.176	5.31		5.49	5.29	0.581	58.1	184.5256
53.21	1.5×10^{-3}	2.176	5.08		5.17	5.08	0.627	62.7	136.4352
46.25	1.5×10^{-4}	1.179	4.9		4.85	4.9	0.634	63.4	74.5584
34.28	1.5×10^{-5}	0.176	4.59		4.53	4.59	0.581	58.1	10.2256

$Sw = 292.6$; $Swx = 615.7976$; $Swx^2 = 102449.9575$; $\bar{X} = \frac{Swx}{Sw} = 2.1046$; When $y = 5$, $x = m = 1.6355$;

$RD_{50} = 0.0004320\ mg/cm^2$; $V = \frac{1}{b^2}\left\{\frac{1}{Sw} + \frac{(m-\bar{x})^2}{Swx^2 - \frac{(Swx)^2}{Sw}}\right\} = 0.0332$

Table 28. Probit analysis of repellency assay data of *Tetradenia riparia* leaf oil

% Repellency	Dose (mg/cm^2)	Log dose + 5	Emperical Probit	Regression Equation	Expected Probit	Working Probit	Weighting Coefficient	Weight	
		x			y			w	wx
87.68	1.5×10^{-1}	4.176	6.18	y = 4.37 + 0.37x	5.92	6.12	0.471	47.1	196.6896
60.82	1.5×10^{-2}	3.176	5.28		5.54	5.26	0.581	58.1	184.5256
54.09	1.5×10^{-3}	2.176	5.1		5.17	5.1	0.627	62.7	136.4352
38.88	1.5×10^{-4}	1.179	4.72		4.80	4.71	0.627	62.7	73.7352
34.75	1.5×10^{-5}	0.176	4.61		4.43	4.62	0.558	55.8	9.8208

$Sw = 286.4$; $Swx = 601.2064$; $Swx^2 = 96884.3874$; $\bar{X} = \frac{Swx}{Sw} = 2.0992$; When y = 5, x = m = 1.7027 ;

$RD_{50} = 0.0005040\ mg/cm^2$; $V = \frac{1}{b^2}\left\{\frac{1}{Sw} + \frac{(m-\bar{x})^2}{Swx^2 - \frac{(Swx)^2}{Sw}}\right\} = 0.0255$

Table 29. Probit analysis of repellency assay data of *Tarchonanthus camphoratus* leaf oil

% Repellency	Dose (mg/cm^2)	Log dose + 5	Emperical Probit	Regression Equation	Expected Probit	Working Probit	Weighting Coefficient	Weight	
		x			y			w	wx
99.69	1.5×10^{-1}	4.176	7.33	y = 4.164 + 0.351x	5.63	6.42	0.558	55.8	233.0208
63.69	1.5×10^{-2}	3.176	5.36		5.28	5.35	0.627	62.7	199.1352
44.83	1.5×10^{-3}	2.176	4.87		4.92	4.87	0.634	63.4	137.9584
29.42	1.5×10^{-4}	1.179	4.45		4.58	4.46	0.601	60.1	70.6776
24.55	1.5×10^{-5}	0.176	4.33		4.23	4.32	0.503	50.3	8.8528

$Sw = 292.3$; $Swx = 649.6448$; $Swx^2 = 118059.7365$; $\bar{X} = \frac{Swx}{Sw} = 2.2225$; When y = 5, x = m = 2.3818 ;

$RD_{50} = 0.0024080\ mg/cm^2$; $V = \frac{1}{b^2}\left\{\frac{1}{Sw} + \frac{(m-\bar{x})^2}{Swx^2 - \frac{(Swx)^2}{Sw}}\right\} = 0.0278$

7.5.2 Mosquito repellency bio-assay of pure compounds

The various test solutions of the pure compounds were prepared using acetone as described above. The highest concentration was, 1% (0.01 g/ml) and not 10% (0.1 g/ml) as was for the case of the essential oils. Therefore, the doses bio-assayed were 1.5×10^{-5}, 1.5×10^{-4}, 1.5×10^{-3} and 1.5×10^{-2} mg/cm^2 corresponding to 0.001, 0.01, 0.1 and 1% solutions respectively. The repellency assay results of the 8 most active standards of the identified compounds are summarised in tables 30 to 37.

Table 30. Repellency assay data of perillaldehyde

Conc. (g/ml)	% PE ± 0.005	Assay	DK	JA	MA	DW	JT	FM	Mean ± 0.005
10^{-4}	33.13	C	24	27	25	30	28	29	27.17
		T	14	18	16	22	20	19	18.17
10^{-3}	42.46	C	28	30	28	33	26	34	29.83
		T	18	17	15	19	14	20	17.17
10^{-2}	61.29	C	34	35	30	29	32	26	31
		T	13	13	12	14	11	9	12
10^{-1}	100	C	36	37	28	36	35	29	33.5
		T	0	0	0	0	0	0	0

Initial: Temp. 26^{O} C % RH. 84 Time. 8.05 a.m Final: Temp. 29^{O} C % RH. 81 Time. 12.55 p.m

Table 31. Repellency assay data of perill alcohol

Conc. (g/ml)	% PE ± 0.005	Assay	DK	JA	MA	DW	JT	FM	Mean ± 0.005
10^{-4}	37.76	C	32	38	26	33	29	30	31.33
		T	20	24	16	22	16	19	19.5
10^{-3}	61.18	C	30	28	31	27	25	29	28.33
		T	10	8	9	10	14	15	11
10^{-2}	71.75	C	24	34	26	34	31	28	29.5
		T	6	9	8	12	8	7	8.33
10^{-1}	100	C	28	27	31	32	29	26	28.83
		T	0	0	0	0	0	0	0

Initial: Temp. 25^{O} C % RH. 82 Time. 8.05 a.m Final: Temp. 28^{O} C % RH. 81 Time. 12.50 p.m

Table 32. Repellency assay data of caryophyllene oxide

Conc. (g/ml)	% PE ± 0.005	Assay	DK	JA	MA	DW	JT	FM	Mean ± 0.005
10^{-4}	33.13	C	20	28	32	33	29	30	28.67
		T	14	18	20	23	19	21	19.17
10^{-3}	41.47	C	32	31	26	28	30	29	29.33
		T	20	17	18	18	16	14	17.17
10^{-2}	50.58	C	28	27	29	26	32	30	28.67
		T	16	13	13	11	18	14	14.17
10^{-1}	100	C	28	20	30	35	29	32	29
		T	0	0	0	0	0	0	0

Initial: Temp. 27^{o} C % RH. 84 Time. 8.15 a.m Final: Temp. 30^{o} C % RH. 81 Time. 1.05 p.m

Table 33. Repellency assay data of verbenol

Conc. (g/ml)	% PE ± 0.005	Assay	DK	JA	MA	DW	JT	FM	Mean ± 0.005
10^{-4}	40.92	C	26	29	32	36	38	28	31.6
		T	18	15	18	20	23	18	18.67
10^{-3}	49.73	C	24	28	29	36	32	38	31.17
		T	8	19	18	16	13	20	15.67
10^{-2}	76.88	C	23	28	34	24	36	28	28.83
		T	2	7	9	7	8	7	6.67
10^{-1}	100	C	26	36	38	25	33	30	31.33
		T	0	0	0	0	0	0	0

Initial: Temp. 25^{o} C % RH. 82 Time. 8.05 a.m Final: Temp. 29^{o} C % RH. 78 Time. 12.55 p.m

Table 34. Repellency assay data of nerolidol

Conc. (g/ml)	% PE ± 0.005	Assay	DK	JA	MA	DW	JT	FM	Mean ± 0.005
10^{-4}	43.9	C	26	32	24	35	27	20	27.33
		T	15	18	14	19	16	15	15.33
10^{-3}	57.33	C	22	31	22	30	22	23	25
		T	12	9	11	14	8	10	10.67
10^{-2}	83.05	C	19	22	18	21	19	19	19.67
		T	2	6	2	3	4	3	3.33
10^{-1}	93.95	C	22	23	21	18	24	22	22
		T	1	1	2	1	2	1	1.33

Initial: Temp. 27^{o} C % RH. 78 Time. 8.05 a.m Final: Temp. 31^{o} C % RH. 81 Time. 12.02 p.m

Table 35. Repellency assay data of geraniol

Conc. (g/ml)	% PE ± 0.005	Assay	DK	JA	MA	DW	JT	FM	Mean ± 0.005
10^{-4}	29.63	C	18	22	17	15	16	20	18
		T	12	16	11	11	13	13	12.67
10^{-3}	51.19	C	26	28	31	30	29	24	28
		T	10	19	14	12	17	10	13.67
10^{-2}	80.77	C	23	26	28	24	25	30	26
		T	4	5	7	3	5	6	5
10^{-1}	100	C	25	32	36	34	28	27	30.33
		T	0	0	0	0	0	0	0

Initial: Temp. 28^{0} C % RH. 84 Time. 8.25 a.m Final: Temp. 32^{0} C % RH. 81 Time. 1.05 p.m

Table 36. Repellency assay data of carveol

Conc. (g/ml)	% PE ± 0.005	Assay	DK	JA	MA	DW	JT	FM	Mean ± 0.005
10^{-4}	34.93	C	24	30	28	31	25	28	27.67
		T	17	19	18	20	11	13	18
10^{-3}	46.29	C	28	27	31	23	26	27	27
		T	16	15	17	12	14	13	14.5
10^{-2}	80.74	C	26	28	23	30	28	26	26.83
		T	8	2	3	7	6	5	5.17
10^{-1}	100	C	28	27	25	36	29	34	29.83
		T	0	0	0	0	0	0	0

Initial: Temp. 28^{0} C % RH. 84 Time. 8.00 a.m Final: Temp. 32^{0} C % RH. 81 Time. 12.50 p.m

Table 37. Repellency assay data of citronellal

Conc. (g/ml)	% PE ± 0.005	Assay	DK	JA	MA	DW	JT	FM	Mean ± 0.005
10^{-4}	27.78	C	30	32	28	32	31	27	30
		T	22	28	15	20	24	21	21.67
10^{-3}	43.16	C	31	30	27	28	26	34	29.33
		T	14	19	17	16	14	20	16.67
10^{-2}	53.76	C	28	33	32	30	32	31	31
		T	11	16	17	14	15	13	14.33
10^{-1}	95.19	C	27	28	33	23	30	25	27.67
		T	1	2	1	0	0	0	0

Initial: Temp. 27^{0} C % RH. 83 Time. 8.05 a.m Final: Temp. 30^{0} C % RH. 84 Time. 12.55 p.m

The RD_{50} values for the above compounds were calculated by probit analysis (Finney, 1971). The results are summarised in tables 38 to 45.

Table 38. Probit analysis of repellency assay results of perillaldehyde

% Repellency	Dose (mg/cm^2)	Log dose + 5	Emperical Probit	Regression Equation	Expected Probit	Working Probit	Weighting Coefficient	Weight	
		x			Y			w	wx
100	1.5×10^{-2}	3.176		Y=4.457 + 0.36x	5.60	6.42	0.558	27.9	88.61
61.29	1.5×10^{-3}	2.176	5.28		5.24	5.29	0.627	31.35	68.218
42.46	1.5×10^{-4}	1.176	4.8		4.88	4.81	0.634	31.7	37.279
33.13	1.5×10^{-5}	0.176	4.56		4.52	4.56	0.581	29.05	5.1128

$Sw = 120$; $Swx = 199{\cdot}22$; $Swx^2 = 13921{\cdot}3234$; $\bar{X} = \frac{Swx}{Sw} = 1{\cdot}6602$; When y = 5, x = m = 1·5083;

$RD_{50} = 0{\cdot}00032\ mg/cm^2$; $V = \frac{1}{b^2}\left\{\frac{1}{Sw} + \frac{(m-\bar{x})^2}{Swx^2 - \frac{(Swx)^2}{Sw}}\right\} = 0{\cdot}0643$

Table 39. Probit analysis of repellency assay results of perill alcohol

% Repellency	Dose (mg/cm^2)	Log dose + 5	Emperical Probit	Regression Equation	Expected Probit	Working Probit	Weighting Coefficient	Weight	
		x			Y			w	wx
100	1.5×10^{-2}	3.176		Y=4.649 + 0.44x	6.05	6.72	0.405	20.25	64.314
71.75	1.5×10^{-3}	2.176	5.55		5.61	5.57	0.558	27.9	60.71
61.18	1.5×10^{-4}	1.176	5.28		5.17	5.29	0.627	31.35	36.868
37.76	1.5×10^{-5}	0.176	4.67		4.73	4.69	0.616	30.8	5.208

$Sw = 110.3$; $Swx = 167.1$; $Swx^2 = 9208.38646$; $\bar{X} = \frac{Swx}{Sw} = 1.5149$; When y = 5, x = m = 0.7977 ;

$RD_{50} = 0.000063\ mg/cm^2$; $V = \frac{1}{b^2}\left\{\frac{1}{Sw} + \frac{(m-\bar{x})^2}{Swx^2 - \frac{(Swx)^2}{Sw}}\right\} = 0.0471$

Table 40. Probit analysis of repellency assay results of caryophyllene oxide

% Repellency	Dose (mg/cm^2)	Log dose + 5	Emperical Probit	Regression Equation	Expected Probit	Working Probit	Weighting Coefficient	Weight	
		x			y			w	wx
100	1.5×10^{-2}	3.176		Y=4.51 + 0.235x	5.26	6.3	0.616	30.8	97.821
50.58	1.5×10^{-3}	2.176	5.03		5.02	5.02	0.637	31.85	69.306
41.47	1.5×10^{-4}	1.176	4.77		4.79	4.78	0.627	31.35	36.868
33.13	1.5×10^{-5}	0.176	4.56		4.55	4.56	0.601	30.05	5.2888

$Sw = 124.05$; $Swx = 209.28$; $Swx^2 = 15759.3664$; $\bar{X} = \frac{Swx}{Sw} = 1.6871$; When $y = 5$, $x = m = 2.0852$;

$RD50 = 0.0012\ mg/cm^2$; $V = \frac{1}{b^2}\left\{\frac{1}{Sw} + \frac{(m-\bar{x})^2}{Swx^2 - \frac{(Swx)^2}{Sw}}\right\} = 0.1462$

Table 41. Probit analysis of repellency assay results of verbenol

% Repellency	Dose (mg/cm^2)	Log dose + 5	Emperical Probit	Regression Equation	Expected Probit	Working Probit	Weighting Coefficient	Weight	
		x			y			w	wx
100	1.5×10^{-2}	3.176		Y=4.579 + 0.48x	6.10	6.72	0.405	20.25	64.314
76.88	1.5×10^{-3}	2.176	5.71		5.62	5.72	0.558	27.9	60.71
49.73	1.5×10^{-4}	1.176	4.97		5.14	4.99	0.634	31.7	37.279
40.92	1.5×10^{-5}	0.176	4.75		4.66	4.77	0.616	30.8	5.4208

$Sw = 110.65$; $Swx = 167.72$; $Swx^2 = 9241.1671$; $\bar{X} = \frac{Swx}{Sw} = 1.5158$; When $y = 5$, $x = m = 0.8771$;

$RD50 = 0.000075\ mg/cm^2$; $V = \frac{1}{b^2}\left\{\frac{1}{Sw} + \frac{(m-\bar{x})^2}{Swx^2 - \frac{(Swx)^2}{Sw}}\right\} = 0.0394$

Table 42. Probit analysis of repellency assay results of nerolidol

% Repellency	Dose (mg/cm^2)	Log dose + 5	Emperical Probit	Regression Equation	Expected Probit	Working Probit	Weighting Coefficient	Weight	
		x			y			w	wx
93.95	1.5×10^{-2}	3.176	6.84	Y=4.644 + 0.575x	6.47	6.54	0.269	13.45	42.717
83.05	1.5×10^{-3}	2.176	5.95		5.89	5.95	0.471	23.55	51.245
57.33	1.5×10^{-4}	1.176	5.18		5.32	5.18	0.616	30.8	36.221
43.9	1.5×10^{-5}	0.176	4.82		4.75	4.85	0.616	30.8	5.4208

$Sw = 98.6$; $Swx = 135.60$; $Swx^2 = 5792.1201$; $\bar{X} = \frac{Swx}{Sw} = 1.3753$; When $y = 5$, $x = m = 0.6191$;

$RD_{50} = 0.000042\ mg/cm^2$; $V = \frac{1}{b^2}\left\{\frac{1}{Sw} + \frac{(m - \bar{x})^2}{Swx^2 - \frac{(Swx)^2}{Sw}}\right\} = 0.0309$

Table 43. Probit analysis of repellency assay results of geraniol

% Repellency	Dose (mg/cm^2)	Log dose + 5	Emperical Probit	Regression Equation	Expected Probit	Working Probit	Weighting Coefficient	Weight	
		x			y			w	wx
100	1.5×10^{-2}	3.176		Y=4.289 + 0.695x	6.50	7.01	0.269	13.45	42.717
80.77	1.5×10^{-3}	2.176	5.84		5.80	5.87	0.503	25.15	54.726
51.19	1.5×10^{-4}	1.176	5.03		5.11	5.03	0.634	31.7	37.279
29.19	1.5×10^{-5}	0.176	4.45		4.41	4.45	0.558	27.9	4.9104

$Sw = 98.2$; $Swx = 139.63$; $Swx^2 = 6233.5888$; $\bar{X} = \frac{Swx}{Sw} = 1.4293$; When $y = 5$, $x = m = 1.0230$;

$RD_{50} = 0.000105\ mg/cm^2$; $V = \frac{1}{b^2}\left\{\frac{1}{Sw} + \frac{(m - \bar{x})^2}{Swx^2 - \frac{(Swx)^2}{Sw}}\right\} = 0.0211$

Table 44. Probit analysis of repellency assay results of carveol

% Repellency	Dose (mg/cm^2)	Log dose + 5	Emperical Probit	Regression Equation	Expected Probit	Working Probit	Weighting Coefficient	Weight	
		x			y			w	wx
100	1.5×10^{-2}	3.176		Y=4.375 + 0.625x	6.36	6.94	0.302	15.1	47.958
80.74	1.5×10^{-3}	2.176	5.84		5.74	5.85	0.532	26.6	57.882
46.29	1.5×10^{-4}	1.176	4.9		5.11	4.91	0.634	31.7	37.279
34.93	1.5×10^{-5}	0.176	4.59		4.49	4.61	0.581	29.05	5.1128

$Sw = 102{\cdot}45$; $Swx = 148{\cdot}23$; $Swx^2 = 7066{\cdot}0905$; $\bar{X} = \frac{Swx}{Sw} = 1{\cdot}4469$; When $y = 5$, $x = m = 1$;

$RD_{50} = 0{\cdot}00010\ mg/cm^2$; $V = \frac{1}{b^2}\left\{\frac{1}{Sw} + \frac{(m-\bar{x})^2}{Swx^2 - \frac{(Swx)^2}{Sw}}\right\} = 0{\cdot}0251$

Table 45. Probit analysis of repellency assay results of citronellal

% Repellency	Dose (mg/cm^2)	Log dose + 5	Emperical Probit	Regression Equation	Expected Probit	Working Probit	Weighting Coefficient	Weight	
		x			y			w	wx
95.19	1.5×10^{-2}	3.176	6.64	Y=4.058 + 0.701x	6.28	6.59	0.336	16.8	53.357
53.76	1.5×10^{-3}	2.176	5.08		5.58	5.03	0.558	27.9	60.71
43.18	1.5×10^{-4}	1.176	4.82		4.88	4.82	0.634	31.7	37.279
27.78	1.5×10^{-5}	0.176	4.39		4.18	4.42	0.503	25.15	4.4264

$Sw = 101{\cdot}55$; $Swx = 155{\cdot}77$; $Swx^2 = 7942{\cdot}0325$; $\bar{X} = \frac{Swx}{Sw} = 1{\cdot}5339$; When $y = 5$, $x = m = 1{\cdot}3438$;

$RD_{50} = 0{\cdot}000221\ mg/cm^2$; $V = \frac{1}{b^2}\left\{\frac{1}{Sw} + \frac{(m-\bar{x})^2}{Swx^2 - \frac{(Swx)^2}{Sw}}\right\} = 0{\cdot}02005$

7.5.3 Repellency bio-assay of the blends of identified compounds

The major compounds identified from the leaf oils of the active plants were taken in the ratio in which they were present in the plants and bio-assayed for their repellency against *Anopheles gambiae* as detailed above. The results obtained are summarised in tables 46 to 53.

Table 46. Repellency assay data of the blend of perillaldehyde: perill alcohol: 1,8-cineole: limonene = 29:4:10:7 from *Conyza newii* oil

Conc. (g/ml)	% PE ± 0.005	Assay	DK	JA	MA	DW	JT	FM	Mean ± 0.005
10^{-5}	24.48	C	25	26	18	30	20	28	24.50
		T	20	24	12	21	14	20	18.50
10^{-4}	43.94	C	26	22	29	18	31	22	24.67
		T	14	10	18	10	19	12	13.83
10^{-3}	75.00	C	26	29	17	15	31	26	24.00
		T	7	6	3	2	10	8	6.00
10^{-2}	100	C	16	10	22	18	24	19	18.17
		T	0	0	0	0	0	0	0

Initial: Temp. 27^{0} C % RH. 83 Time. 8.05 a.m Final: Temp. 30^{0} C % RH. 84 Time. 12.55 p.m

Table 47. Repellency assay data of the blend of perillaldehyde: perill alcohol: 1,8-cineole = 29:4:7 from *Conyza newii* oil

Conc. (g/ml)	% PE ± 0.005	Assay	DK	JA	MA	DW	JT	FM	Mean ± 0.005
10^{-5}	26.08	C	28	14	22	24	19	31	23.00
		T	12	9	18	20	16	27	17.00
10^{-4}	46.62	C	24	28	26	23	19	28	24.67
		T	14	10	14	16	12	13	13.17
10^{-3}	78.93	C	18	24	22	19	24	26	22.17
		T	5	6	4	2	3	8	4.67
10^{-2}	100	C	21	19	24	26	24	18	22.00
		T	0	0	0	0	0	0	0

Initial: Temp. 28^{0} C % RH. 80 Time. 8.15 a.m Final: Temp. 33^{0} C % RH. 84 Time. 12.58 p.m

Table 48. Repellency assay data of the blend of perillaldehyde: perill alcohol: limonene = 29:4:10 from *Conyza newii* oil

Conc. (g/ml)	% PE ± 0.005	Assay	DK	JA	MA	DW	JT	FM	Mean ± 0.005
10^{-5}	19.85	C	27	22	15	16	30	26	22.67
		T	22	18	14	10	26	19	18.17
10^{-4}	31.81	C	21	27	18	21	26	19	22.00
		T	16	18	10	18	16	12	15.00
10^{-3}	63.94	C	18	15	12	27	19	31	20.33
		T	7	6	3	10	6	12	7.33
10^{-2}	100	C	19	20	29	17	26	21	22
		T	0	0	0	0	0	0	0

Initial: Temp. 26° C % RH. 73 Time. 8.00 a.m Final: Temp. 32° C % RH. 84 Time. 12.45 p.m

Table 49. Repellency assay data of the blend of fenchone: limonene: 1,8-cineole = 64:2:1.5 from *Tetradenia riparia* oil

Conc. (g/ml)	% PE ± 0.005	Assay	DK	JA	MA	DW	JT	FM	Mean ± 0.005
10^{-5}	13.04	C	21	23	18	26	31	19	23.00
		T	20	19	15	22	28	16	20.00
10^{-4}	34.86	C	15	31	26	17	20	23	22.00
		T	12	24	18	13	14	15	14.33
10^{-3}	55.73	C	23	20	24	15	19	21	20.33
		T	12	9	10	6	8	9	9.00
10^{-2}	69.03	C	18	17	22	24	17	15	18.83
		T	5	6	8	7	5	4	5.83
10^{-1}	91.42	C	21	19	23	27	20	18	21.33
		T	2	1	2	3	2	1	1.83

Initial: Temp. 26° C % RH. 78 Time. 8.15 a.m Final: Temp. 31° C % RH. 82 Time. 1.05 p.m

Table 50. Repellency assay data of the blend of camphor: 1,8-cineole: α-terpinene = 49:9:3 from *Plectranthus marrubioides* oil

Conc. (g/ml)	% PE ± 0.005	Assay	DK	JA	MA	DW	JT	FM	Mean ± 0.005
10^{-5}	11.05	C	23	18	21	25	18	22	21.17
		T	21	17	20	22	15	18	18.33
10^{-4}	21.24	C	18	13	19	22	24	17	18.83
		T	16	10	14	15	20	14	14.83
10^{-3}	46.38	C	14	19	23	17	28	24	20.83
		T	9	9	16	10	13	10	11.17
10^{-2}	73.39	C	24	26	18	16	21	19	20.67
		T	3	8	4	9	4	5	5.50
10^{-1}	90.77	C	28	19	13	23	21	26	21.67
		T	1	3	1	2	3	2	2.00

Initial: Temp. 27° C % RH. 83 Time. 8.05 a.m Final: Temp. 30° C % RH. 84 Time. 12.55 p.m

Table 51. Repellency bio-assay data of the blend of camphor: camphene: 1,8-cineole = 40:9:2 from *Lippia ukambensis* oil

Conc. (g/ml)	% PE ± 0.005	Assay	DK	JA	MA	DW	JT	FM	Mean ± 0.005
10^{-5}	16.11	C	24	22	21	18	15	24	20.66
		T	18	16	19	16	13	22	17.33
10^{-4}	29.21	C	16	19	21	17	18	22	18.83
		T	10	12	14	10	14	20	13.33
10^{-3}	40.60	C	19	21	26	18	25	24	22.17
		T	16	10	14	11	12	16	13.17
10^{-2}	53.91	C	24	19	29	21	15	20	21.33
		T	9	13	10	14	5	8	9.83
10^{-1}	74.08	C	21	18	19	24	18	22	20.33
		T	5	8	6	3	7	2	5.17

Initial: Temp. 28° C % RH. 75 Time. 8.00 a.m Final: Temp. 30° C % RH. 82 Time. 12.40 P.M

Table 52. Repellency assay data of the blend of camphene:α-pinene: α-fenchyl alcohol: 1,8-cineole:α-terpineol:terpen-4-ol = 16:16:14:7:4:3 from *Tarchonanthus camphoratus* oil

Conc. (g/ml)	% PE ± 0.005	Assay	DK	JA	MA	DW	JT	FM	Mean ± 0.005
10^{-5}	15.61	C	22	28	21	15	19	23	21.33
		T	18	21	20	13	16	20	18.00
10^{-4}	33.96	C	18	14	20	19	22	13	17.67
		T	12	10	15	10	15	8	11.67
10^{-3}	41.83	C	26	27	24	19	20	25	23.50
		T	10	14	19	18	8	13	13.67
10^{-2}	57.73	C	19	25	21	16	12	23	19.33
		T	4	6	9	7	8	15	8.17
10^{-1}	96.62	C	24	19	21	17	15	23	19.83
		T	0	1	0	1	2	0	0.67

Initial: Temp. 27° C % RH. 75 Time. 8.00 a.m Final: Temp. 31° C % RH. 82 Time. 12.40 p.m

Table 53. Repellency assay data of the blend of limonene oxide: *cis*-verbenol: verbenone = 39:11:6 from *Lippia javanica* oil

Conc. (g/ml)	% PE ± 0.005	Assay	DK	JA	MA	DW	JT	FM	Mean ± 0.005
10^{-5}	16.67	C	21	24	26	17	20	18	21.00
		T	20	19	21	14	16	15	17.50
10^{-4}	38.19	C	23	20	19	27	28	16	20.50
		T	15	12	10	9	20	10	12.67
10^{-3}	42.19	C	18	21	23	26	19	21	21.33
		T	10	11	14	16	10	13	12.33
10^{-2}	84.28	C	22	18	24	21	17	19	20.17
		T	2	4	6	3	1	3	3.17
10^{-1}	95.24	C	20	24	18	16	22	26	21.00
		T	1	0	2	0	1	2	1.00

Initial: Temp. 28° C % RH. 75 Time. 8.05 a.m Final: Temp. 31° C % RH. 82 Time. 12.50 p.m

7.5.4 Mosquito repellency bio-assay of formulated compounds

The compounds that showed good repellent activity were formulated in various carrier media and bio-assayed once again to ascertain the longevity of the protection. Several bases, oils, and gels were investigated. These included aqueous base, Tween 80, Tween 60, and emulsion gel. This was necessary since a carrier medium that gives a homogeneous mixture is required for such formulations. The compounds were therefore mixed with various media and left to stand. If the two separated after some time then the medium was declared unsuitable. Formulated compounds (10%) were assayed for their repellent activity against *A. gambiae* after 0, 2, 4, 6 and 8 hrs after application and their protective efficacies (PE) calculated as previously explained. The oils were eventually formulated in an aqueous base. The results obtained are summarised in table 12.

7.5.5 Mosquitocidal assays (WHO, 1996)

7.5.5.1 Fumigation bio-assay of essential oils

For preliminary screening 30 female *A. gambiae* mosquitoes were introduced into a small cage measuring 20 x 20 x 35.5 cm and fumigated with 1 ml of 0.1 g/ml (10%) solution of plant extract on a small Whatman filter paper (diameter 7 cm) placed in a petri-dish (diameter 8 cm), and then covered with a wire gauze. The control was similarly set but with acetone only. Glucose solution (6%) in a bottle (20 ml) with a rolled rectangular filter paper (5 x 10 cm) in it was provided to the insects in each cage to serve as food. The number of dead mosquitoes in both cages was recorded separately within 6 hrs at interval of 30 mins. The percentage insecticidal (PI) activity was calculated using the formula:

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

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. Extracts that showed high insecticidal activity were exposed to detailed bio-assay where 10 replicates were done. Probit analysis (Busvine, 1971 and Finney, 1971) of the results obtained was done to get the LD_{50} values of the oils. These are summarised in tables 54 to 59.

Table 54. Probit analysis of mosquitocidal assay results of *Conyza newii* leaf oil

Mosq. Used	% Mortality	Conc. g/ml	Log dose +5	Emperical Probit	Regression Equation	Expected Probit	Working Probit	Weighting Coefficient	Weight	
			x			y			w	wx
30	100	1×10^{-1}	4	_	y = -3.363 + 2.427x	6.35	6.87	0.336	10.08	40.32
30	92	8×10^{-2}	3.9031	6.41		6.11	6.35	0.405	12.15	47.7427
30	74	6×10^{-2}	3.7782	5.64		5.81	5.63	0.503	15.09	57.013
30	52	4×10^{-2}	3.6021	5.05		5.38	5.03	0.601	18.03	64.9459
30	44	2×10^{-2}	3.301	4.85		4.65	4.85	0.616	18.48	61.0025

$Sw = 73.83$; $Swx = 271.0372$; $Swx^2 = 15094.8249$; $\bar{X} = \frac{Swx}{Sw} = 3.6711$; When $y = 5$, $x = m = 3.4458$;

$LC_{50} = 0.0279$ g/ml ; $V = \frac{1}{b^2}\left\{\frac{1}{Sw} + \frac{(m - \bar{x})^2}{Swx^2 - \frac{(Swx)^2}{Sw}}\right\} = 0.0023$

Table 55. Probit analysis of mosquitocidal assay results of *Lippia javanica* leaf oil

Mosq. Used	% Mortality	Conc. g/ml	Log dose +5	Emperical Probit	Regression Equation	Expected Probit	Working Probit	Weighting Coefficient	Weight	
			x			y			w	wx
30	100	1×10^{-1}	4	_	y = -5.096 + 2.664x	5.56	6.47	0.532	15.96	63.48
30	68	8×10^{-2}	3.9031	5.47		5.30	5.46	0.616	18.48	72.1293
30	39	6×10^{-2}	3.7782	4.72		4.97	4.73	0.637	19.11	72.2014
30	33	4×10^{-2}	3.6021	4.56		4.50	4.56	0.581	17.43	62.7846
30	10	2×10^{-2}	3.301	3.72		3.70	3.98	0.336	10.08	33.2741

$Sw = 81.06$; $Swx = 304.2294$; $Swx^2 = 19540.2954$; $\bar{X} = \frac{Swx}{Sw} = 3.7531$; When $y = 5$, $x = m = 3.7898$;

$LC_{50} = 0.0616$ g/ml ; $V = \frac{1}{b^2}\left\{\frac{1}{Sw} + \frac{(m - \bar{x})^2}{Swx^2 - \frac{(Swx)^2}{Sw}}\right\} = 0.0017$

Table 56. Probit analysis of mosquitocidal assay results of *Plectranthus marruboides* leaf oil

mosq. Used	% Mortality	Conc. g/ml	Log dose +5	Emperical Probit	Regression Equation	Expected Probit	Working Probit	Weighting Coefficient	Weight	
			x			y			w	wx
30	100	1×10^{-1}	4	_	y = -1.564 + 1.823x	5.73	6.47	0.532	15.96	63.84
30	78	8×10^{-2}	3.9031	5.77		5.55	5.76	0.558	16.74	65.3379
30	53	6×10^{-2}	3.7782	5.08		5.32	5.07	0.616	18.48	69.8211
30	48	4×10^{-2}	3.6021	4.95		5.00	4.95	0.637	19.11	68.8361
30	32	2×10^{-2}	3.301	4.53		4.45	4.53	0.581	17.43	57.5364

$Sw = 87.72$; $Swx = 325.3716$; $Swx^2 = 21268.4188$; $\bar{X} = \frac{Swx}{Sw} = 3.7092$; When $y = 5$, $x = m = 3.6007$;

$LC_{50} = 0.0399$ g/ml; $V = \frac{1}{b^2}\left\{\frac{1}{Sw} + \frac{(m - \bar{x})^2}{Swx^2 - \frac{(Swx)^2}{Sw}}\right\} = 0.0034$

Table 57. Probit analysis of mosquitocidal assay results of *Lippia ukambensis* leaf oil

Mosq. Used	% Mortality	Conc. g/ml	Log dose +5	Emperical Probit	Regression Equation	Expected Probit	Working Probit	Weighting Coefficient	Weight	
			x			y			w	wx
30	100	1×10^{-1}	4	_	y = -5.731 + 2.809x	5.51	6.38	0.581	17.43	69.72
30	58	8×10^{-2}	3.9031	5.2		5.23	5.2	0.627	18.81	73.4173
30	46	6×10^{-2}	3.7782	4.9		4.88	4.9	0.634	19.02	71.8614
30	28	4×10^{-2}	3.6021	4.42		4.39	4.42	0.558	16.74	60.2992
30	7	2×10^{-2}	3.301	3.52		3.54	3.52	0.269	8.07	26.6391

$Sw = 80.07$; $Swx = 301.9370$; $Swx^2 = 19760.6743$; $\bar{X} = \frac{Swx}{Sw} = 3.7709$; When $y = 5$, $x = m = 3.8202$;

$LC_{50} = 0.0661$ g/ml; $V = \frac{1}{b^2}\left\{\frac{1}{Sw} + \frac{(m - \bar{x})^2}{Swx^2 - \frac{(Swx)^2}{Sw}}\right\} = 0.0016$

Table 58. Probit analysis of mosquitocidal assay results of *Tetradenia riparia* leaf oil

Mosq. Used	% Mortality	Conc. g/ml	Log dose +5	Emperical Probit	Regression Equation	Expected Probit	Working Probit	Weighting Coefficient	Weight	
			x			y			w	wx
30	100	1×10^{-1}	4	_	y = -5.724 + 2.823x	5.57	6.42	0.558	16.74	66.96
30	64	8×10^{-2}	3.9031	5.36		5.29	5.36	0.616	18.48	72.1293
30	43	6×10^{-2}	3.7782	4.82		4.94	4.82	0.634	19.02	71.8614
30	31	4×10^{-2}	3.6021	4.5		4.44	4.51	0.558	16.74	60.2992
30	8	2×10^{-2}	3.301	3.59		3.59	3.59	0.302	9.06	29.9071

$Sw = 80{\cdot}04$; $Swx = 301{\cdot}157$; $Swx^2 = 19380{\cdot}7665$; $\bar{X} = \frac{Swx}{Sw} = 3{\cdot}7626$; When y = 5, x = m = 3·7988;

$LC_{50} = 0{\cdot}0629$ g/ml; $V = \frac{1}{b^2}\left\{\frac{1}{Sw} + \frac{(m-\bar{x})^2}{Swx^2 - \frac{(Swx)^2}{Sw}}\right\} = 0{\cdot}0016$

Table 59. Probit analysis of mosquitocidal assay results of *T. camphoratus* leaf oil

Mosq. Used	% Mortality	Conc. g/ml	Log dose +5	Emperical Probit	Regression Equation	Expected Probit	Working Probit	Weighting Coefficient	Weight	
			x			y			w	wx
30	100	1×10^{-1}	4	_	y = -5.763 + 2.885x	5.78	6.53	0.503	15.09	60.36
30	72	8×10^{-2}	3.9031	5.58		5.50	5.58	0.581	17.43	68.031
30	54	6×10^{-2}	3.7782	5.1		5.14	5.1	0.634	19.02	71.8614
30	32	4×10^{-2}	3.6021	4.53		4.63	4.53	0.601	18.03	64.9459
30	12	2×10^{-2}	3.301	3.82		3.76	3.83	0.37	11.1	36.6411

$Sw = 80{\cdot}67$; $Swx = 301{\cdot}8394$; $Swx^2 = 18996{\cdot}1475$; $\bar{X} = \frac{Swx}{Sw} = 3{\cdot}7417$; When y = 5, x = m = 3·7307;

$LC_{50} = 0{\cdot}0538$ g/ml; $V = \frac{1}{b^2}\left\{\frac{1}{Sw} + \frac{(m-\bar{x})^2}{Swx^2 - \frac{(Swx)^2}{Sw}}\right\} = 0{\cdot}0015$

7.5.5.2 Fumigation bio-assay of pure compounds

The pure compounds identified from the oils of the six active insecticidal plants were subjected to mosquitocidal assay by fumigation method as detailed above. The bio-assay data of the two active compounds were analysed (Busvine, 1971) to get the LD_{50} values, which are summarised in tables 60 and 61.

Table 60. Probit analysis of the fumigation assay data of perillaldehyde

mosq. Used	% Mortality	Conc. g/ml	Log dose +5	Emperical Probit	Regression Equation	Expected Probit	Working Probit	Weighting Coefficient	Weight	
			x			y			w	wx
30	100	1×10^{-2}	3	_	y = -3.534 + 3.93x	8.25	8.6	0.006	0.18	0.54
30	100	8×10^{-3}	2.903	_		7.88	8.21	0.019	0.57	1.6547
30	88	6×10^{-3}	2.778	6.18		7.38	2.4	0.062	1.86	5.1671
30	72	4×10^{-3}	2.602	5.58		6.69	4.19	0.208	6.24	16.2365
30	45	2×10^{-3}	2.301	4.87		5.51	4.82	0.581	17.43	40.1064

$Sw = 26.28$; $Swx = 63.7047$; $Swx^2 = 1901.8774$; $\bar{X} = \frac{Swx}{Sw} = 2.4240$; When $y = 5$, $x = m = 2.1715$;

$LC_{50} = 0.001484$ g/ml ; $V = \frac{1}{b^2}\left\{\frac{1}{Sw} + \frac{(m-\bar{x})^2}{Swx^2 - \frac{(Swx)^2}{Sw}}\right\} = 0.002466$

Table 61. Probit analysis of the fumigation assay of data perill alcohol

mosq. Used	% Mortality	Conc. g/ml	Log dose +5	Emperical Probit	Regression Equation	Expected Probit	Working Probit	Weighting Coefficient	Weight	
			x			y			w	wx
30	100	1×10^{-2}	3	_	y = -3.738 + 3.422x	6.53	7.01	0.269	8.07	24.21
30	95	8×10^{-3}	2.903	6.64		6.19	6.53	0.37	11.1	32.2233
30	65	6×10^{-3}	2.778	5.39		5.77	5.32	0.503	15.09	41.92
30	45	4×10^{-3}	2.602	4.87		5.17	4.87	0.627	18.81	48.9436
30	26	2×10^{-3}	2.301	4.36		4.14	4.38	0.471	14.13	32.5131

$Sw = 67.2$; $Swx = 179.8101$; $Swx^2 = 6834.3348$; $\bar{X} = \frac{Swx}{Sw} = 2.6757$; When $y = 5$, $x = m = 2.5535$;

$LC_{50} = 0.003576$ g/ml ; $V = \frac{1}{b^2}\left\{\frac{1}{Sw} + \frac{(m-\bar{x})^2}{Swx^2 - \frac{(Swx)^2}{Sw}}\right\} = 0.001271$

7.5.5.3 Tarsal contact bio-assay of essential oils

In this bioassay two petri-dishes (diameter 8 and 10 cm) were used. A small hole (diameter 0.5 cm) was made at the centre of the big petri-dish, and a Whatman filter paper (diameter 7 cm) placed in the small dish (diameter 8 cm). A solution (1 ml) of 1 mg/ml essential oil in acetone was spread evenly on the filter paper and allowed to dry for 18 hours. The small petri-dish (diameter 7 cm) was covered with the big one (diameter 10 cm) and 10 non-starved female mosquitoes (> 2 days old) introduced 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 cage with food (6% glucose solution). The number of dead mosquitoes within 24 hrs at interval of 2hrs (WHO, 1996) was recorded. The control experiment was similarly set up but with 1 ml of acetone. The results revealed that, no essential oil had tarsal contact activity.

7.5.5.4 Tarsal contact bio-assay of chloroform extracts

The chloroform extracts were also subjected to tarsal contact mosquitocidal assay as previously explained.

7.5.5.5 Tarsal contact bio-assay of pure compounds

The pure compounds identified in the oils of the six insecticidal plants were also tested for their tarsal contact mosquitocidal activity as described above. The bio-assay of the 2 active ones were analysed to obtain their LD_{50} values. These results are summarised in tables 62 and 63.

Table 62. Probit analysis of the tarsal contact assay data of perillaldehyde

Mosq. Used	% Mortality	Conc. mg/ml	Log dose +5	Emperical Probit	Regression Equation	Expected Probit	Working Probit	Weighting Coefficient	Weight	
			x			y			w	wx
10	100	1×10^{0}	5	_	y = -27.223 + 6.61x	5.83	6.53	0.503	5.03	25.15
10	100	9×10^{-1}	4.954	_		5.52	6.38	0.581	5.81	28.7827
10	70	8×10^{-1}	4.903	5.52		5.18	5.51	0.627	6.27	30.7418
10	50	7×10^{-1}	4.845	5		4.8	5	0.627	6.27	30.3782
10	30	6×10^{-1}	4.778	4.48		4.36	4.48	0.558	5.58	26.6612
10	20	5×10^{-1}	4.698	4.16		3.83	4.24	0.37	3.7	17.3826

$Sw = 32.66$; $Swx = 159.0965$; $Swx^2 = 4341.8360$; $\bar{x} = \frac{Swx}{Sw} = 4.8713$; When $y = 5$, $x = m = 4.8749$;

$LC_{50} = 0.7496$ mg/ml ; $V = \frac{1}{b^2}\left\{\frac{1}{Sw} + \frac{(m-\bar{x})^2}{Swx^2 - \frac{(Swx)^2}{Sw}}\right\} = 0.00070008$

Table 63. Probit analysis of the tarsal contact assay data of perill alcohol

Mosq. Used	% Mortality	Conc. mg/ml	Log dose +5	Emperical Probit	Regression Equation	Expected Probit	Working Probit	Weighting Coefficient	Weight	
			x			y			w	wx
10	100	1×10^{0}	5	_	y = -42.345 + 9.63x	5.87	6.59	0.471	4.71	23.55
10	70	9×10^{-1}	4.954	5.52		5.42	5.52	0.601	6.01	29.7735
10	40	8×10^{-1}	4.903	4.75		4.93	4.75	0.634	6.34	31.085
10	30	7×10^{-1}	4.845	4.48		4.37	4.48	0.558	5.58	27.0351
10	10	6×10^{-1}	4.778	3.72		3.73	3.72	0.336	3.36	16.054
10	0	5×10^{-1}	4.698	_		2.96	2.49	0.11	1.1	5.1678

$Sw = 27.1$; $Swx = 132.6655$; $Swx^2 = 3422.6809$; $\bar{x} = \frac{Swx}{Sw} = 4.8954$; When $y = 5$, $x = m = 4.9098$;

$LC_{50} = 0.8124$ mg/ml ; $V = \frac{1}{b^2}\left\{\frac{1}{Sw} + \frac{(m-\bar{x})^2}{Swx^2 - \frac{(Swx)^2}{Sw}}\right\} = 0.0003968$

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APPENDICES

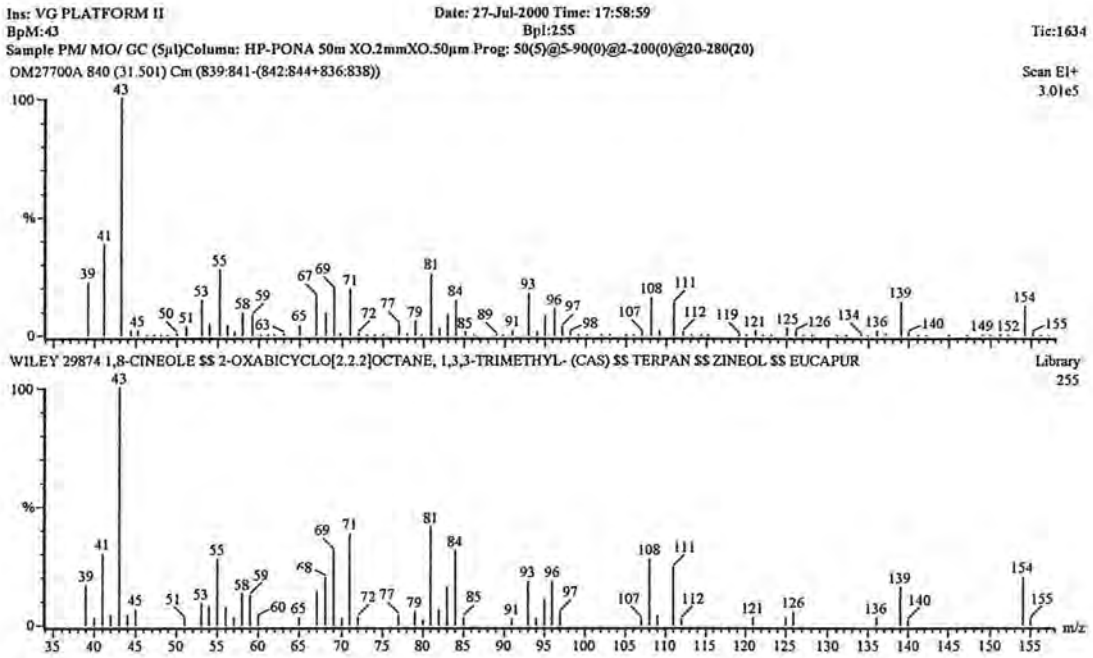

Fig. 16 MS of 1,8-cineole

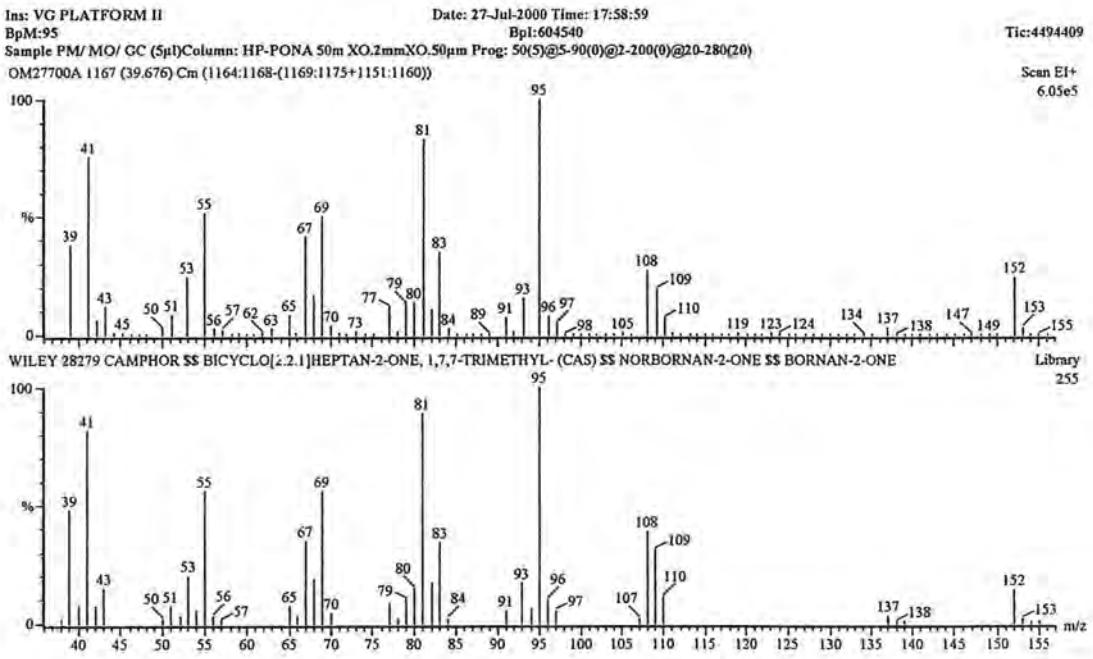

Fig. 17 MS of Camphor

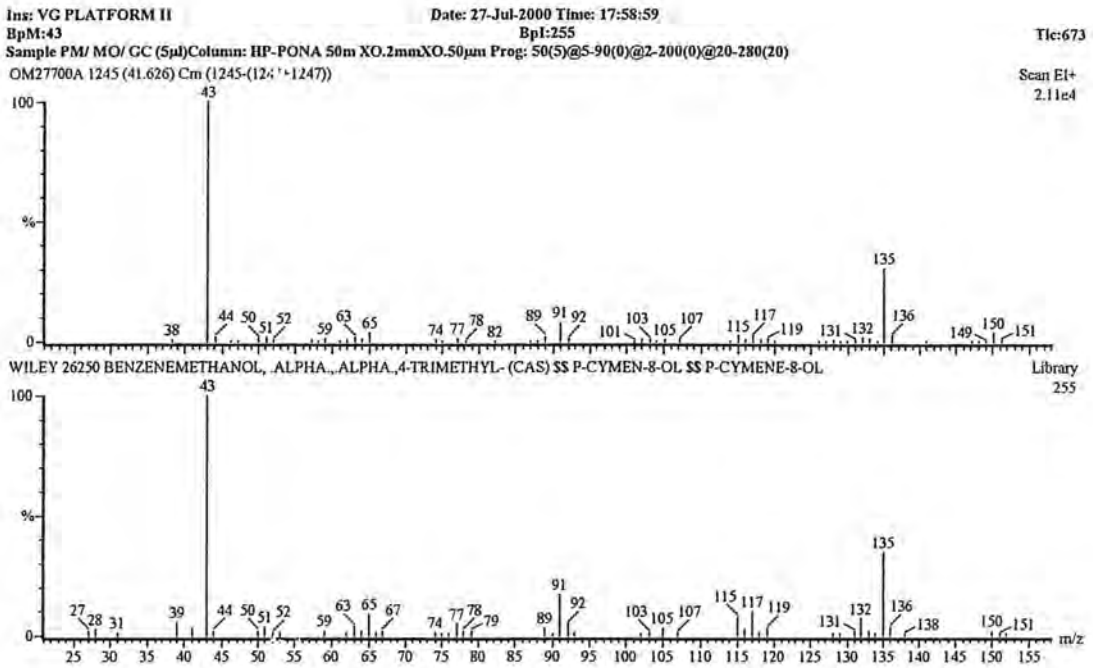

Fig. 18 MS of *p*-Cymen-3-ol

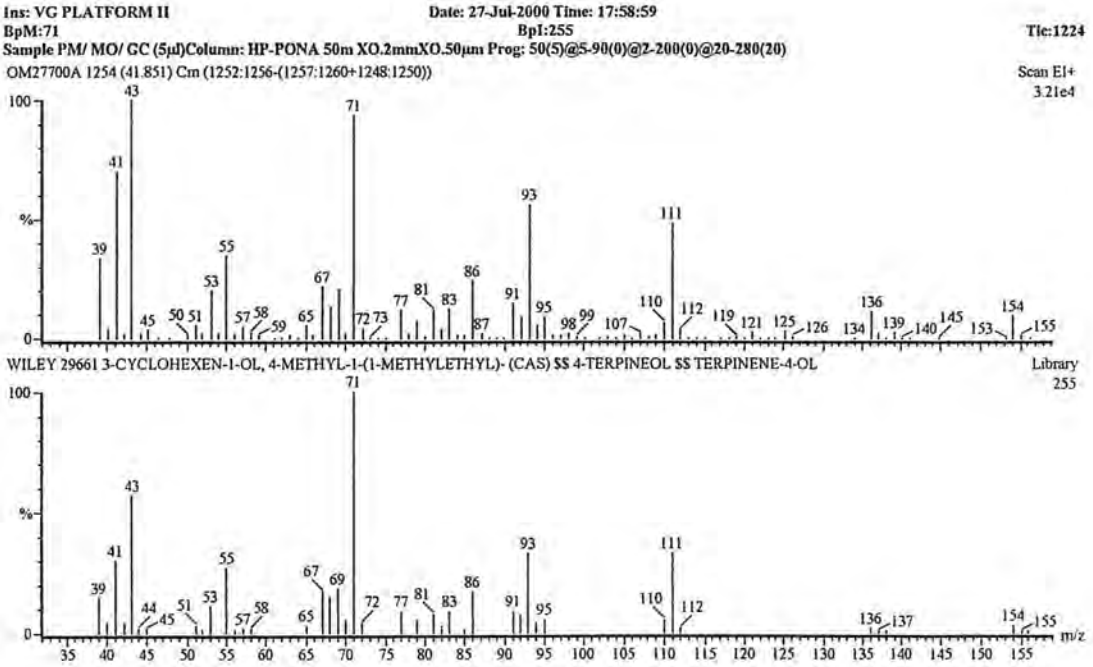

Fig. 19 MS of Terpen-4-ol

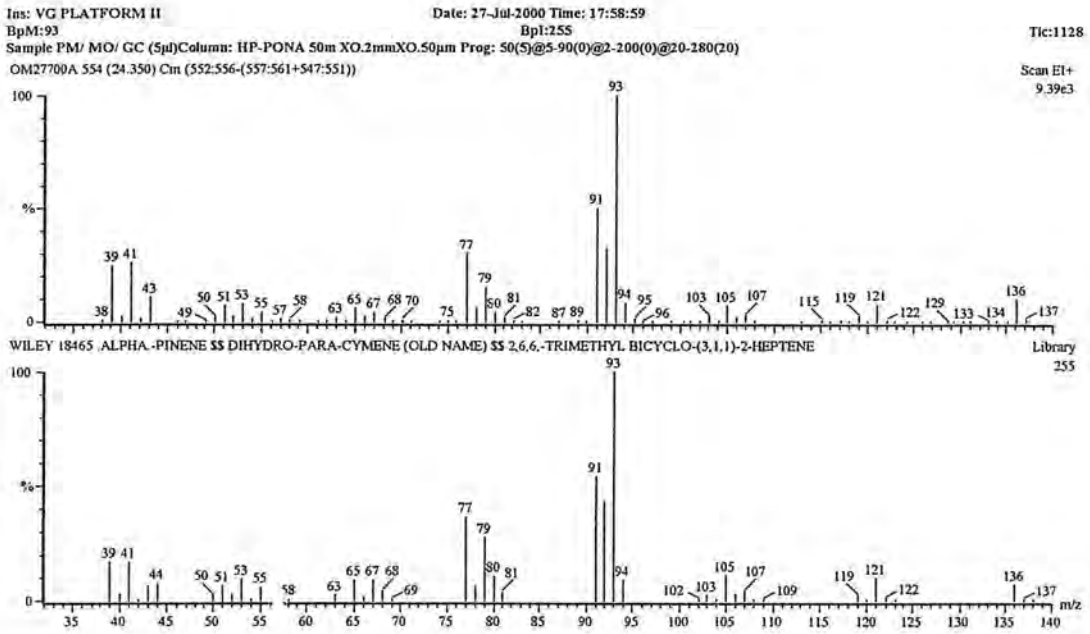

Fig. 20 MS of α-pinene

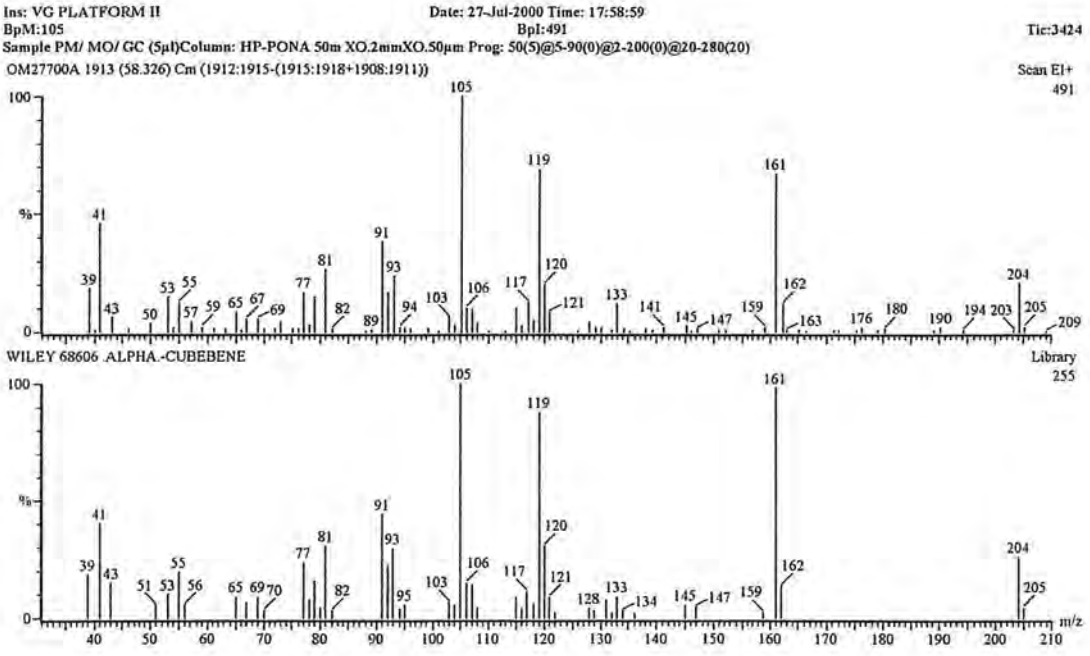

Fig. 21 MS of α-cubebene

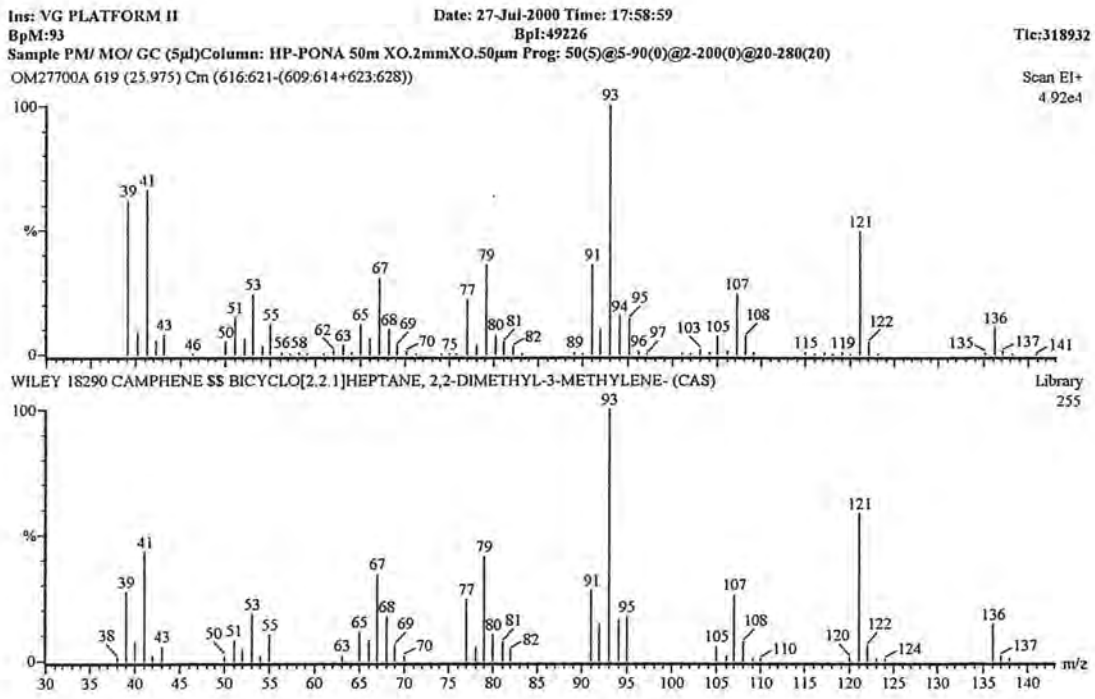

Fig. 22 MS of Camphene

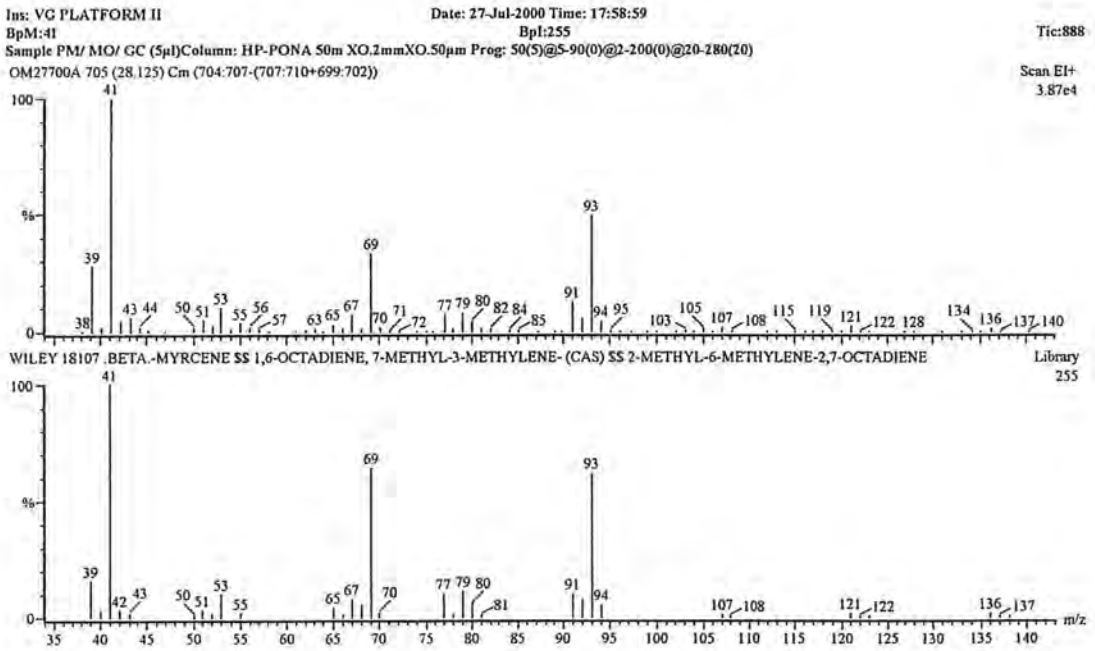

Fig. 23 MS of β-myrcene

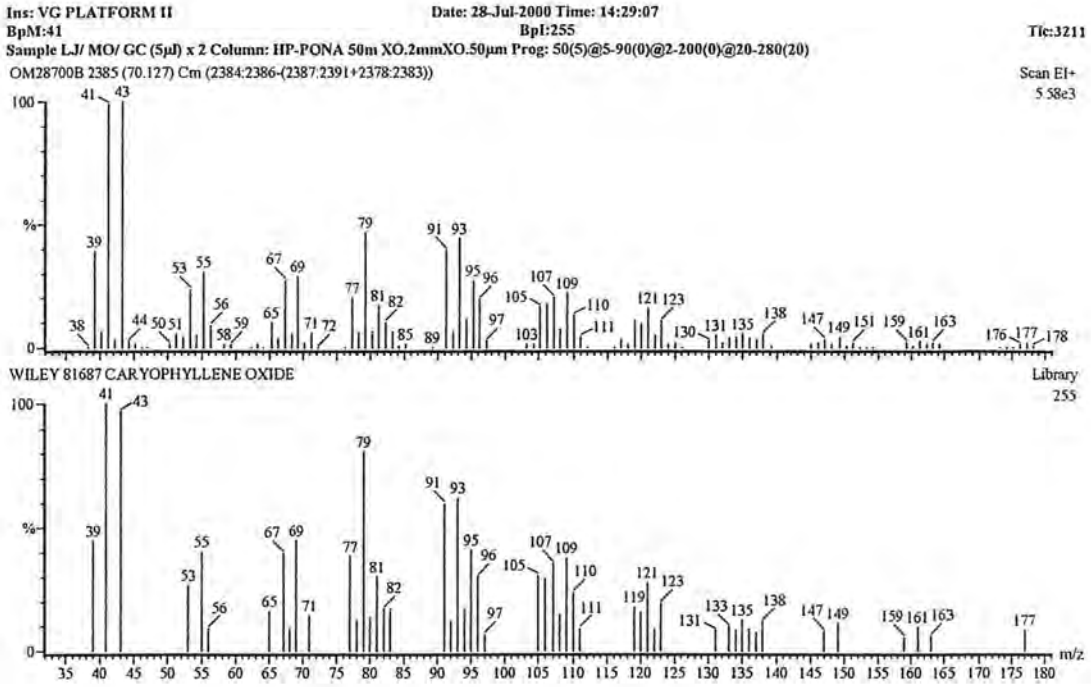

Fig. 24 MS of Caryophyllene oxide

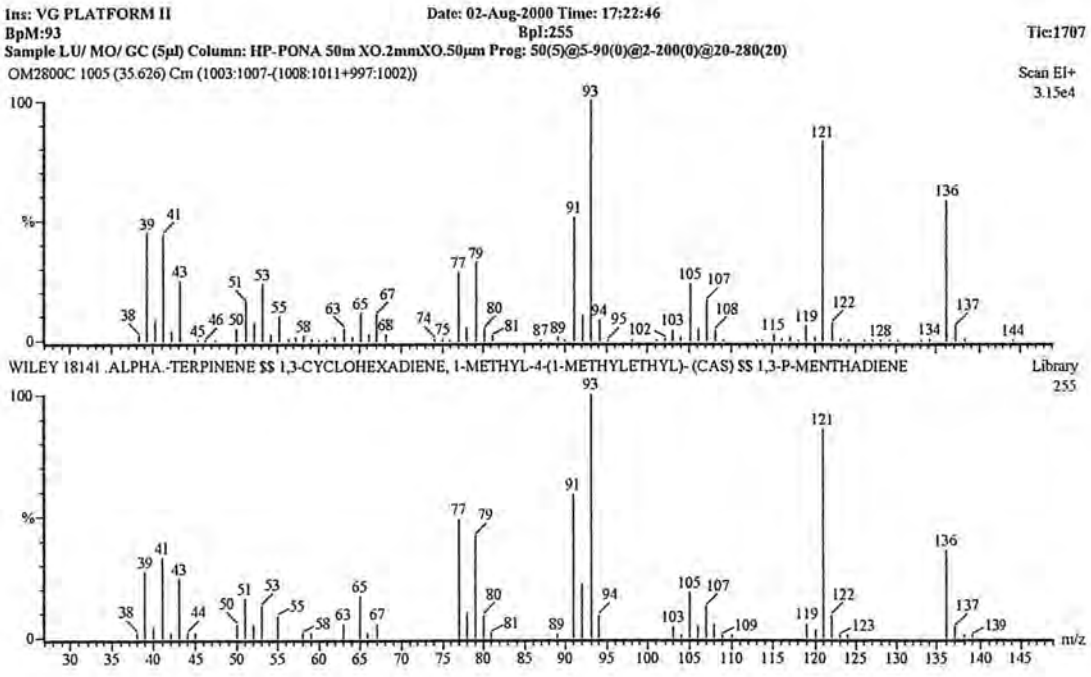

Fig. 25 MS of α-terpinene

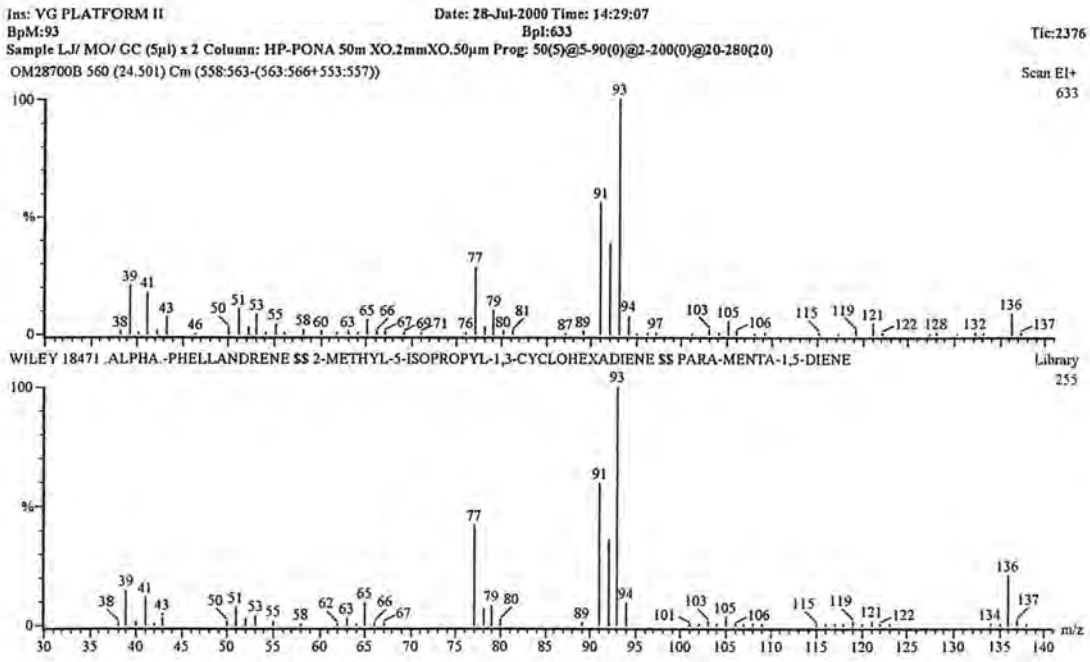

Fig. 26 MS of α-phellandrene

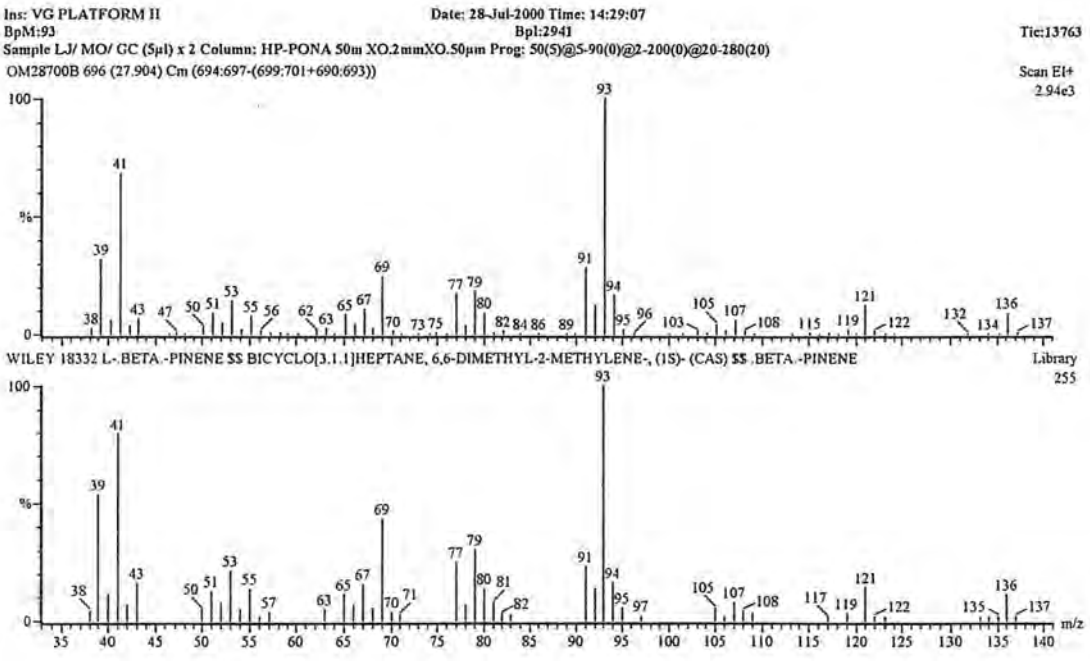

Fig. 27 MS of β-pinene

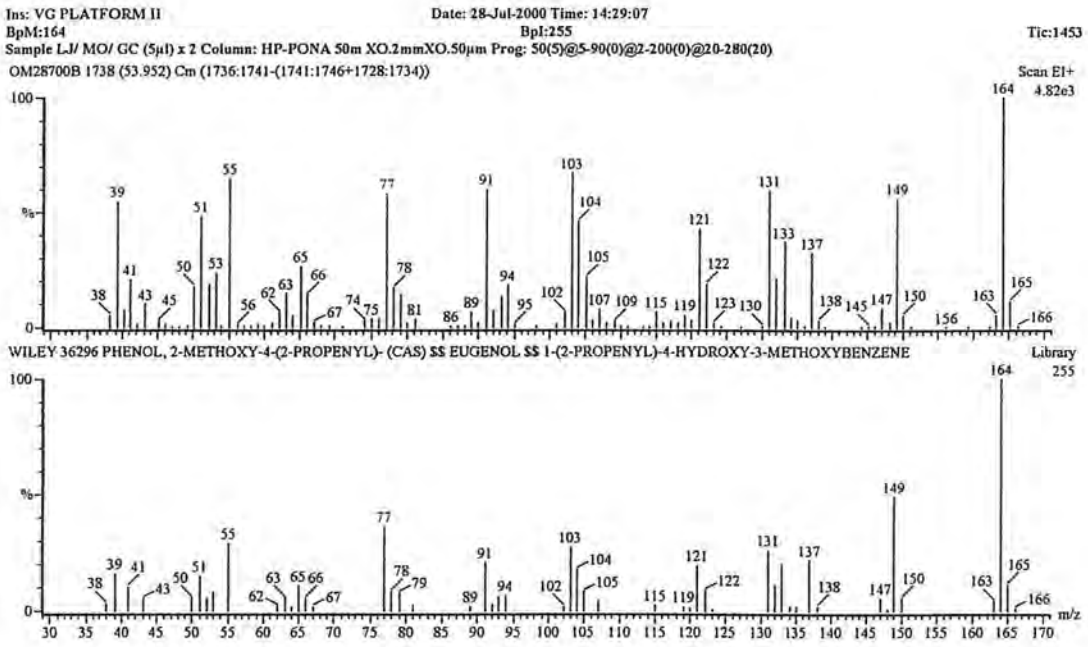

Fig. 28 MS of Eugenol

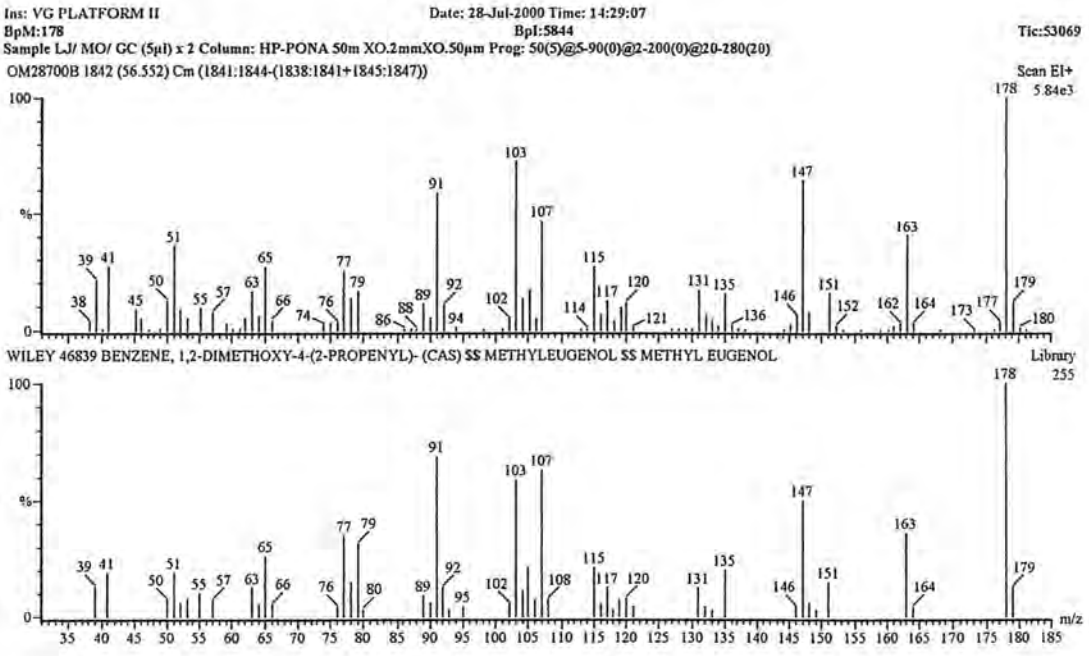

Fig. 29 MS of Methyl eugenol

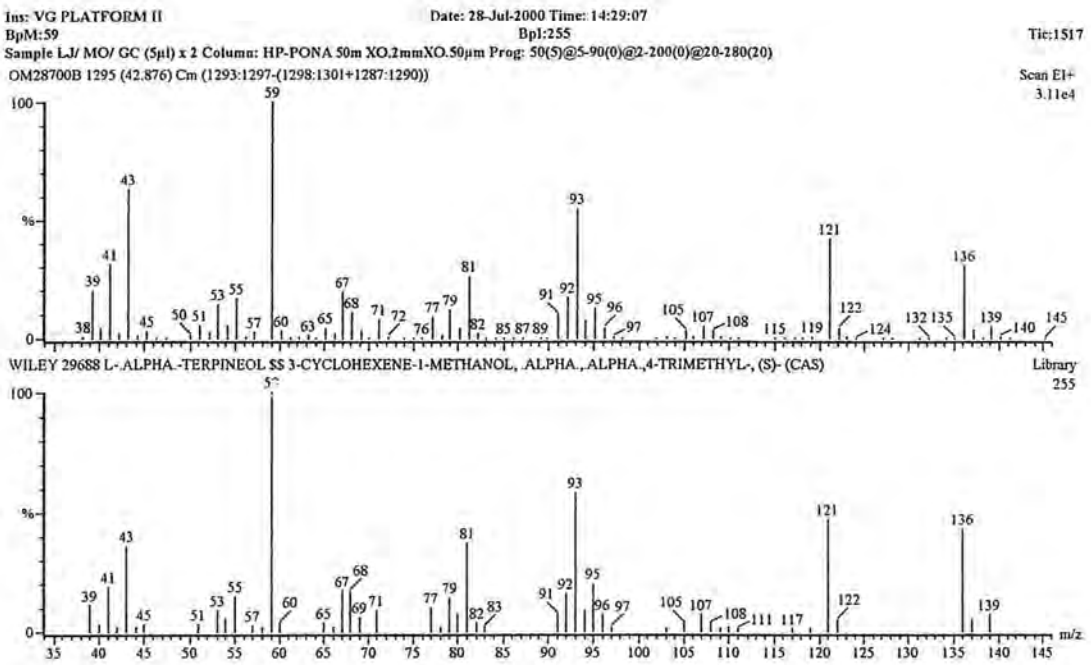

Fig. 30 MS of α-terpineol

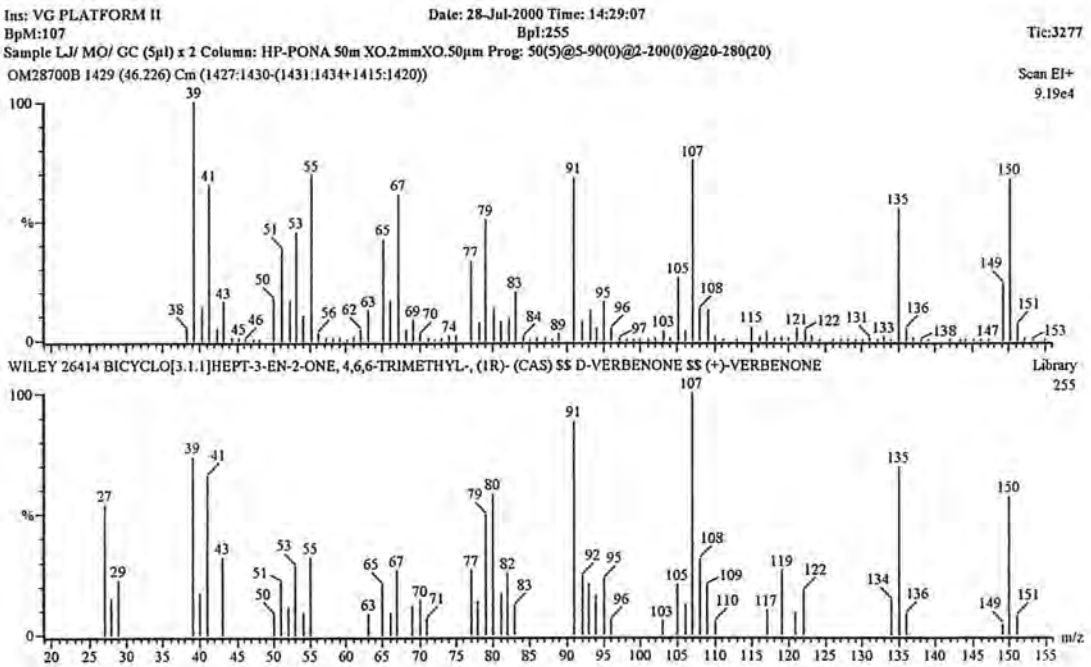

Fig. 31 MS of Verbenone

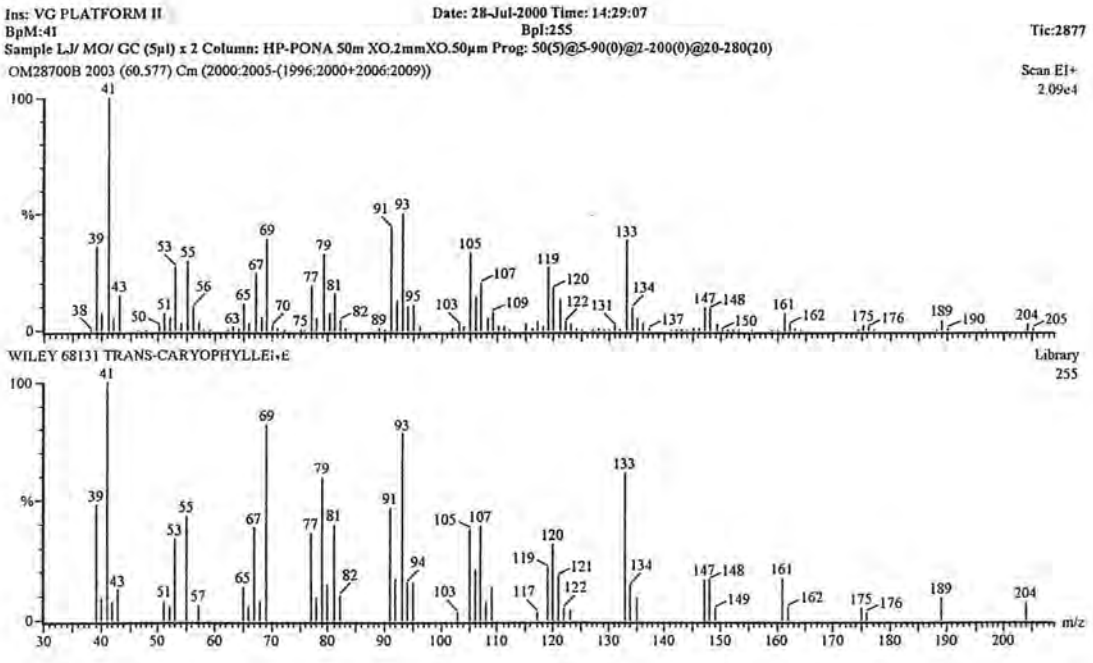

Fig. 32 MS of trans-Caryophyllene

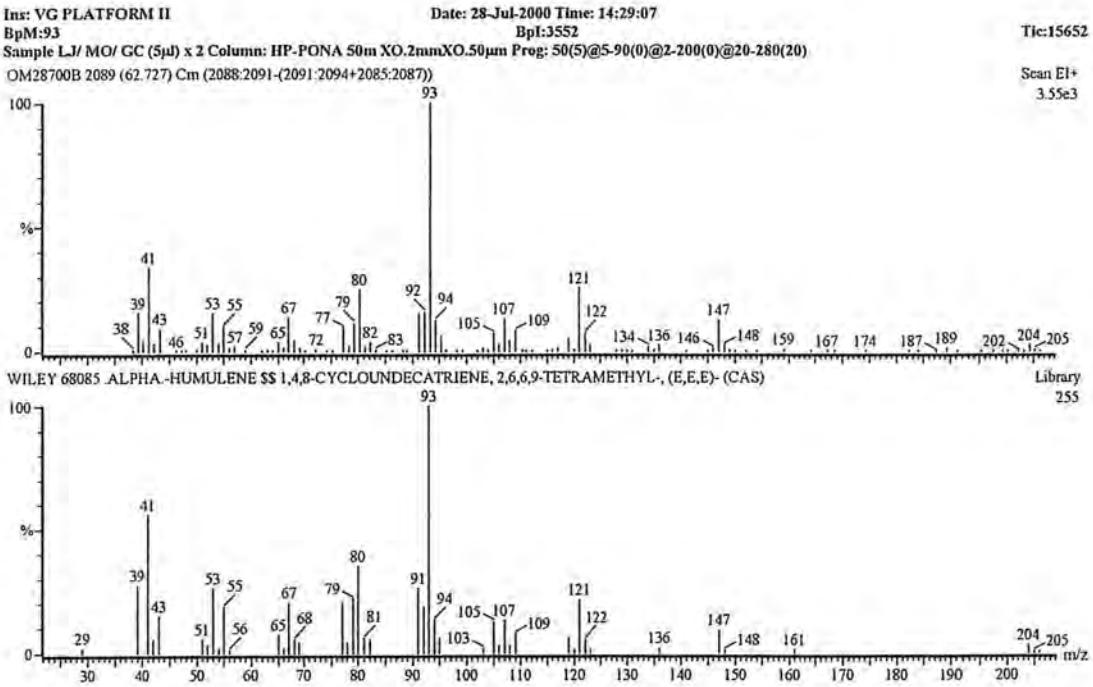

Fig. 33 MS of α-caryophyllene

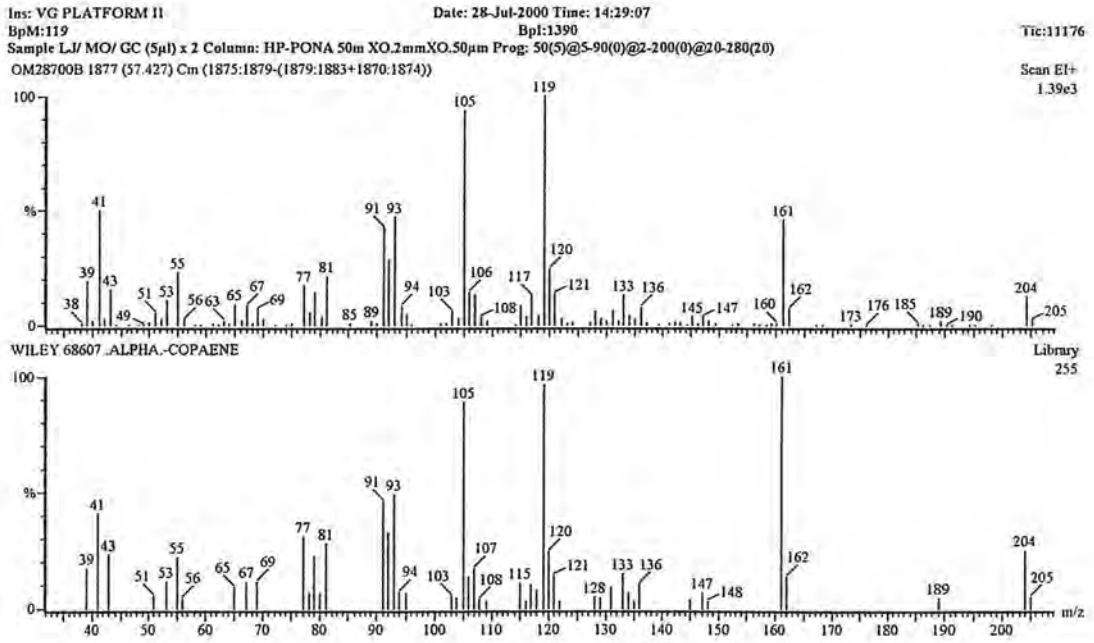

Fig. 34 MS of α-copaene

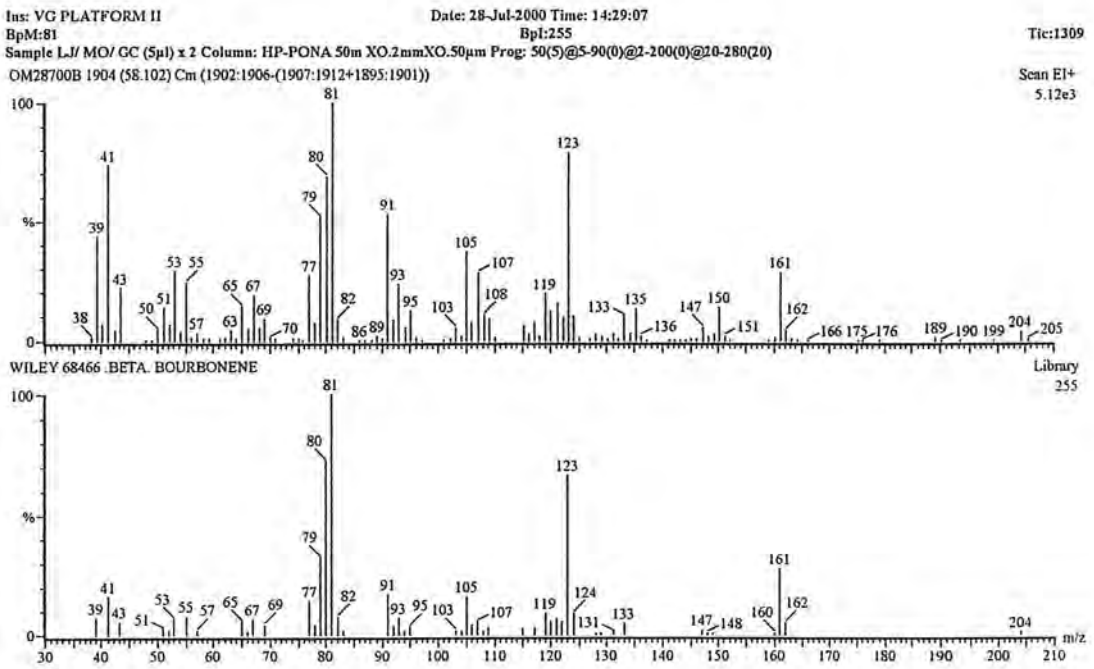

Fig. 35 MS of β-bourbonene

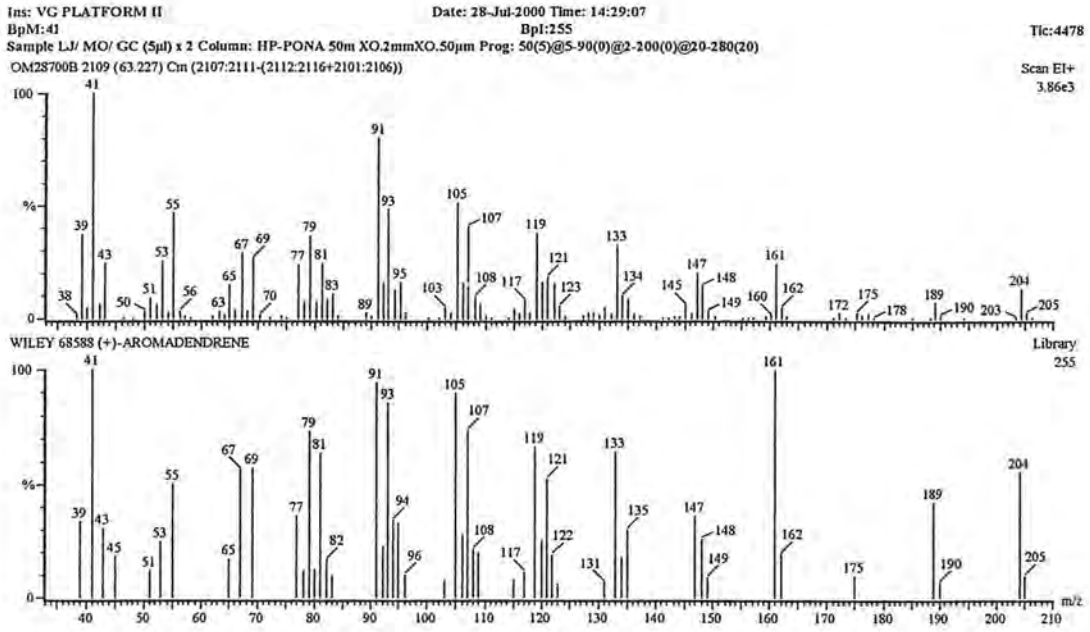

Fig. 36 MS of Aromadendrene

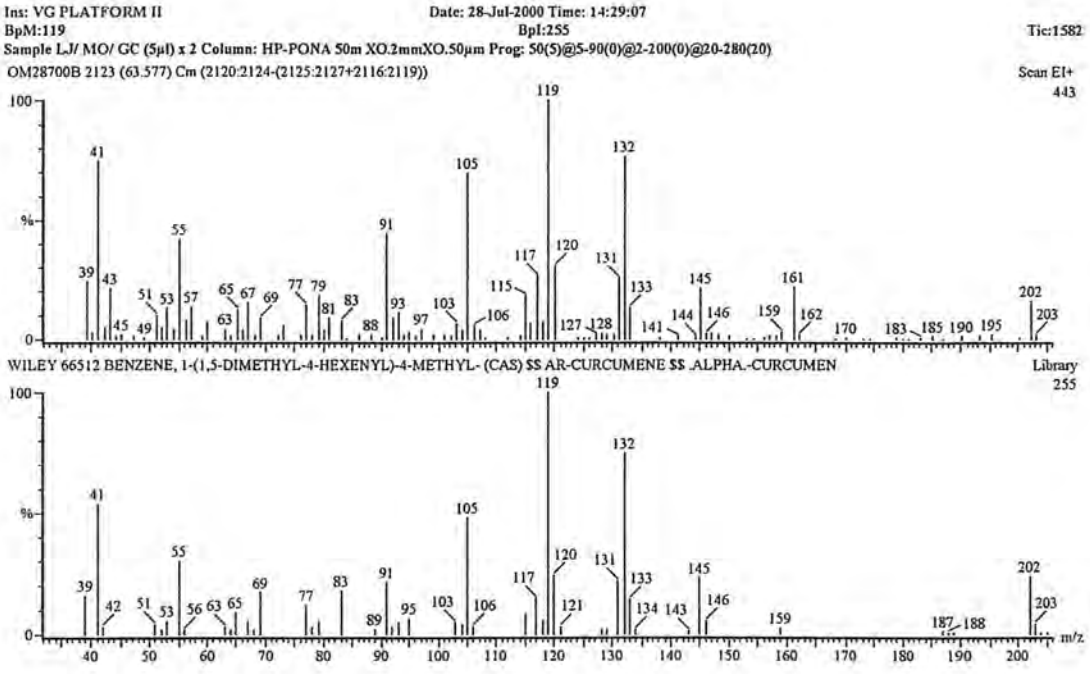

Fig. 37 MS of α-curcumen

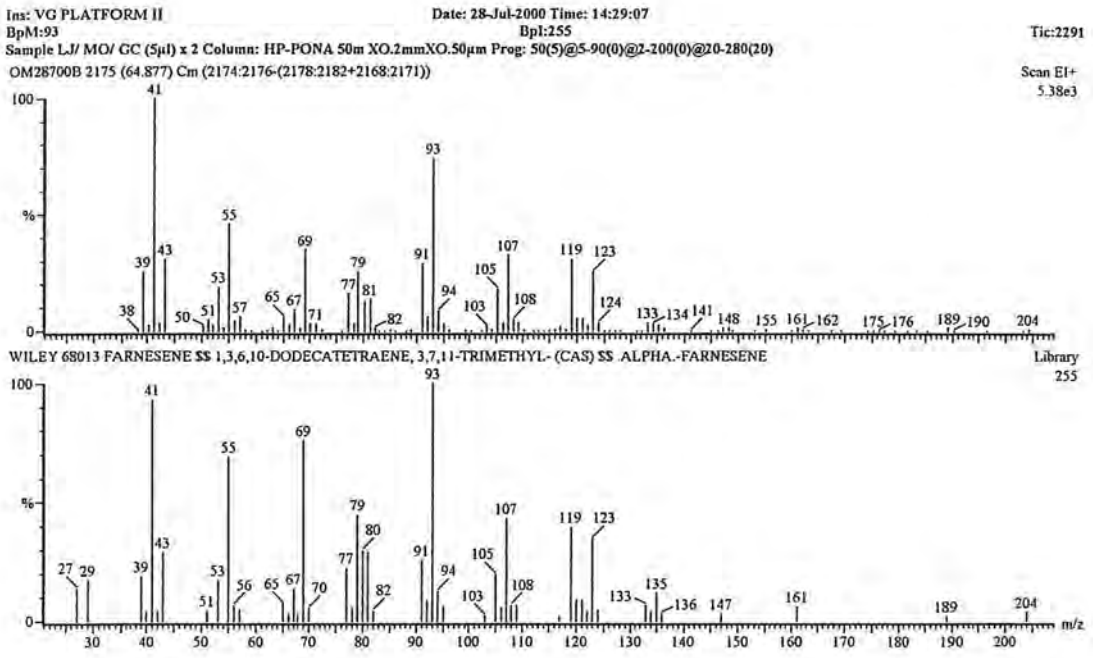

Fig. 38 MS of α-farnesene

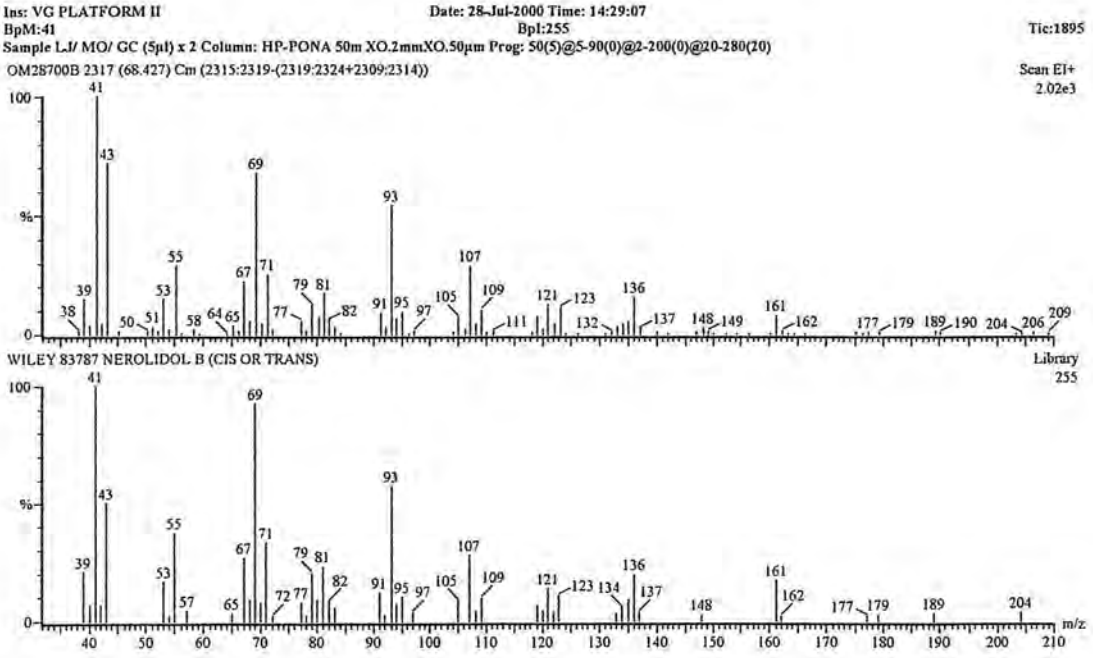

Fig. 39 MS of Nerolidol

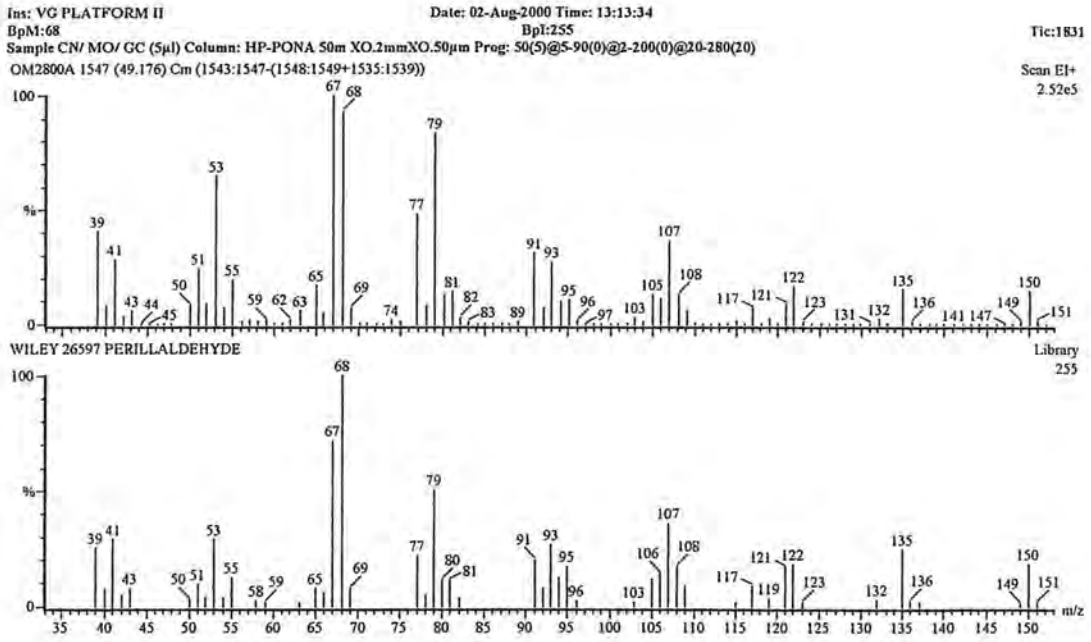

Fig. 40 MS of Perill anhydride

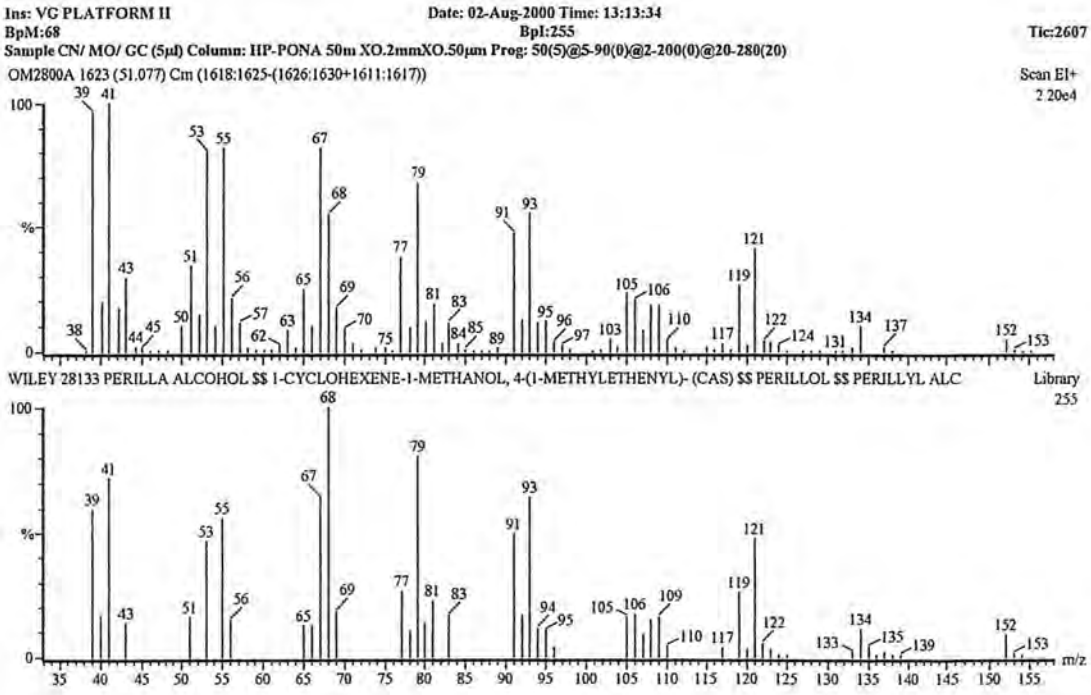

Fig. 41 MS of Perill alcohol

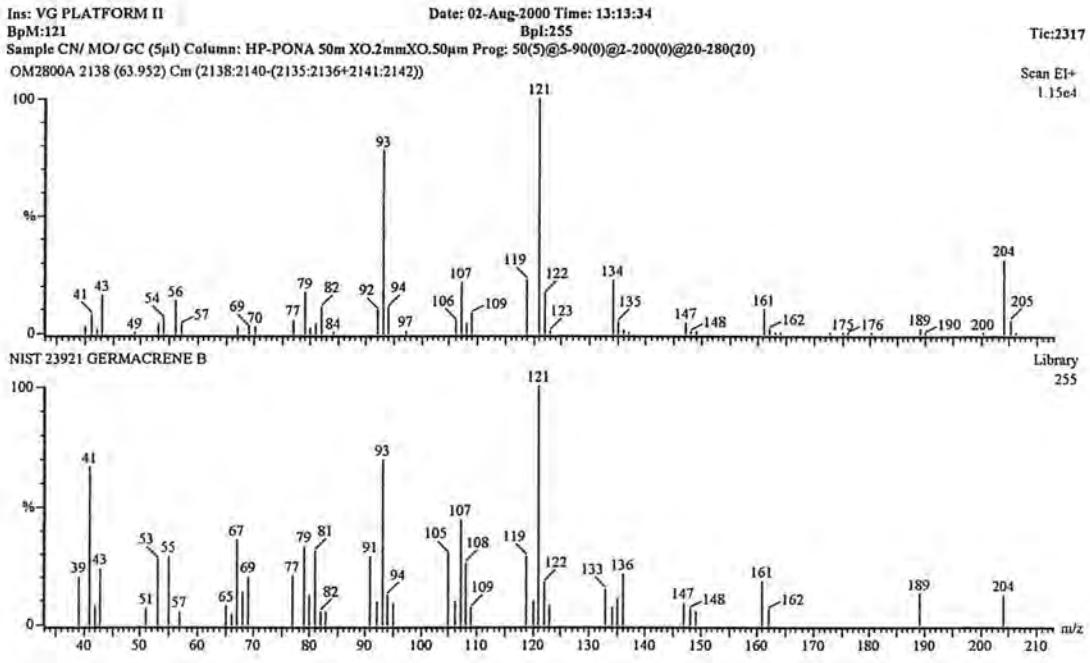

Fig. 42 MS of Germacrene B

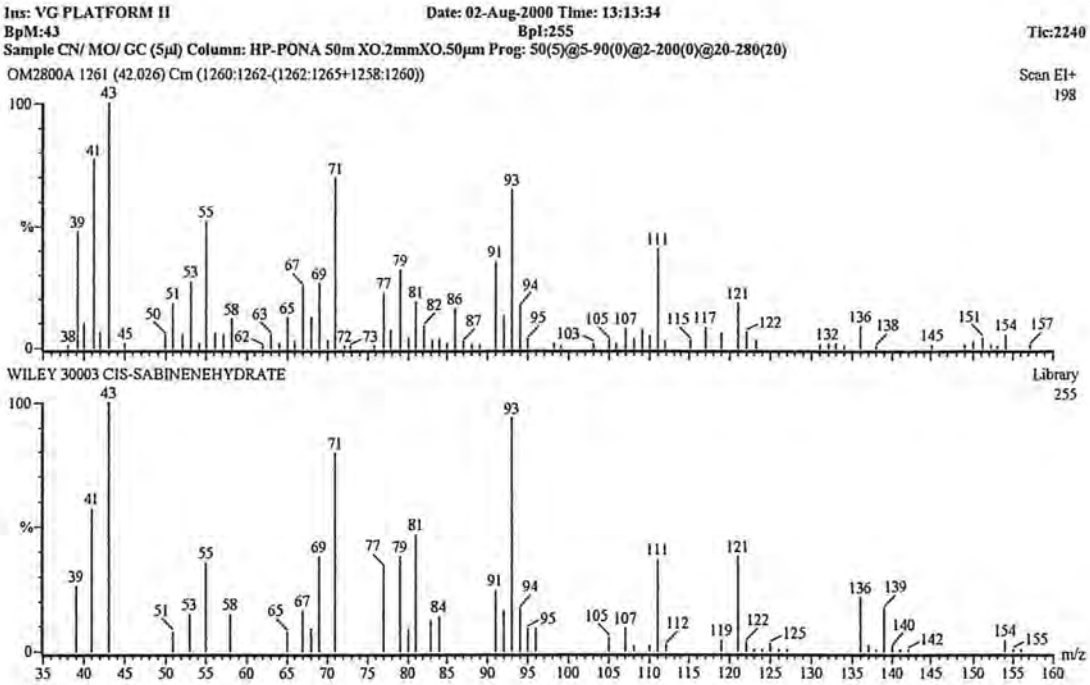

Fig. 43 MS of cis-Sabinenehydrate

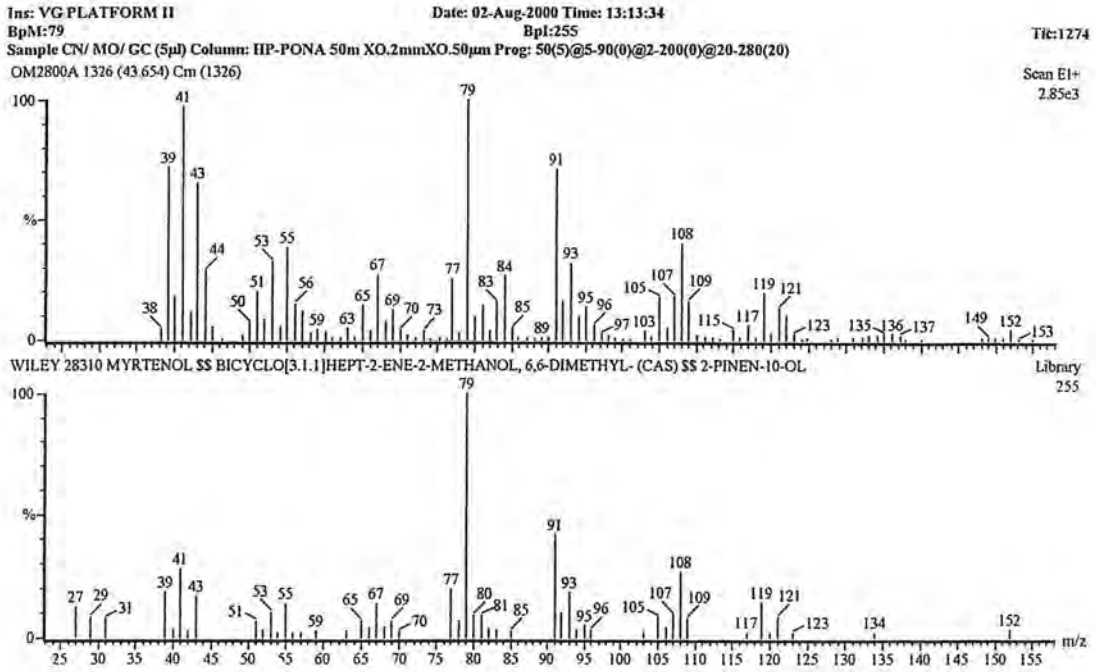

Fig. 44 MS of Myrtenol

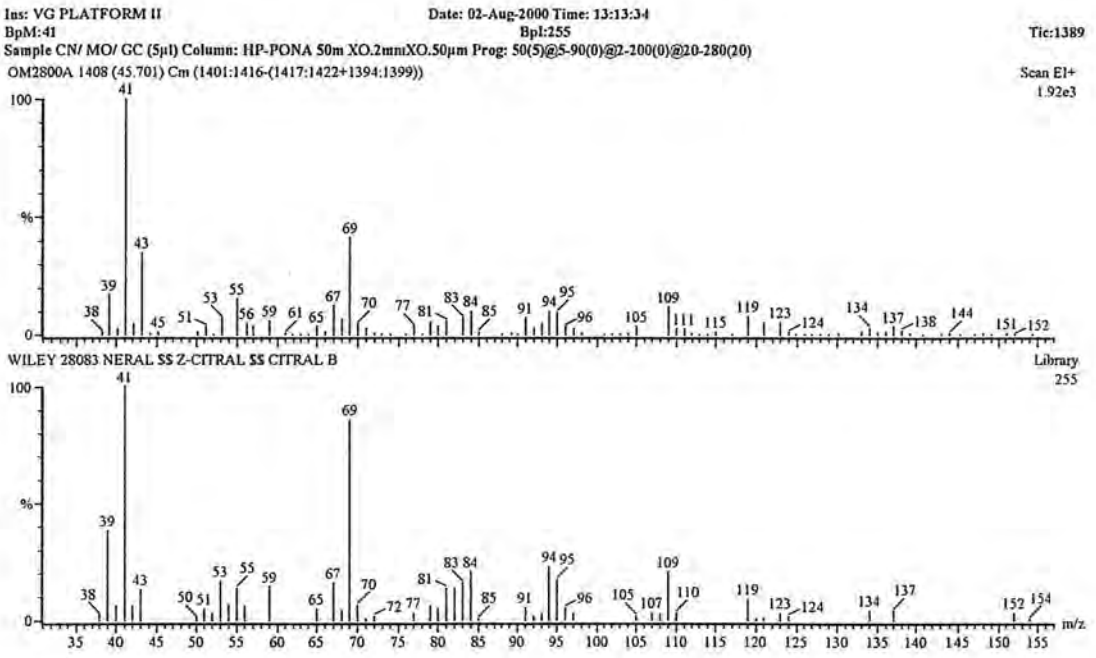

Fig. 45 MS of Neral

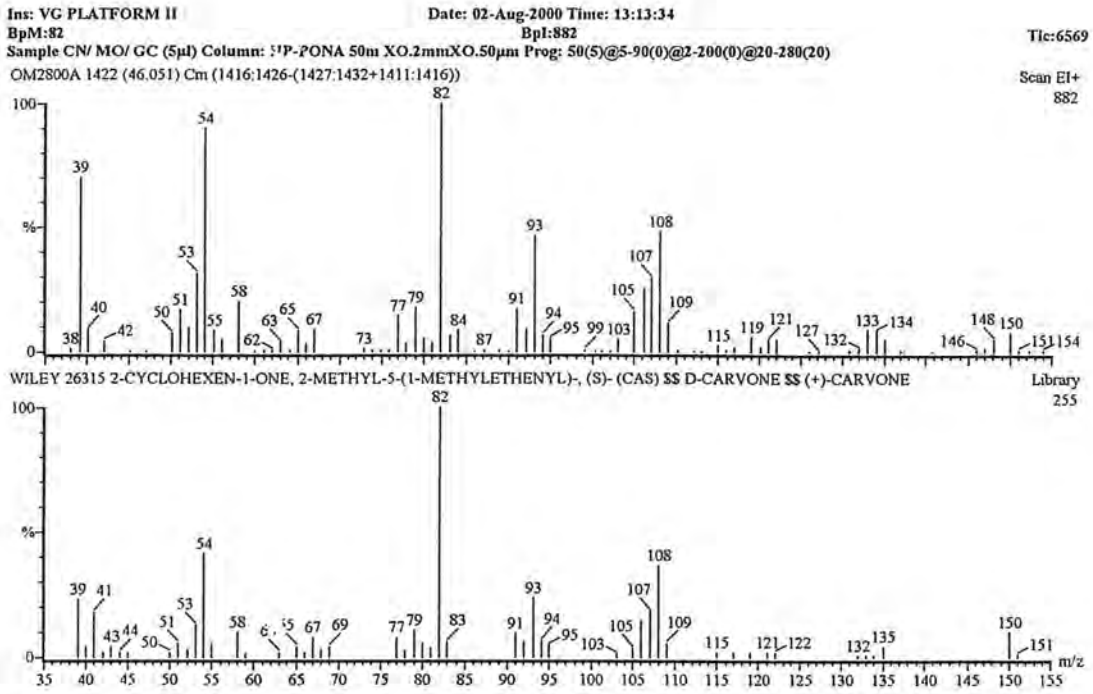

Fig. 46 MS of Carvone

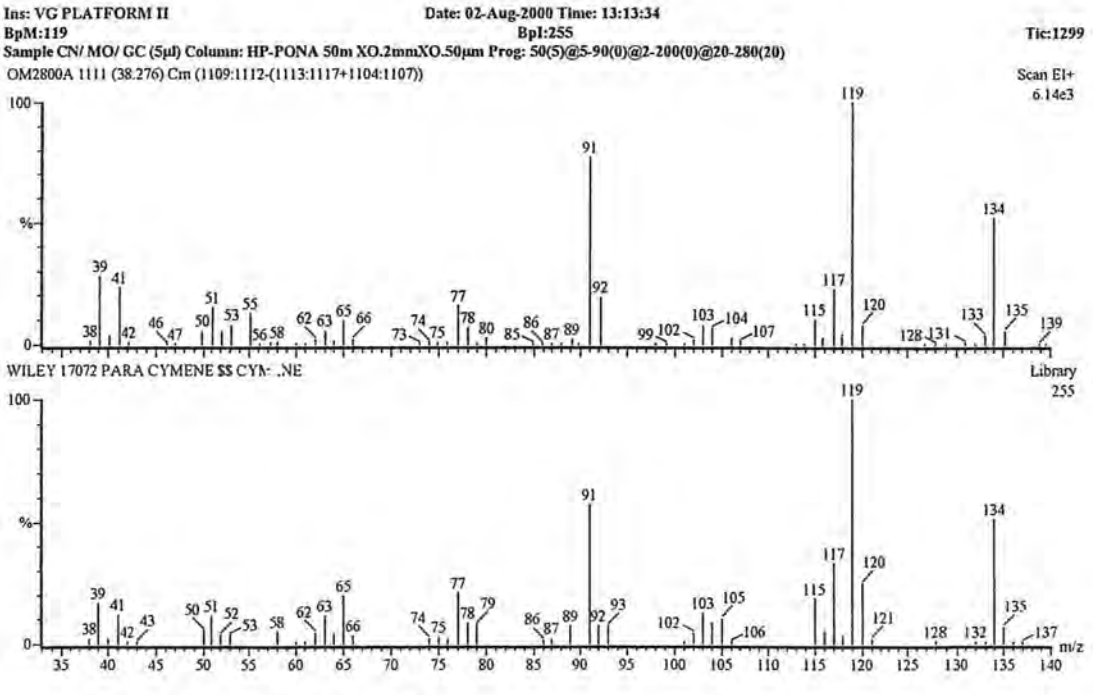

Fig. 47 MS of *p*-Cymene

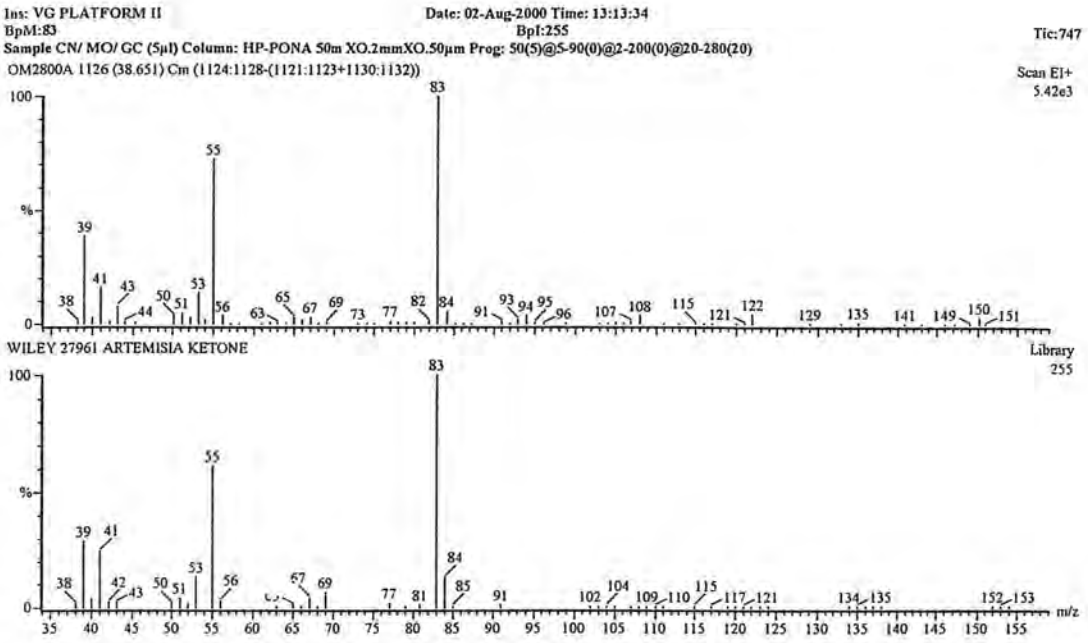

Fig. 48 MS of Artemisia ketone

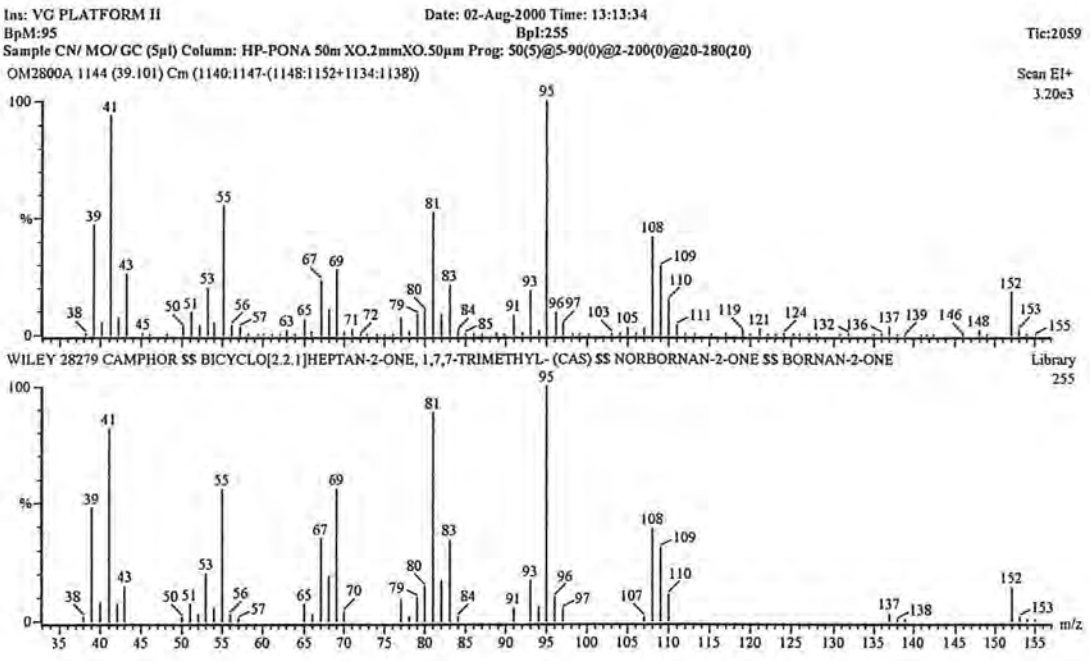

Fig. 49 MS of Camphor

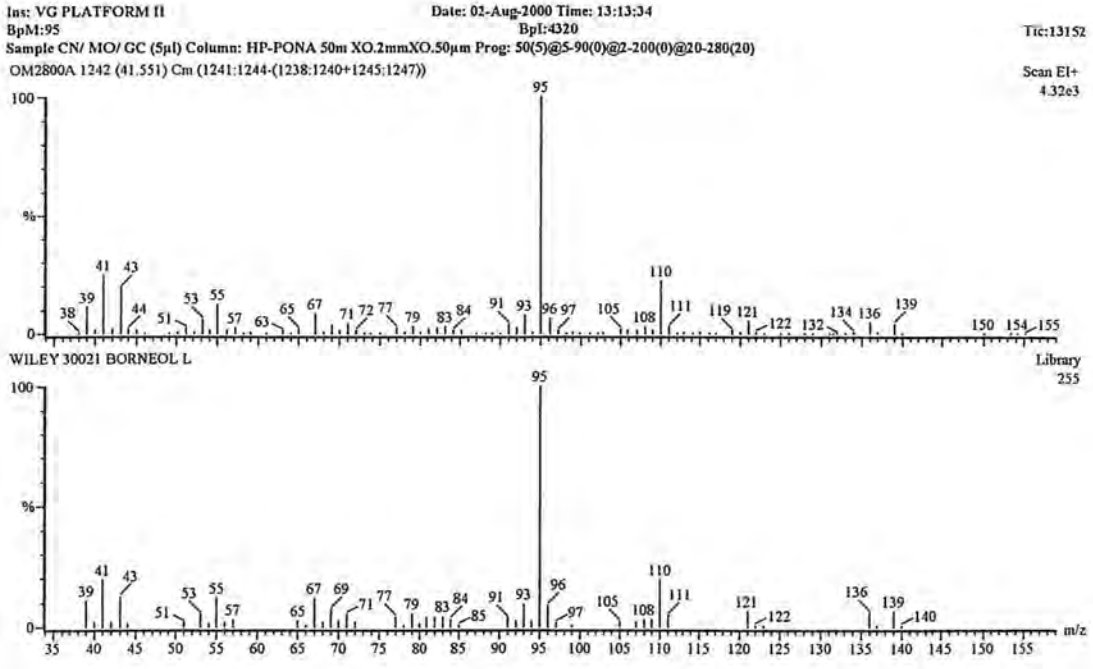

Fig. 50 MS of Borneol

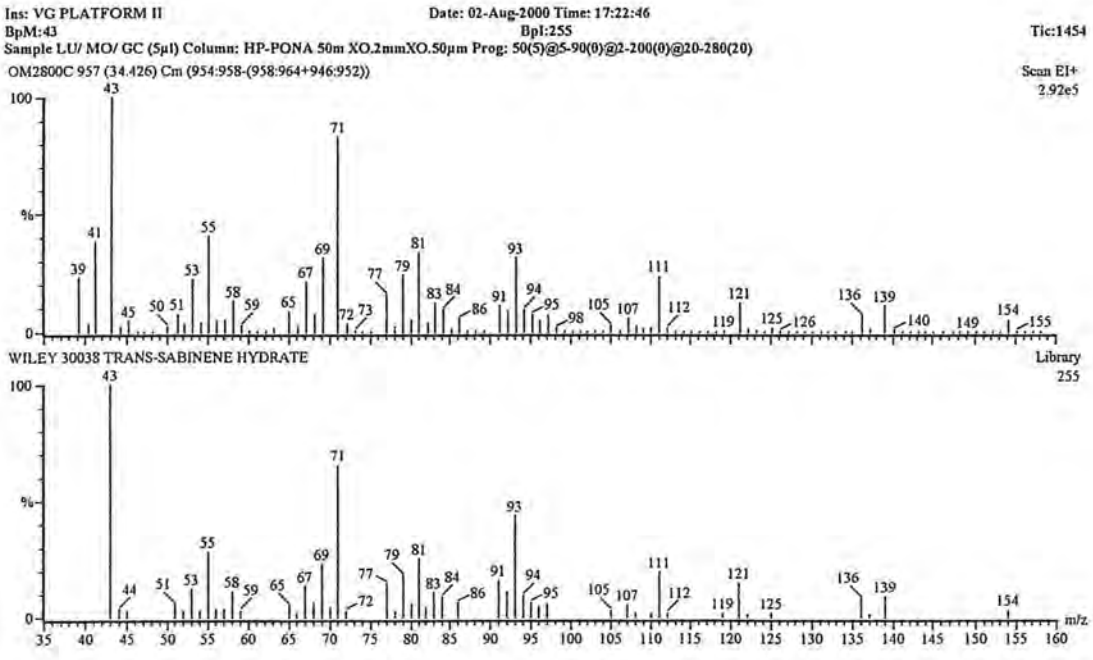

Fig. 51 MS of trans-Sabinenehydrate

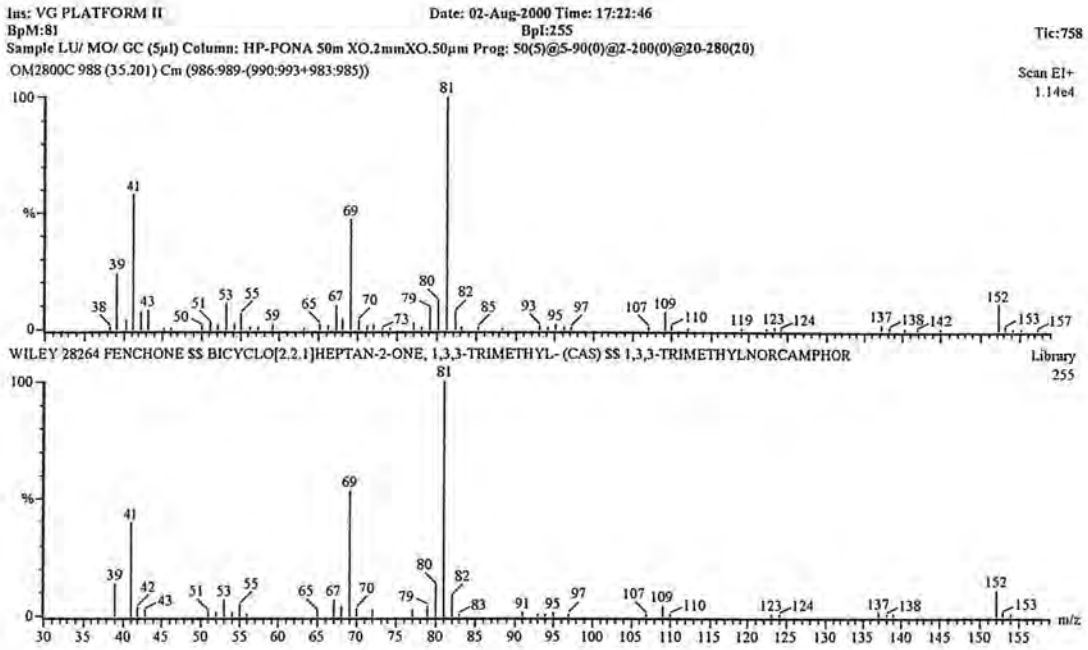

Fig. 52 MS of Fenchone

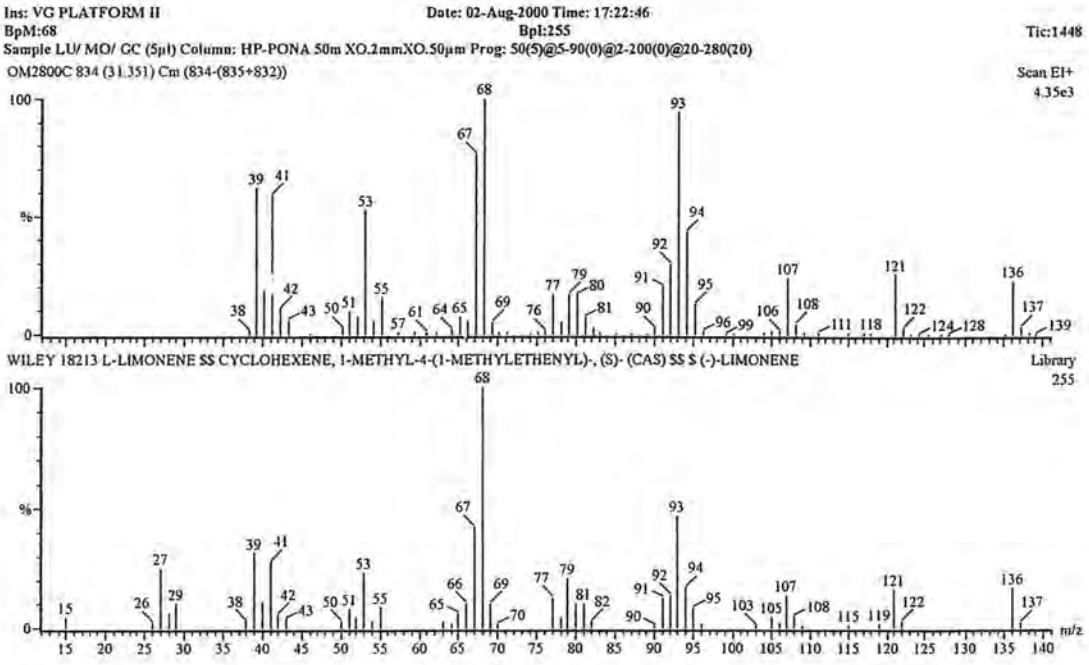

Fig. 53 MS of Limonene

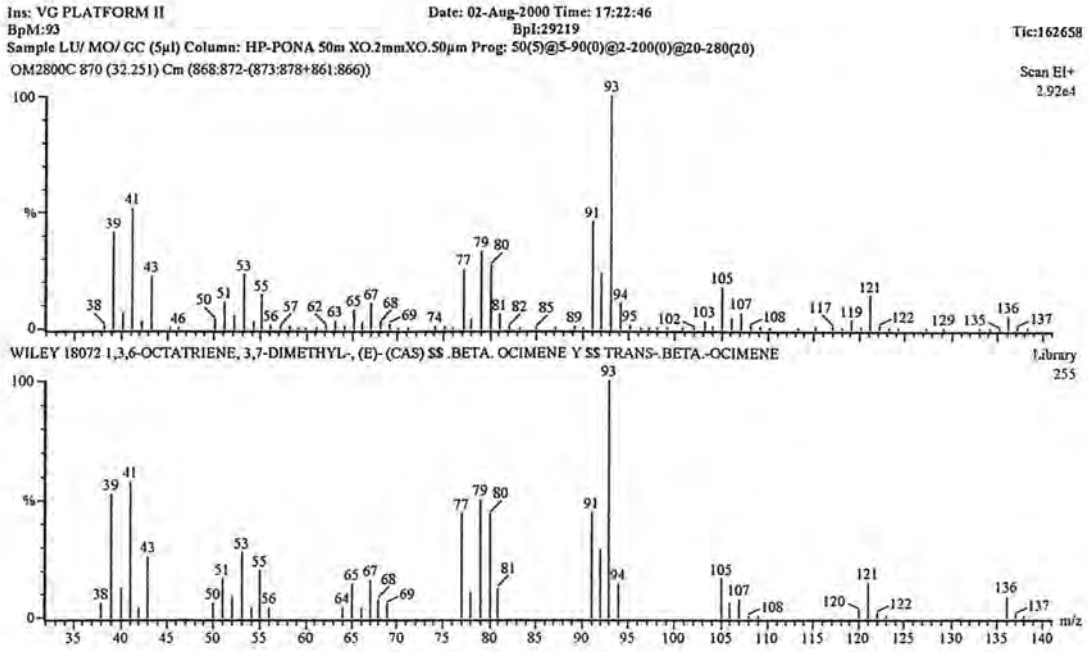

Fig. 54 MS of trans-β-ocimene

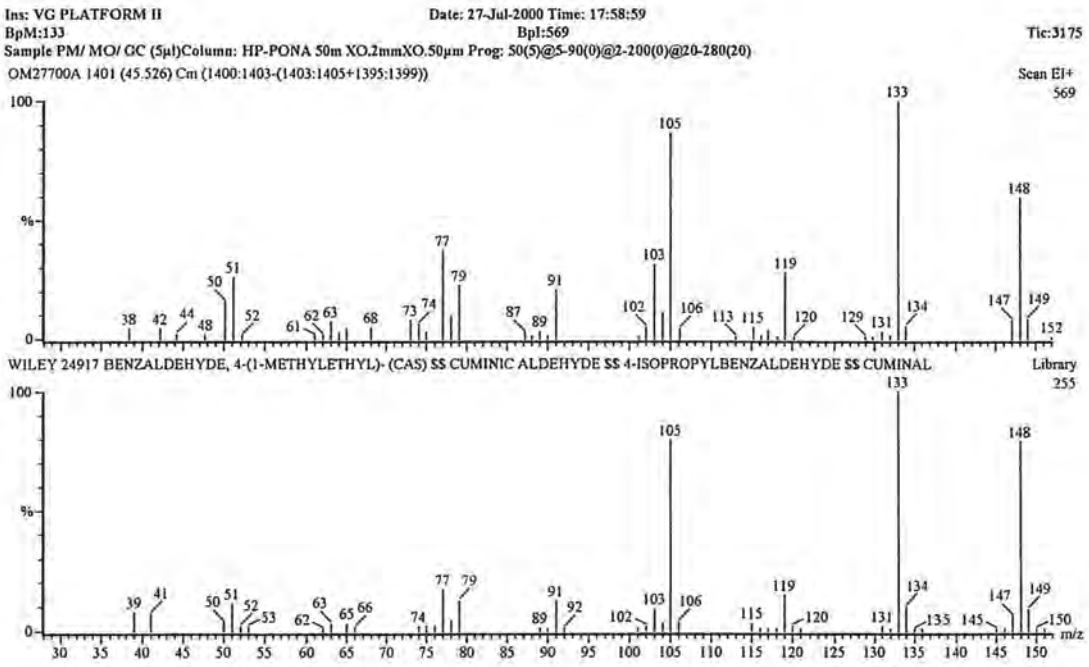

Fig. 55 MS of Cuminal

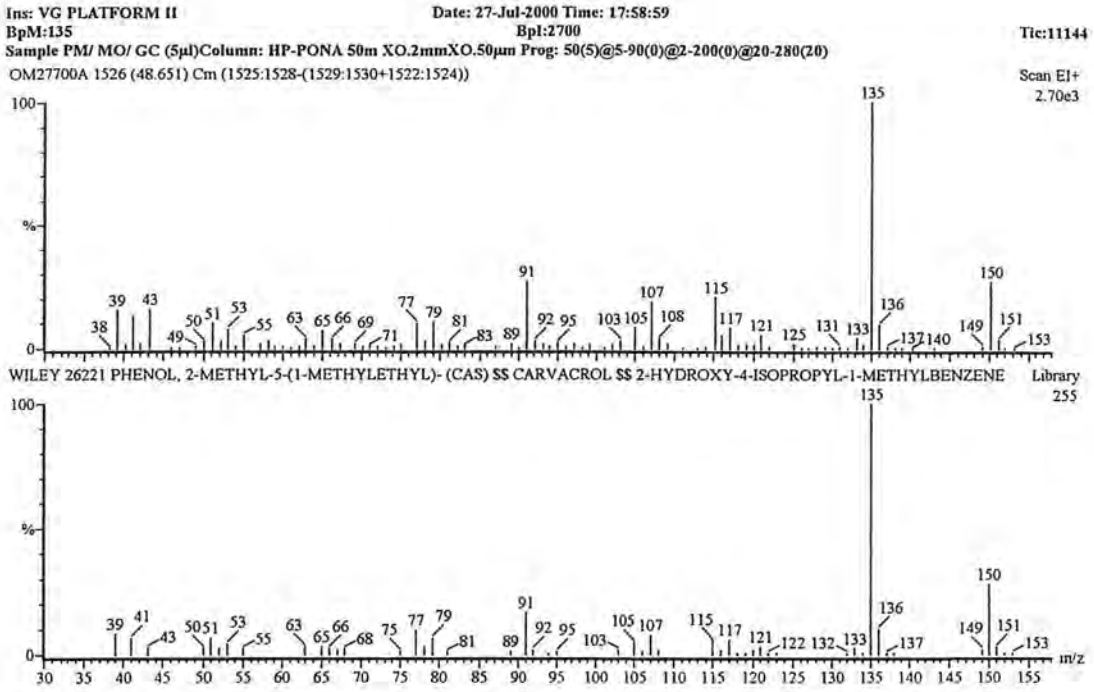

Fig. 56 MS of Carvacrol

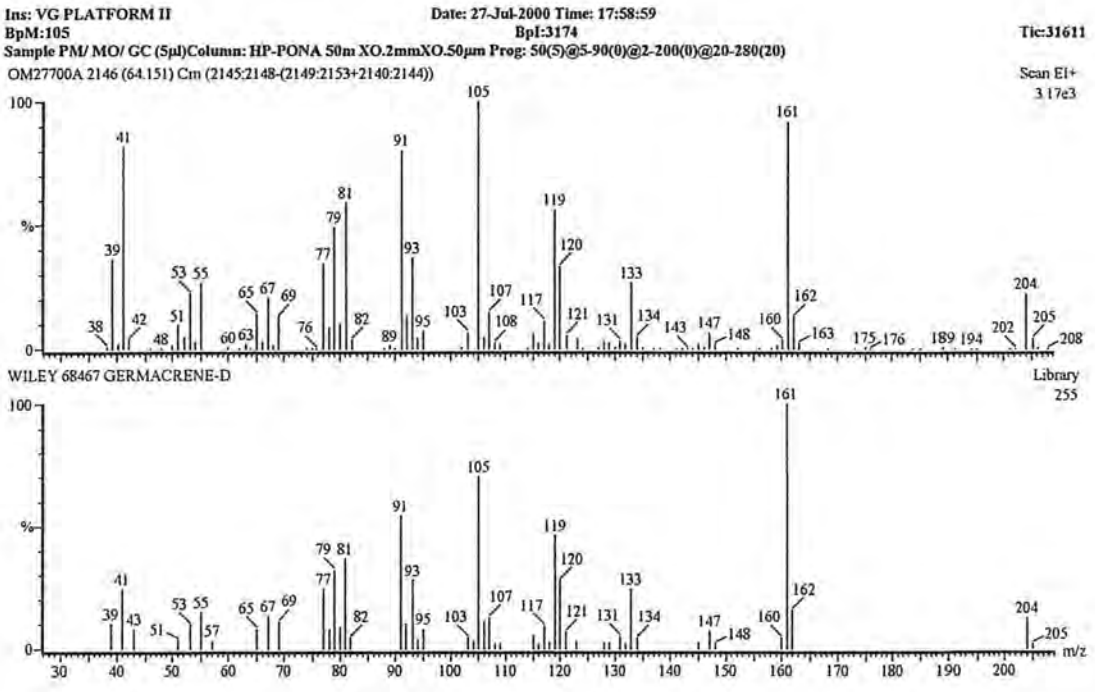

Fig. 57 MS of Germacene D

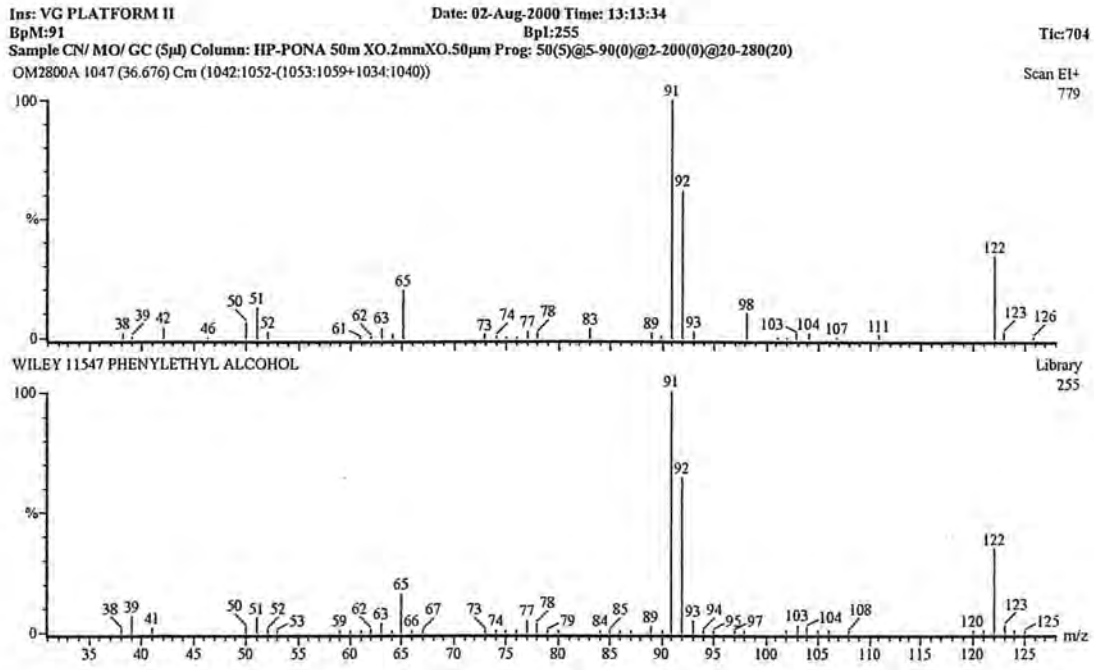

Fig. 58 MS of Phenylethyl alcohol

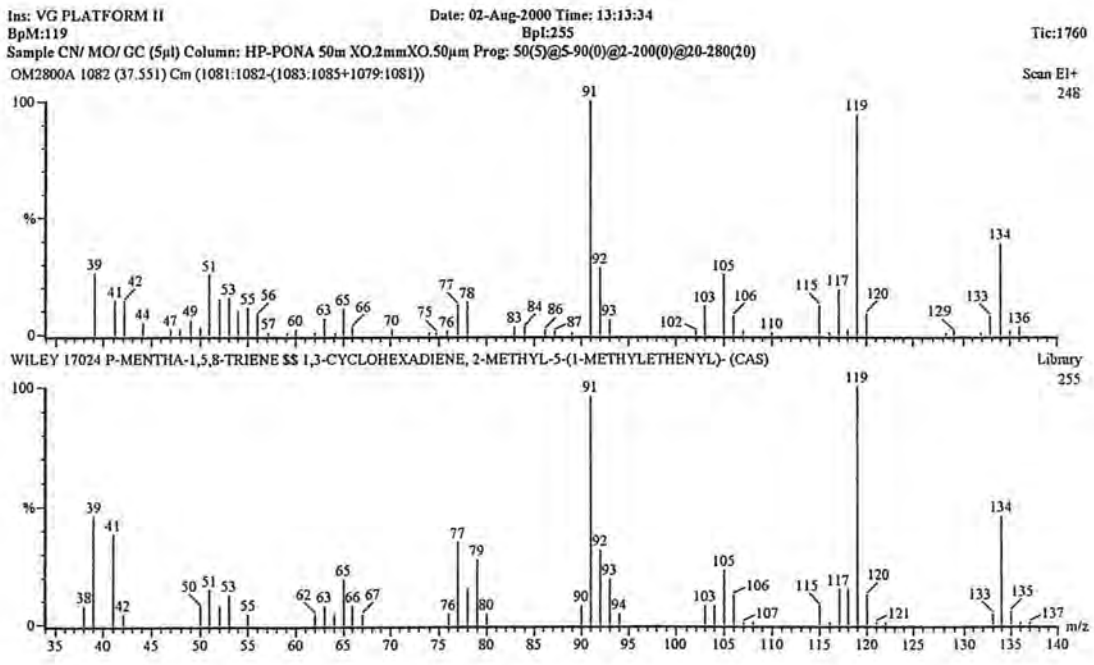

Fig. 59 MS of *p*-Mentha-1,5,8-triene