

**ISOLATION AND CHARACTERIZATION OF ESSENTIAL  
OILS FROM *Ocimum americanum*, *Lantana camara*, *Lantana  
trifolia* AND *Tephrosia vogelii***

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**A thesis submitted to the Graduate School in partial fulfilment for the requirements of  
the Master of Science Degree in Chemistry of Egerton University**

**EGERTON UNIVERSITY**

**October 2007**

## DECLARATION AND RECOMMENDATION

### DECLARATION

This thesis is my original work and has not been submitted in this or any other University.

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### RECOMMENDATION

This work has been submitted with our approval as the University Supervisors.

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## **DEDICATION**

With honour, I dedicate this thesis to my parents, Mr. Mohamed S. Bendera and Ms. Saum Twahir.

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I am grateful to my supervisors, Dr. J. C. Matasyoh and Dr. T. Kinyanjui for the quality and consistency of their supervision, the endless feedback on the progress of the study and stimulating ongoing discussions throughout the course of this research project.

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## ABSTRACT

The botanical insecticides are generally pest-specific, relatively harmless to non-target organisms and biodegradable. Unlike conventional insecticides which are based on a single active ingredient, plant-derived insecticides comprise of an array of chemical compounds which act concertedly on both behavioural and physiological processes. Thus the chances of pest developing resistance to such substances are less likely. Essential oils are good example of plant compounds with such properties. Toxicity of a large number of essential oils and their constituents has been evaluated against several stored-product pests. Consequently, determination of the extraction yield of the essential oils and comparison of the chemical composition of essential oil of different plant species and chemotypes is an important step in promoting and understanding their use as insecticides. This study involved characterisation of essential oils of *Lantana camara*, *Lantana trifolia*, *Ocimum americanum* and *Tephrosia vogelii*, which have known repellent, insecticidal and antifeedant activities against various insect pests. The fresh leaves of *L. camara*, *L. trifolia* and *T. vogelii* were collected and hydro-distilled for 3h in a modified Clevenger apparatus. The essential oil from the leaves of *L. lantana* and *T. vogelii* was obtained in a yield of 0.63% w/w and 0.056%, respectively, after drying over anhydrous Na<sub>2</sub>SO<sub>4</sub>. The leaves and flowers of *L. trifolia* gave 0.060% and 0.064% of essential oil respectively. The leaves of *O. americanum* were collected, air-dried under shade to constant weight and ground to a fine powder. The essential oil obtained by steam distillation using a modified clevenger apparatus gave 4% based on dry weight. The main components of the essential oil from *L. camara* were β-caryophyllene (21.35%), α-humulene (8.73%), sabinene (9.15%), 1, 8-cineole (5.54%). *L. trifolia* essential oil had germacrene D as the main component in leaves (23.14%) and flowers (24.88%). Other components in essential oil from the leaves' were *cis*-ocimene (7.27%), β-caryophyllene (6.32%); and β-caryophyllene (11.93%) and α-humulene (4.65%) in flowers. In *O. americanum* essential oil the major components were terpinen-4-ol (43.21%) and 1, 8-cineole (16.13%) while in *T. vogelii* essential oil nerolidol (7.73%), β-caryophyllene (6.89%), caryophyllene oxide (6.22%) and germacrene D (6.20%) were the main components. A number of compounds in the oils are known to exhibit insecticidal and pharmacological properties, and are used in the cosmetic, flavour and fragrance industries

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## LIST OF ABBREVIATION

AICAD	African Institute for Capacity Development
EI Mode	Electron Impact Mode
GC-MS	Gas Chromatography- Mass Spectroscopy
GC-MSD	Gas Chromatography- Mass Spectroscopy Detector
KI	Kovats' Index
MS	Mass Spectroscopy
MTBE	Methyl Tertiary Butyl Ether
RA	Relative Abundance
RT	Retention Time

## CHAPTER ONE: INTRODUCTION

### 1.1 Background Information

Plant materials have played an important role in traditional methods of field crop and stored grain protection against insect pest infestation since time immemorial (Regnault-Roger & Hamraoui, 1993). Several thousand of plant species worldwide have been found to exhibit insecticidal and repellent properties. Tropical plants have been singled out as rich sources of plant-based pesticides, which maybe useful in insect pest control (Dales, 1996 & Facknath, 1994). In traditional Kenyan medicinal and agricultural practices, much use is made of local trees, shrubs and herbs, particularly from Lamiaceae, Rutaceae, Fabaceae, Verbenaceae and Maliaceae (Dale, 1996; Masinde, 1996; Ogendo *et al.*, 2003). Their use as insecticides has been problematic because the plant materials are mixed with the stocks for short periods, necessitating regular replacement. The practice is time-consuming and painstaking process. Therefore efforts should be directed at finding economical and sustainable traditional/cultural methods of grain storage.

Dales (1996) reported that wide-scale commercial use of plant extracts as insecticides began in the 1850's with the introduction of nicotine, rotenone, *derris* dust and pyrethrum. The most economically important natural plant compounds used in commercial insect control are the pyrethrins from the flower heads of pyrethrum, *Chrysanthemum cinerariaefolium* Vis. (Schery, 1954). Pyrethrins are unstable ultra-violet light and are rapidly metabolised thus limiting their potency and application (Casida, 1983). The limitations gave impetus for the synthesis of analogues, (pyrethroids) (Yamamoto, 1970). Unfortunately, several insects have exhibited resistance to pyrethroids (Corbett *et al.*, 1984). Nicotine, isolated from *Nicotiana* species is insecticidal but its use in insect control has dropped steadily because of the high cost of production, disagreeable odour, extreme mammalian toxicity and instability in the environment which limits the insecticidal activity (Schmeltz, 1971). Rotenone was found to be unstable in the environment and very toxic to fish (Peveling *et al.*, 1994). This has limited its use as an insecticide.

Synthetic insecticides have been used widely and successfully to control insects in grain storage (Pierce & Schimidt, 1992), where it has been observed that pests and diseases cause 30-80% pre and post-harvest losses in yields. Insect pest attack alone accounts for 20-60% grain loss

in storage (Saxena *et al.*, 1990). But it has been observed that improving post-harvest protection measures at farm level in developing countries could increase food supply by 30-40%, since; up to 90% of cereals produced are intended for human consumption (NRI, 1999). Most insecticidal compounds in use fall within four main classes: the organochlorines, the organophosphates, the carbamates and the pyrethroids. Out of these the major classes in use today are organophosphates and carbamates (Ware, 1982 & Dorow, 1993). The use of organochlorine insecticides has been banned in developed countries and alternative methods of insect pest control are being investigated (Franzen, 1993).

In Kenya, the adoption rate of recommended synthetic insecticides in traditional farming communities has remained remarkably low (Ogendo *et al.*, 2003). This reluctance to adopt the insecticide, probably influenced by lack of financial resources and toxicity concerned, offers a stimulating challenge to scientists in search for safer and natural pesticides that are readily available, cost effective, less detrimental to the environment and compatible with the resource poor farmers' circumstances. This opens the door for plant-based alternatives, which have proven useful for the survival of mankind over several generations. A few examples of plants used by traditional people with different cultures to repel and control insects include wormwood (*Artemisia absinthium*), basil (*Ocimum basilicum*), eucalyptus (*Eucalyptus globulus*), tansy (*Tanacetom vulgan*), tephrosia (*Tephrosia vogelii* Hook), rue (*Rutagra veolens*), Mexican marigold (*Tagetes minuta*), wild sage (*Lantana camara*) and rosemary (*Rosmarinus officinalis*) (Oliver-Bever, 1986; Ogendo *et al.*, 2003). It has been observed that volatile compounds present in many spices and herbs possess insecticidal activities (Shaaya *et al.*, 1991).

The botanical insecticides are generally pest-specific and are relatively harmless to non-target organisms, biodegradable and harmless to the environment (Facknath, 1994). Unlike conventional insecticides which are based on a single active ingredient, plant-derived insecticides comprise of an array of chemical compounds which act concertedly on both behavioural and physiological processes. Thus the chances of pests developing resistance to such substances are less likely (Saxena, 1987). Essential oils are good example of plant compounds with such properties. Toxicity of a large number of essential oils and their constituents has been evaluated against several stored-product insects.

The essential oils of basil (*Ocimum* species) and *Eucalyptus* species showed insecticidal activity against *Sitophilus zeamais*, *Sitophilus oryzae*, *Tribolium confusum* Du Val and

*Callosobruchus maculatus* Fab. (Tapondjou *et al.*, 2005; Kéita *et al.*, 2001; Bekele, 1994). Fumigant toxicity and reproductive inhibition induced by a number of essential oils and their respective monoterpenoid components has been evaluated against the bean weevil (*Acanthoscelides obtectus* Say) (Regnault-Roger & Humaraoui, 1995). Toxicity of eugenol, isoeugenol, methyleugenol and linalool were observed on several Coleoptera that damage post harvest products (Huang *et al.*, 2002; Weaver *et al.*, 1991). In contrast, the toxicity of conventional synthetic insecticides is mainly restricted to neuro-muscular function (Ware, 1982).

The conventional synthetic insecticides also require special safety procedures and equipment during production and application (Ware, 1982). Despite precautions, exposure to humans, the environment (Franzen, 1993) and food (FAO, 1992), synthetic insecticides are expensive and in many cases only produce moderate results along with major ecological damage (Franzen, 1993). In contrast, the low toxicity of botanical insecticides makes processing and application of the product, inexpensive and in many cases, the materials is locally available and affordable.

Based on all these factors, it has been observed that determination of the extraction yield of essential oils and comparing the chemical composition of the different plant species and chemotypes is an important step in promoting the use of essential oils as insecticides. The chemical composition could also help in determining the active ingredients in these oils. Therefore, this study aims at characterisation of essential oils of *Lantana camara*, *Lantana trifolia*, *Ocimum americanum* and *Tephrosia vogelii*, which have known repellent, insecticidal and antifeedant activities against various insect pests.

## **1.2 Statement of the Problem**

New environmentally-safe and readily available natural insecticides in local insecticidal plants have not been isolated and characterized.

## **1.3 Objectives**

The general objective of this study is to elucidate the oil composition from anti-insect plants: *O. americanum*, *L. camara*, *L. trifolia* and *T. vogelii*.

### **1.3.1 Specific objectives**

- To extract essential oils from *O. americanum*, *L. camara*, *L. trifolia* and *T. vogelii*.
- To characterize components of the insecticidal essential oils using gas chromatography-mass spectroscopy.

## **1.4 Justification**

The poor storage facilities used by traditional farmers in developing countries are unsuitable for effective conventional chemical-based pest control, as most storage types are open to re-infestation by pests. Stored-product insects destroy between 20-60% of the stored grains. The control of these insects relies heavily on the use of synthetic insecticides and fumigants which has led to environment pollution, insecticide resistance which has resulted in pest resurgence and lethal effects to non-target organisms in addition to direct toxicity to users. The cost of synthetic insecticides is beyond the reach of most farmers therefore, the possibility of using plant materials (extracts and derived compounds) as insecticides giving alternatives to the synthetic chemicals is attractive.

## CHAPTER TWO: LITERATURE REVIEW

### 2.1 The Genus *Lantana*

The genus *Lantana* belongs to the family Verbenaceae, which consists of 3000 species in 75 genera. The medium sized aromatic shrubs with quadrangular stems, sometimes having prickles are found in the tropics and sub-tropics. Their posture may be sub-erect, scrambling or occasionally clambering.

The common species are *L. camara*, *L. trifolia*, *L. rhodesiensis* and *L. viburnoide* (Dale & Greenway, 1961). These species are differentiated by the colour of their flowers, whereby *L. camara* flowers have flat top that is pink, yellow or white showy with mono-seeded greenish-blue or black fruits. *L. trifolia* has leaves in threes and pink or mauve flowers, *L. rhodesiensis* has crimson or purple flowers, while *L. viburnoide* has leaves which are opposite with white flowers (Dale & Greenway, 1961). Different parts of the plants are used in traditional systems of medicine for the treatment of various human ailments. The fresh leaves are crushed and boiled in sufficient amount of water for topical application in curing dermatitis, eczema, tinea, traumatic injury and wound bleeding, for haemoptysis of pulmonary tuberculosis, dried flowers are boiled in water (Qingrong & Guangcheng, 1993).

Two species of *Lantana* are a subject of this study, *L. camara* (Figure 2) and *L. trifolia* (Figure 1). This is because of their frequent occurrence as wild shrubs in Kenya. There is no much information on *L. trifolia* but *L. camara* has been widely studied.



Figure 1: *Lantana trifolia*



Figure 2: *Lantana camara*

### 2.1.1 *Lantana camara*

It has been recorded that different parts of the plant are rich sources of various bioactive principles. In Africa infusion of the leaves are used against rheumatism, asthma, coughs and colds. The whole plant and its infusion are also considered to be antipyretic, diaphoretic and anti-malarial (Watt & Breyer-Brandwijk, 1962; Oliver-Bever, 1982). The leaves and flowering tops are given as a Febrifuge and diaphoretic, it is used to treat yellow fever and has been reported to contain a quinine-like alkaloid, lantamine (Burkill, 1995).

The plant is also known to be toxic to grazing animals, which on ingestion of the leave develop hepatotoxicity and photosensitization (Sharma & Sharma, 1989). The toxicity is believed to be due to the presence of lantadene A and lantadene B in the plant (Khan *et al.*, 2003). The extract of *L. camara* roots have been reported to exhibit antimalarial activity against *Plasmodium falciparum* (Weenen, 1990) and as a potential source of oleanolic acid, a hepatoprotective agent (Misra *et al.*, 1997).

Laboratory and field evaluations have been conducted on the efficacy of aqueous and organic extracts, and topical application of ground powders from roots, leaves, stems and flowers on several insect pests. Aqueous extracts of *L. camara* reported significant anti-feedant activity against the jutestem weevil, *Apion corchori*; the jute semilooper, *Anonus sabulifera*; and the bihar hairy caterpillar, *Spilosoma oblique* (Sharma *et al.*, 1992). Similarly aqueous extracts

exhibited strong antifeedant effect on *Plutella xylostella* (Lepidoptera) but were not repellent. In the same study the volatile oil from flowers and leaves was found, based on LD<sub>50</sub> values, to be moderately toxic to *Sitophilus zeamais* (Rejesus & Tantengco, 1985). Kulkarni & Joshi (1998), using leaf (methanol and petroleum ether) extracts of *L. camara* reported significant antifeedant activity against Sissoo defoliator, *Plecoptera reflexa* and Maharukha webworm, *Attera fabriciella*. Alcoholic extracts produced 96% short-term repellency against the German cockroach, *Blattella germanica* (Scheffler & Dombrowski, 1993). Similarly leaf extracts have been reported to have insecticidal and growth regularly effects on leaf miners, *Linomyza* spp (Facknath, 1994).

Extensive study of the essential oils of *L. camara* has been done. The isolation and identification of six novel compounds from *L. camara* from Brazil: 1, 12-epoxycadina-3, 11-dienes; italicen-15-al (2, 11-cycloacor-3-en-15-al); 6, 10-epoxybisabolene-12-al and *epi-α*-bisabolone; *ar*-curcumen-15-al and its 10, 11-epoxides, methyl(E)-*trans-α*-bevgamota-2,10-dien-12-olate and its 2, 3-epoxide; and helifolen-12-al have been reported (Weyerstahl *et al.*, 1999)

Essential oils of *L. camara* from India was reported to have Germacrene D as the major compound in the leaves together with  $\gamma$ -elemene,  $\beta$ -caryophyllene,  $\beta$ -elemene,  $\alpha$ -copaene,  $\alpha$ -cadinene while in the flower oil  $\beta$ -clemene was a major compound together with germacrene D,  $\alpha$ -copaene,  $\beta$ -caryophyllene and  $\gamma$ -elemene (Khan *et al.*, 2002). Essential oil of fruits and stems from northern India are dominated by palmitic acid, stearic acid and germacrene D while the stem by palmitic acid and stearic acid. Khan *et al.*, (2003)

The essential oil *L. camara* from Nigeria has  $\beta$ -caryophyllene as major compound together with  $\alpha$ -humulene, sabinene, germacrene D and cubebol (Oyededeji *et al.*, 2003). The essential oil of *L. camara* from South China was characterised by a high percentage of sesquiterpenes. The main components detected were germacrene D (15.85%),  $\beta$ -caryophyllene (12.35%),  $\alpha$ -humulene (9.31%) and germacrene B (6.19%). The oxygenated monoterpene 1, 8-cineole (4.61%) was reported in relatively high amounts compared to other monoterpenes.

*L. camara* leaf oil which is rich in sesquiterpenes:  $\gamma$ -cadinene,  $\alpha$ -selinene,  $\beta$ -gurjunene, eudesma-3, 11-diene,  $\delta$ -cadinene and Isolongifolene, constituting over half of the oil (65%) has been reported (Misra & Laatsch 2000).

### **2.1.2 *Lantana trifolia***

It is an erect suffrutex growing to a height of 3m with tetragonal stem and branches, pubescent in their upper parts and thornless. The leaves are opposing and petiolate and generally arranged in threes. The inflorescences are spiked with pink or mauve lilac flowers, pink in the centre and white or mauve at the outer edge. The dupes are mid to dark purple (Chalchat *et al.*, 1998). Little work has been done on the chemical composition of the essential oil of the *L. trifolia* growing on the African continent.

The only reported work was done on this species growing in Rwanda (Chalchat *et al.*, 1998). There is no record of the traditional and cultural uses of *L. trifolia* in Kenya but it is used as an anti-inflammatory medicinal plant in Venezuela. Extraction of this plant growing in South America has shown anti-inflammatory, anti-nociceptive (Silva *et al.*, 2005) and anti-pyretic activities (Uzeategui *et al.*, 2004). The chemical composition of volatile oils of the *L. trifolia* from Kenya is being reported for the first time.

## **2.2 The Genus *Ocimum***

The genus *Ocimum* belongs to family Lamiaceae. They are collectively known as Basil and are acclaimed for its diversity. It undergoes abundant cross-pollination resulting in large numbers of sub-species and varieties, which differ in essential oil composition and morphological characteristics. It comprises of about 60 species and numerous varieties of herbs and shrubs from tropical and sub tropical regions of Asia, Africa, Central and South America, but the center of diversity appears to be Africa (Paton, 1992). It represents an important source of essential oils and aroma compounds used in foods, perfumery and cosmetic industry (Simon *et al.*, 1984, Simon *et al.*, 1990).

Some *Ocimum* species are used in traditional medicine for different applications, and the extract of the plants have been shown to contain biologically active constituents that are insecticidal, nematicidal, fungistatic or anti-microbial (Simon *et al.*, 1990, Albuguerque, 1996). The common species, which include *O. americanum*, *O. suave*, *O. basilicum* and *O. gratissimum*, have been used traditionally against pests like *Sitophilus zeamais* (Bekele, 1994 & Bekele *et al.*, 1996). Kéita *et al.*, (2000a) reported *O. basilicum* and *O. gratissimum* to be potential insecticides. The leaves and seeds are rich in essential oils, which are repellent, toxic or growth inhibitory to many insects (Grange & Ahmed, 1988). In field and laboratory experiments,

treatment with seed extracts against bugs in turnips, *Bragada cruciferarum*; the American ball worm, *Helicoverpa armigera*; and the aphid, *Aphis gossypii* increased mortality between 50-90% (Pandey & Verma, 1982; Pandey *et al.*, 1983a; 1983b).

There are usually considerable variations in the amount of the major components within this species. The variation in chemical composition of basil oil is thought to be due to polymorphism caused by inter-specific hybridization or the biosynthetic pathways of major compounds in the oil (Lawrence, 1988). In this study Lawrence found that the main constituents of the essential oil of basil are produced by two different biochemical pathways, the phenylpropanoids (eugenol, methyl chavicol, methyl cinnamate) by the shikimic acid pathway and the terpenes (linalool, geraniol, myrcene) by the mavalonic acid pathway.

Several aroma compounds are found in chemotypes of basil, which are commonly known by names based on geographical origins such as Egyptian, French, European or Re-Union basil (Simon *et al.*, 1990). The European type, a sweet basil, is considered to have the highest quality aroma, containing linalool and methylchavicol as the major constituents. The Egyptian basil is similar to European but contains a higher percentage of methylchavicol. The Re-Union type, from the Comoro islands and more recently from Madagascar, Thailand and Vietnam is characterized by high concentration methylchavicol (Marotti *et al.*, 1996). Methyl cinnamate-rich basil has been commercially produced in Bulgaria (Simon *et al.*, 1990), India, Guatemala and Pakistan (Marotti *et al.*, 1996). Basil from Java (Simon *et al.*, 1990), Russia and North Africa (Marotti *et al.*, 1996) was found to be rich in eugenol.

### **2.2.1 Essential oil components from various *Ocimum* species**

Chemical analysis of the essential oils of various *Ocimum* species has been reported. The oils from *O. suave* revealed that the principal compound is eugenol, which was reported to have repellence on mosquitoes (Chogo & Crank, 1981). *Ocimum basilicum* L. occurs everywhere in several forms, varieties and chemotypes whose essential oils present marked variation on chemical composition. Analysis of *O. basilicum*, *O. basilicum* var. *minimum* L. and *O. basilicum* var. *purpuracens* Benth. from in North-eastern Brazil revealed that *O. basilicum* var. *minimum* represented a true estragole chemotype while the other two species analysed considered were as linalool chemotype (De Vasconcelos *et al.*, 2003).

The *Ocimum* species from Bangladesh have been analysed and various volatile oil components reported (Mondello *et al.*, 2002). The oil from *O. basilicum* var. *purpuracens* was classified as a monoterpene alcohol-type, based on the monoterpene content (64.5%). Oil obtained from *O. sanctum* L. green was classified as eugenol/sesquiterpene-type based on the content of eugenol (41.7%) and the sesquiterpene components (45.9%), while the oil from *O. sanctum* purple was classified as eugenol-type based on the content of eugenol (77.5%).

A new chemotype of *O. basilicum* L. from Nigeria was found to contain methylchavicol (60.3%) as the major component. Other notable components were 1, 8-cineole (3.1%) and  $\beta$ -pinene (4.8%). This was the third chemotype to be reported (Kasali *et al.*, 2005). The volatile constituents from *Ocimum micranthum*, cultivated in the state of Para, Brazil (Rosas *et al.*, 2005) was found to contain *E*-methyl cinnamate (34.6-56.7%), carvone (10.4-16.1%), limonene (8.1-10.3%) and linalool (4.1-9.4%). *Ocimum* species in the Republic of Guinea, contained linalool (69%), eugenol (10%), *trans*-bergamotene(3%) and thymol (2%) as major constituent in *O. basilicum*; thymol (46%), *p*-cymene (12%) and *trans*-sabinene hydrate (17%) for *O. gratissimum* while *p*-cymene (59%), thujene (10%), myrecene (7%) and thymol (7%) for *O. suave* (Kéita *et al.*, 2000b). This study is going to focus on chemical composition of *O. americanum* (Figure 3), which is abundant in most parts of Kenya.

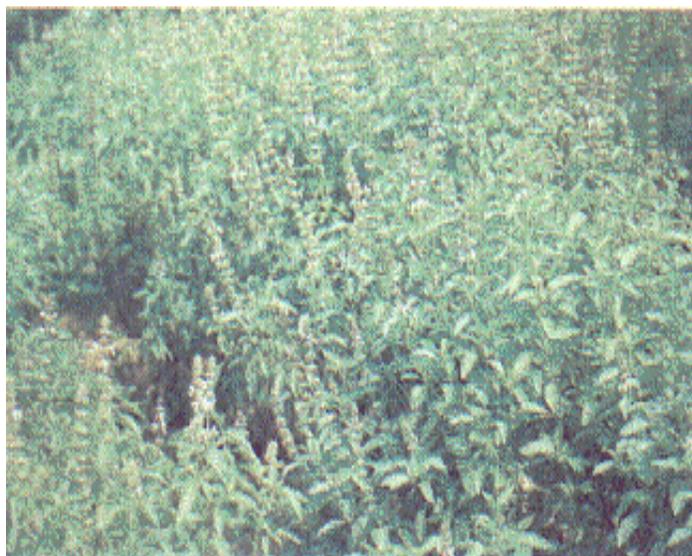


Figure 3: *Ocimum americanum*

### 2.2.2 *Ocimum americanum*

*Ocimum americanum* L. (syn *O. canum* Sims) belongs to the family Lamiaceae (Labiatae) and commonly known as hoary, American or camphor basil. It is an erect branching annual or short-lived perennial shrub with square stems covered by short hairs curved downwards. The leaves are pelliptic-ovate and usually hairy, 7 cm long with small flowers (3-4 mm) broad in fruit. It is common in disturbed bush-land and annual grassland where pools of water gather, growing in altitude range of 0- 1850m (Agnew & Agnew, 1994).

*Ocimum americanum* L. is used in the traditional system of medicine to treat conjunctivities, malaria and headache. The essential oils of *O. americanum* and *O. basilicum* were found to kill insects and to protect stored cereals from damage by cowpea weevil, *Callosobruchus maculatus* (Kéita *et al.*, 2000). Seyoum *et al.*, (2002a) reported that thermal expulsion and or direct burning of *O. americanum*, *O. kilimandscharicum* and *O. suave* were effective in repelling *Anopheles gambiae* mosquitoes in experimental huts within a screen-walled greenhouse. Consequently, Seyoum *et al.*, (2002b) demonstrated that potted *O. americanum* repelled *Anopheles gambiae* in experimental huts under semi-filled conditions. Furthermore, *O. americanum* volatile oil was shown to repel *Aedes aegypt*, *Anopheles dirus* and *Culex quinquefasciatus*, under cage conditions for up to 8 hours (Jawatsin *et al.*, 2001).

Previous studies on this plant report the occurrence of seven chemotypes in view of the qualitative and quantitative differences in the chemical composition of its essential oils. The essential oil of the chemotype from Africa, the Commodore Islands and the French Congo has methyl cinnamate as the major constituents. Whereas the oil from plants growing in the USA and Nigeria showed eugenol in high amounts, and the oil of plants from Somalia, Zimbabwe and India have camphor as the main constituent. Other chemotypes of *americanum* contain mainly fenchone, limonene, methylchavicol and  $\alpha$ -terpineol (De Vasconcelos *et al.*, 2003; Chagonda *et al.*, 2000; Demissew, 1993). The essential oil of the plant from northeastern Brazil has (*E*)-methyl cinnamate as the major component of the leaves and inflorescence. Eugenol was found in high contents in the roots but absent in the other parts (De Vasconcelos *et al.*, 2003). Two chemotypes of *O. americanum* oil from Bangladesh: the citral type based on the high contents of neral and geranial, and the camphor type, based on the high content of camphor have been reported (Mondello *et al.*, 2002).

Essential oils, flavones and triterpenic acids isolated from *O. americanum* from Somalia, where it is used for flavouring foods and in traditional medicine, have been reported (Xaasan *et al.*, 1981; Xaasan *et al.*, 1980). In South Africa, it is known as camphor basil (Xaasan & Roberts, 1990). In Zimbabwe, its traditional uses range from flavour and fragrance, to insect repellence and preservation of corpses (Gelfand *et al.*, 1985, Watt & Breyer-Brandwijk, 1962; Lukwa, 1994).

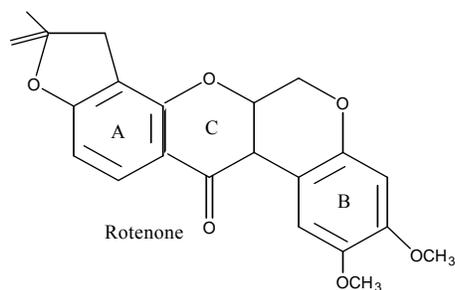
It is also grown in parts of India for flavour and fragrance industry and as a source of natural camphor, since the plant was introduced there from Kenya in the Second World War (Chagonda *et al.*, 2000). However, there is little data available about the chemical characterisation of the essential oil of the *Ocimum* species from Kenya. This paper deals with the results of chemical analysis of the oil obtained from the leaves of *Ocimum americanum* L. growing in western Kenya.

### **2.3 The Genus *Tephrosia***

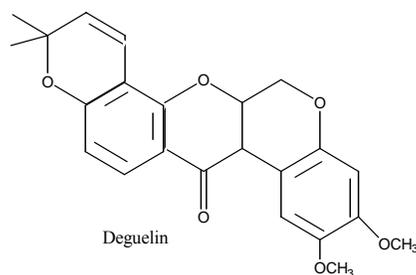
The genus *Tephrosia* belongs to the family Fabaceae/Leguminosae, which is known to be a rich source of flavonoids besides the rotenoids an interesting class of compounds showing mainly ichthiotoxic activity as fish poison. Rotenone (structure 1) was the first rotenoid identified and has been used as insecticides before the advent of the organosynthetic insecticides. Tephrosin (structure 3) isolated from *Amorpha* species exhibit anti-tumour activity including skin cancer. Recently, anticarcinogenic activity has been attributed to rotenoids (Li *et al.*, 1993).

A great variety of rotenoids are accumulated in the leaves of these plants. The rotenoids: 1, 2, 3 and 4 are composed of an isoflavone nucleus with an isoprene moiety attached to C-8 of ring A. These compounds are classified with the isoflavonoids, the end product of the phenylpropanoid pathway, which start with L-phenylalanine from the Shikimate pathway (Lambert *et al.*, 1993).

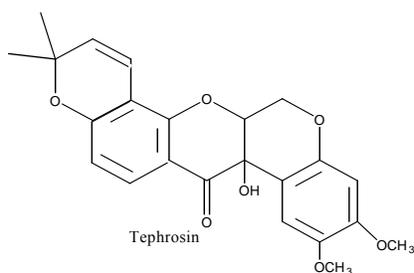
The flavonoid is an integral part of the plant kingdom, present in all photosynthesizing cells. The different biological activities, including anti-oxidant, anti-microbial, carcinogenic, cytotoxic and mutagenic properties make them interesting compounds for research. Some flavonoids and biflavonoids, isolated from Mexican tephrosia species: *T. tepicana*, *T. crassifolia*, *T. vividiflora*, *T. abbottiae*, *T. lelocarpa* and *T. madrensis*, showed different photosynthetic reaction (Céspedes *et al.*, 2001).



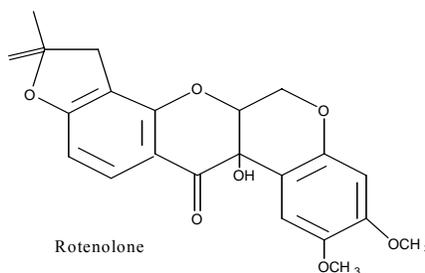
(1)



(2)



(3)



(4)

Two other species are known to have a rich source of rotenoids: *T. candida* and *T. vogelii*. Three compounds belonging to a new class of spirorotenoides isolated from *T. candida* and *T. vogelii* has been shown to have toxicity on *Tilapia nilotica* (Andrei *et al.*, 2002; Ibrahim *et al.*, 2000). Petitjean *et al.* (1993) have identified four species of *Tephrosia* used as fish poisons from Malagasy, but *T. linearis*, which is toxic to fish was also pointed out to be a danger to man and warm blooded animals. This species has abortive properties on pregnant cows.

### 2.3.1 *Tephrosia vogelii*

*Tephrosia vogelii* (figure 5) belongs to the family Leguminaceae/Fabaceae/Papilionaceae with 400 species (Dale & Greenway, 1961). It is an annual or perennial shrub or herb with erect or prostrate stems arising from a woody crown, woody roots and grey hairy twigs. The leaves are imparipinnate with numerous petioluled leaflet (often >3) usually silky pubescent beneath. Their inflorescence consists of several clustered flowers with 4-6 flowers in each cluster, and is usually red, purple or white coloured in leaf opposed, auxiliary or terminal racemes. The fruits consist of pods, which are sessile, 8 cm long, covered with silky hairs, obliquely narrowed and beaked at the apex, dehiscent and many seeded (non-septet within or thinly septet) (Allen, 1981; Sommerlate, 1990). *Tephrosia* species are adapted to sandy soils and are generally found in

grassy fields, thickets and along roadsides throughout warm regions of both hemispheres (Allen, 1981).



Figure 4: *Tephrosia vogelii*

The shrubby plant is indigenous to Africa but distributed to many other parts in the tropics where it is used as shelter, cover crops, fish poison and as a non-residual pesticide (Blommert, 1950). It was introduced to the United States in the 1960s for the purpose of commercial production of rotenone and regarded as a more promising plant than *Derris* and *Lonchocarpus* spp, which were the main sources of pesticides (Barnes & Freyre, 1966). In Eastern and Southern Africa, *T. vogelii* and other related species have been grown in small plantation by small scale farmers for use in crop protection (Watt & Breyer-Brandwijk, 1962). The crude powder of *T. vogelii* is reported to have repellent activity against *Sitophilus zeamais* (Ogendo *et al.*, 2003).

Three major constituents have been isolated and identified from the leaves of *T. vogelii*. These include rotenone (1), tephrosin (3) and deguelin (2). The principal active ingredient among the three is rotenone, the other two constituents are found to be toxic to both fish and mammals (Lambert *et al.*, 1993). Farmers in Malawi and Zambia use tephrosia as a dip to protect cattle from ticks and as a fish poison (CMMYT, 1991; Kaposhi, 1992). In Congo, *Tephrosia* leaves powder is used to protect stored products. When mixed with groundnuts at a ratio of 1:40, the powder exhibited 98.8% mortality of the groundnut borer, *Caryedon serratus*, after 13 days (Delobel & Malonga, 1987). It was also suggested that live *T. vogelii* plants could effectively

repel mole rats thereby reducing damage caused to field crops. Similarly powdered (sun-dried) leaves can effectively control maize weevils, the larger grain borer, cowpea, and bean bruchids in grain storage (Ayoub, 1999).

Among all the *Tephrosia* species, *T. vogelii* (Figure 4) has been chosen for this study because of its frequent occurrence in Kenyan flora. It has been locally use as fish poison and also reported to have potent insecticidal activities (Ogendo *et al.*, 2003). Due to the fact that essential oil from different types of plants has been used in the control of pest, this study was done to identify various oil components, which might have contributed to the activity against the insect pests.

## 2.4 Essential Oils

Essential oils are volatile plant oils; steam distillable, which are composed of terpenoids: monoterpenes, sesquiterpene and diterpenes; plus various alcohol, ketones and aldehydes terpenoids; with frequently occurring aromatic compounds (eugenol, safrole) arising from the phenylpropanoid pathway. In some species, alkanes and aliphatic alcohol, ketones and aldehydes may be obtained (Adams, 1995). Occasionally, compounds containing nitrogen (methyl anthranilate in lemon oils) and sulphur atoms (dimethylsulphide in onions) may be present in the oils (Williams, 1996).

Essential oils are secondary metabolites of plants known to have several important functions including protection against predators (micro-organisms, fungi, insects and herbivores), attraction of pollinators and dispersal of diaspores, inhibitors of seed germination and plant growth. The oils are formed in special cells or groups of cells like the cell cytosol, vacuoles of epidermal cells, and mesophyll cells of petals and glandular cells. They are generally found to predominate in one particular part or organ of the plant, such as leaves - citronella oil, flowers - bergamot oil, roots - vetiver oil, Rhizome - ginger oil, wood - sandalwood oil, bark - cinnamon tree oil, and fruit and seeds - nutmeg oil. They are still used as raw materials in many fields, including perfumery, cosmetics, aromatherapy, phytotherapy, spices and nutrition (Lawrence, 1988).

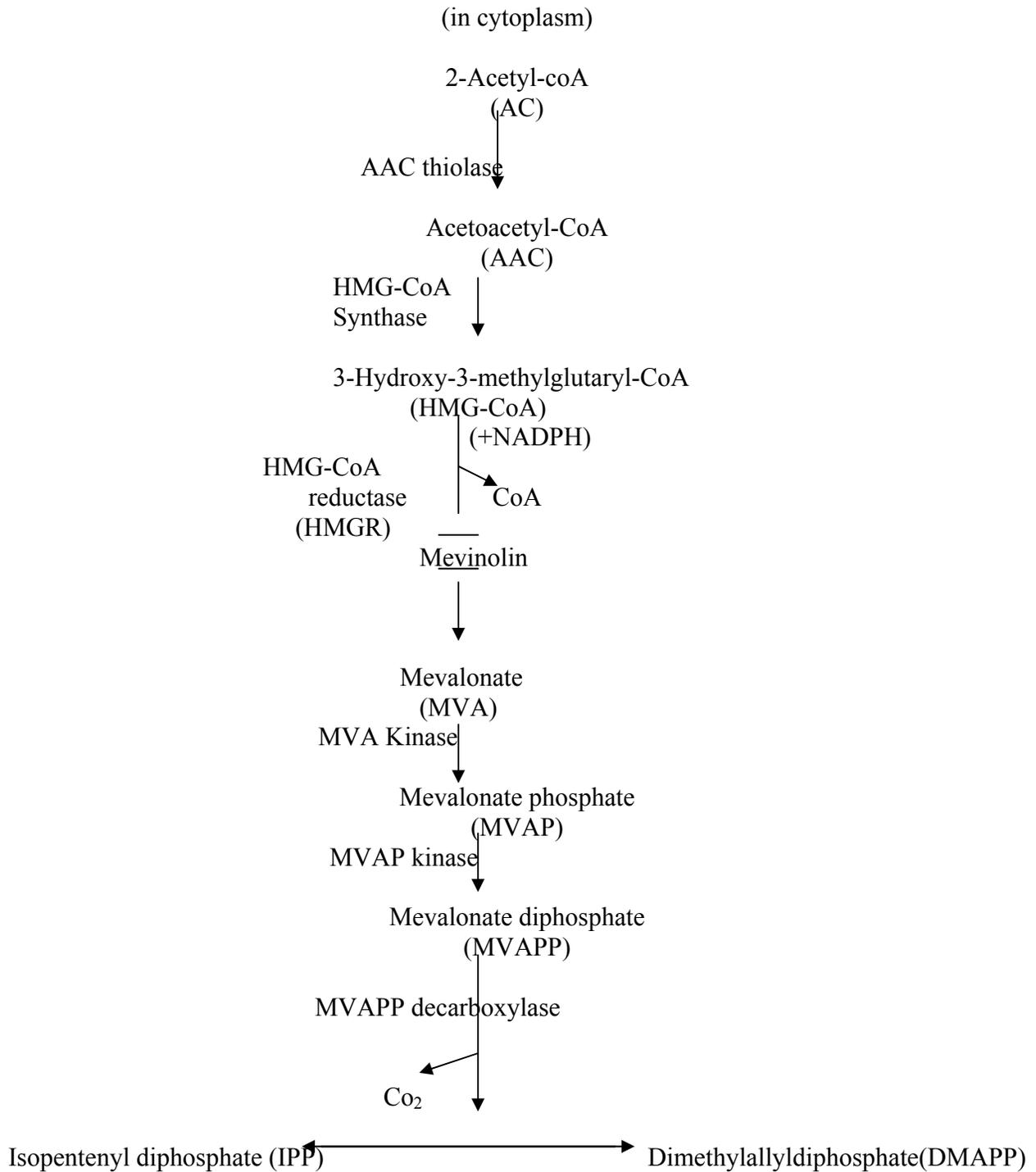
A thorough knowledge of essential oil composition allows a better and specifically directed application. The composition is dependent on characteristics such as the geographical location from which the plant is obtained, seasonal variations and climate, production technique and

purity. The effect of plant maturity at the time of oil production and the existence of chemotypic differences can also drastically affect the composition of essential oil suggesting that the ecological conditions and/or physiological states could interfere with the presence of biologically active compounds in the plant (Lahlou & Berrada, 2003). These variations are of distinct importance for the derived natural products, because the value of an essential oil is directly related to its chemical composition.

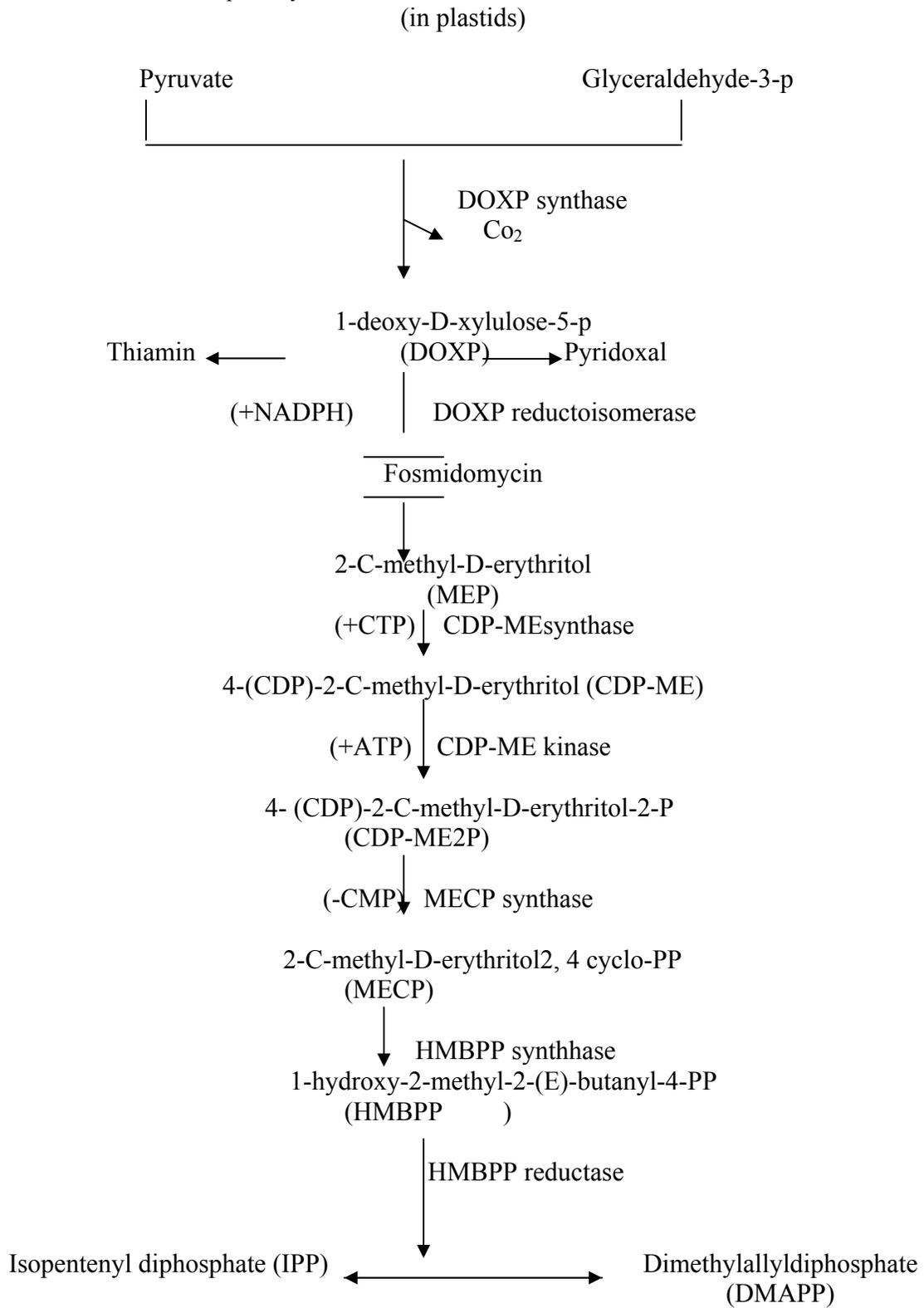
#### **2.4.1 Biosynthesis of essential oils**

Essential oils in plants are produced via two different biochemical pathways; the phenylpropanoids (methylchavicol, eugenol, methyleugenol, methyl cinnamate) by the shikimic acid pathway and the terpenes (linalool, myrcene and geraniol) by the mevalonic acid pathway (Williams, 1996). It has been demonstrated that monoterpenoid essential oil components are not always biosynthesized via the mevalonic acid pathways, but can also be formed by another route involving glyceraldehyde-3-phosphate and pyruvate. The two terpenoid biosynthetic pathways are shown (Lichtenthaler, 1999 and Rohmer, 1999).

Scheme 1: Acetate-mevalonate pathway



Scheme 2: Non-Mevalonate pathway



#### 2.4.2 Mode and mechanism of action - Insecticidal activity

Few reports on essential oils and fragrance compounds mentions their mode and mechanisms of action on biological organisms or tested animals (Lahlou, 2004). Most insecticides act by ingestion, contact and/or via the respiratory tract. Two asarone isomers isolated from the essential oil of *Acorus calamus* L. rhizomes (Araceae) have different mode of action (Koul *et al.*, 1990). *Cis* - asarone is toxic in addition to strong anti-feedant activity, whereas the *trans* - asarone acts only as an anti-feedant with no appreciable toxicity. In all the cases penetration of the insecticide through the insect cuticle acts as the corresponding binding sites, especially on the receptor proteins in target insects (neurological action compounds) e.g. the antagonist of NMDA-sensitive glutamate receptors (Lahlou, 2004).

Some essential oil constituents have synergistic as well as antagonistic actions. Balsamita major (costmary) essential oil has insecticidal activity against aphids due to synergistic effect of L-carvone and pyrethrin I (William *et al.*, 1999). Some essential oils have acute toxicity, repellent action, feeding inhibition, or harmful effects on the reproductive system of insects. Pine oil, a by-product of the sulphate wood pulping industry, contains  $\alpha$ -terpineol as one of the major constituent and repels several species of tree bark beetle (Prates *et al.*, 1998).

## CHAPTER THREE: METHODOLOGY

### 3.1 Extraction of essential oils

There are four general conventional methods of extraction of essential oils known. These include expression, steam distillation, extraction with volatile solvents, and resorption in purified fats. The separation of individual components is usually accomplished by vacuum fractionation and chromatographic methods (Raphael, 1991).

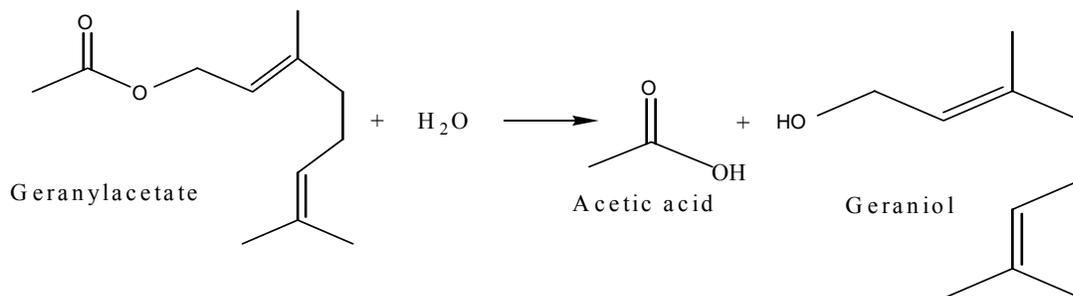
#### 3.1.1 Distillation processes

Basically, distillation involves heating a liquid or solid material to a temperature sufficiently high to produce a vapour, and then cooling the vapour to cause it to condense to a liquid or solid distillate. Two main types of distillation are applied to the production of essential oils. These are hydro distillation and steam distillation.

In steam distillation, steam from a separate boiler is ejected into the distillation vessel through jets in a ring-shaped pipe secured beneath a perforated support, upon which rest the charge of material to be distilled. Consequently, in water distillation, the water in the distillation vessel is heated by a steam coil or directly and there is no jet in this coil to provide steam for the process. A modern theory of steam distillation proposes that injected steam condense around the margins of essential oil droplets, which have diffused from oil glands in particles of the charge onto the surface of the particles. In condensing, the steam gives up latent heat, which vapourises the oil. Presumably the same thing happens in water distillation by condensation of steam within bubbles on particles of the charge as the water boils. This theory was put forward to explain why essential oils do, in fact, distil in steam efficiently when their low volatility suggests that they should not.

The condition of steam distillation promote a much faster rate of distillation of an essential oil than those pertaining to water distillation, because the flow of steam from the boiler is much faster than that produced by boiling water in the vessel. The advantage is that the essential oil being distilled is exposed to the risk of hydrolysis for only relatively short period of time. Thus an essential oil such as geranyl acetate in lavender oils, which would be seriously damaged by the long contact with hot water and steam necessary for water distillation (scheme 3), survive

steam distillation with minimum hydrolysis of the esters, which greatly contributes to the quality of the oil (Williams, 1996).



Scheme 3: Hydrolysis of an essential oil

### 3.1.2 Extraction with volatile solvents

Solvent extraction is applied to those parts of the plants whose essential oils are too far degraded by distillation, of which jasmine flowers are examples. The sample materials are mixed with purified volatile hydrocarbon solvent such as hexane which dissolves essential oil from the oil glands, together with natural wax and small amounts of pigment. The extract is filtered and the filtrate subjected to low pressure distillation to recover the solvent for further use. The residue contains 55% of essential oil and is normally known as the concrete (Williams, 1996).

### 3.1.3 Expression process

Expression process is normally applied to release of essential oil from the oil cells of the outer coloured rind of the citrus fruits and to the production of vegetable oils. The oils are expressed mechanically by either scarification or compression of the outer rind. During production the constituents of expressed oil come into contact with water, air containing oxygen and enzymes capable of accelerating enormously oxidative and hydrolytic reactions. Thus, effective proportions of anti-oxidants are added to the product oil immediately following expression to minimize the onset of oxidation during transport and storage (Williams, 1996).

### 3.1.4 Adsorption in purified fats (enfleurage)

The extraction of aromatic plant matter with hot fat and the absorption of flower fragrances by enfleurage are both absolute processes of historical interest. The fat is usually warmed to about 50 °C and then the flower petals are spread on the surface of the fat until the latter is

saturated. The fat is then digested with ethanol and any fat that dissolves is being removed by cooling to 20 °C. The essential oils obtained always contain a number of terpenoids, which can be separated by fractional distillation (Williams, 1996).

### 3.2 Plant Material

The leaves of *L. camara* and *T. vogelii* were collected from Suba and Rachuonyo Districts of Western Kenya while the aerial parts of the *L. trifolia* were collected from the Botanical garden of Egerton University, Kenya in the month of April 2005. The leaves of *O. americanum* were collected in the month of August 2003 from Suba and Rachuonyo Districts of Western Kenya. The plants were identified by the Botany Department, Egerton University, Kenya, where a voucher specimen was deposited.

### 3.3 Isolation procedure

The fresh plant material leaves of *L. camara* and *T. vogelii* were separated and hydro-distilled for 3h in a modified Clevenger apparatus. The essential oil was obtained in 0.63% and 0.056% w/w (fresh leaves) yield respectively, after drying over anhydrous Na<sub>2</sub> SO<sub>4</sub>.

The *L. trifolia* plant material was separated into leaves and flowers, and the essential oil was obtained in a yield of 0.060% and 0.064% respectively.

The leaves of *O. americanum* were air-dried under shade to constant weight and ground to a fine powder using a laboratory electric mill. The essential oil obtained had a pale yellow colour with a yield of 4% w/w on a dry weight basis.

$$\% \text{ Yield} = \frac{\text{Wt of oil}}{\text{Wt of leaves}} \times 100\%$$

### 3.4 GC-MS Analysis

Samples of essential oils were diluted in MTBE (1:100) and analysed on an Agilent GC-MSD apparatus equipped with an Rtx-5SIL MS ('Restek') (30 × 0.25 mm film thickness i.d., 0.25 μm) fused-silica analytical capillary column. Helium (0.8 ml/min) was used as a carrier gas. Samples were injected in the split mode at a ratio of 1:10- 1:100. The injector was kept at 250 °C and the transfer line at 280 °C. The column was maintained at 50 °C for 2 min and then programmed to 260 °C at 5 °C /min and held for 10 min at 260 °C. The MS was operated in the EI mode at 70 eV, in *m/z* range 42-350. The identification of the compounds was performed by comparing their retention indices and mass spectra with those found in literature (Adams, 1995 & 2001) and those supplemented by Wiley and QuadLib 1607 GC-MS libraries.

## CHAPTER FOUR: RESULTS

Table 1: Monoterpenes

Compounds	<i>Lantana camara</i>			<i>Lantana trifolia</i>				<i>Ocimum americanum</i>			<i>Tephrosia vogelii</i>		
	KI	RT	% RA ▼	KI	RT	% RA ◇	% RA ▼	KI	RT	% RA ▼	KI	RT	% RA ▼
$\alpha$ -Thujene	935	5.39	0.21	-	-	-	-	935	5.36	0.23	-	-	-
$\alpha$ -Pinene	938	5.57	1.30	938	5.56	0.51	0.77	938	5.55	0.46	938	5.57	2.74
Camphene	952	5.97	0.71	952	5.96	0.98	1.61	-	-	-	-	-	-
Sabinene	975	6.57	9.15	-	-	-	-	-	-	-	975	6.55	0.24
2- $\beta$ -Pinene	978	6.69	0.96	978	6.68	-	0.42	980	6.66	0.39	980	6.68	0.22
$\beta$ -Myrcene	990	6.98	0.71	990	6.98	-	0.36	990	6.96	0.58	990	6.98	0.35
$\delta$ -3-Carene	1010	7.52	1.17	-	-	-	-	-	-	-	-	-	-
$\alpha$ -Terpinene								1018	7.71	0.42	-	-	-
o-Cymene	1022	7.96	0.51	-	-	-	-	-	-	-	-	-	-
p-Cymene	-	-	-	1025	7.95	0.52	0.52	1025	7.94	0.29	-	-	-
<i>dl</i> -Limonene	1030	8.09	0.92	1030	8.09	1.36	3.41	1030	8.07	0.8	1030	8.09	1.58
$\beta$ -Phellandrene				1031	8.13	0.32	-	-	-	-	-	-	-
<i>cis</i> -Ocimene	1037	8.28	0.30	1037	8.28	3.29	7.27	-	-	-	-	-	-
<i>trans</i> - $\beta$ -Ocimene	1050	8.58	0.29	1050	8.58	0.63	1.42	-	-	-	-	-	-
$\gamma$ -Terpinene	-	-	-	-	-	-	-	1062	8.90	2.59	-	-	-
$\alpha$ -Terpinolene	-	-	-	-	-	-	-	1089	9.68	0.34	-	-	-

◇- Flower      ▼ - Leaves

Table 2: Oxygenated Monoterpenes

Compounds	<i>Lantana camara</i>			<i>Lantana trifolia</i>				<i>Ocimum americanum</i>			<i>Tephrosia vogelii</i>		
	KI	RT	% RA ▼	KI	RT	% RA ◊	% RA ▼	KI	RT	% RA ▼	KI	RT	% RA ▼
1,8-Cineole	1033	8.18	5.54	1033	8.16	0.43	-	1033	8.15	16.13	-	-	-
<i>cis</i> -Sabinene hydrate	1068	9.28	0.97	-	-	-	-	1068	9.26	1.94	-	-	-
Linalool	-	-	-	1098	10.16	1.07	3.88	1098	10.12	1.42	-	-	-
<i>trans</i> -Sabinene hydrate	-	-	-	-	-	-	-	1109	10.16	1.48	-	-	-
<i>cis</i> - $\beta$ -Terpineol	1120	10.18	0.74	-	-	-	-	1120	10.85	0.46	-	-	-
<i>trans-p</i> -Menth-2-en-1-ol	-	-	-	1133	10.89	3.77	2.32	-	-	-	-	-	-
1-Terpeneol	-	-	-	1134	11.42	2.42	1.27	-	-	-	-	-	-
<i>trans</i> -Pinene hydrate	-	-	-	-	-	-	-	1137	11.39	0.37	-	-	-
Camphor	1147	11.52	1.30	1147	11.52	0.43	-	1147	11.52	0.71	-	-	-
1-Borneol	1165	12.29	0.46	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	1170	12.21	0.38	-	-	-
4-Terpeneol	1177	12.54	0.97	1177	12.52	0.47	-	1177	12.52	43.21	-	-	-
$\alpha$ -Terpineol	1189	12.98	0.65	1189	12.98	0.32	0.37	1189	12.95	4.01	-	-	-
<i>cis</i> -Piperitol	-	-	-	1193	13.03	1.02	0.59	-	-	-	-	-	-
<i>trans</i> -Piperitol	-	-	-	1205	13.4	2.74	2.47	-	-	-	-	-	-
Piperitone	-	-	-	1252	14.66	0.48	0.46	-	-	-	-	-	-
Bornyl acetate	-	-	-	1285	15.53	0.86	1.87	-	-	-	-	-	-
Myrtenyl acetate	-	-	-	-	-	-	-	1312	16.60	0.26	-	-	-
$\beta$ -Ionone	-	-	-	-	-	-	-	-	-	-	1485	20.86	0.37

◊- Flower      ▼ - Leaves

Table 3: Sesquiterpenes

Compounds	<i>Lantana camara</i>			<i>Lantana trifolia</i>				<i>Ocimum americanum</i>			<i>Tephrosia vogelii</i>		
	KI	RT	% RA ▼	KI	RT	% RA ◊	% RA ▼	KI	RT	% RA ▼	KI	RT	% RA ▼
$\alpha$ -Cubebene	-	-	-	-	-	-	-	-	-	-	1351	18.12	2.69
$\alpha$ -Copaene	1376	18.12	0.83	1376	18.12	1.98	2.58	-	-	-	-	-	-
$\beta$ -Bourbonene	-	-	-	1384	18.33	0.64	0.91	-	-	-	-	-	-
$\beta$ -Cubebene	1390	18.45	0.38	-	-	-	-	-	-	-	1390	18.44	0.72
$\beta$ -Elemene	1392	18.5	1.07	1392	18.5	2.19	2.31				1392	18.5	2.53
Zingiberene	-	-	-	-	-	-	-	-	-	-	-	18.84	0.39
<i>cis</i> - $\alpha$ -Bergamotene	-	-	-	-	-	-	-	-	-	-	1415	19.14	0.96
$\beta$ -Caryophyllene	1418	19.38	21.35	1418	19.34	11.93	6.32	1420	19.26	3.06	1418	19.34	6.89
Epi-Bicyclosquiphellandrene	-	19.59	0.79	-	-	-	-	-	-	-	-	-	-
<i>epi</i> -bicyclophellandrene	-	-	-	1423	19.58	0.69	0.53	-	-	-	-	-	-
<i>trans</i> - $\alpha$ -Bergamotene	-	-	-	-	-	-	-	1430	19.61	2.68	1440	19.67	1.94
<i>cis</i> - $\beta$ -Farnesene	-	-	-	-	-	-	-	-	-	-	1445	19.86	0.39
<i>trans</i> - $\beta$ -Farnesene	-	-	-	-	-	-	-	-	-	-	1452	20.18	1.18
$\alpha$ -Humulene	1454	20.29	8.73	1454	20.27	4.65	2.52	-	-	-	1454	20.27	2.34
Aromadendrene	1461	20.39	0.32	1461	20.39	2.23	2.42	1438	19.67	0.67	-	-	-
$\gamma$ -Muurolene	1477	20.78	0.40	-	-	-	-	-	-	-	-	-	-
Germacrene D	1487	20.94	1.97	1487	20.99	24.88	23.14	-	-	-	1487	20.95	6.2
$\beta$ -Selinene	-	-	-	1493	21.16	-	0.71	-	-	-	1490	21.16	3.73
Bicyclogermacrene	1494	21.33	4.86	1494	21.32	2.36	1.85	-	-	-	-	-	-
$\alpha$ -Muurolene	1499	21.39	0.47	1499	21.39	1.09	1.21	-	-	-	-	-	-

δ-Guaiene	-	-	-	-	-	-	-	1500	21.42	1.43	-	-	-
β-Bisabolene	-	-	-	-	-	-	-	-	-	-	1508	21.62	1.67
α-Farnesene	-	-	-	1508	21.52	0.63	-	-	-	-	-	-	-
Germacrene A	1510	21.62	0.30	1510	21.62	0.54	0.30	-	-	-	-	-	-
γ-Cadinene	-	-	-	1513	21.76	0.34	0.43	-	-	-	-	-	-
δ-Cadinene	-	-	-	1524	21.85	1.17	1.91	-	-	-	-	-	-
α-Cadinene	-	-	-	1538	21.9	2.08	1.77	-	-	-	1538	21.86	2.1
Seline-3,7(11)-diene	1542	21.85	1.38	-	-	-	-	-	-	-	-	-	-
Germacrene B	-	-	-	1556	22.92	0.77	0.91	-	-	-	1556	22.34	3.72
Thujopsene (12)	-	-	-	-	-	-	-	-	-	-	-	22.46	1.56

◇ - Flower    ▼ - Leaves

Table 4: Oxygenated Sesquiterpenes

Compounds	<i>Lantana camara</i>			<i>Lantana trifolia</i>				<i>Ocimum americanum</i>			<i>Tephrosia vogelii</i>		
	KI	RT	% RA ▼	KI	RT	% RA ◇	% RA ▼	KI	RT	% RA ▼	KI	RT	% RA ▼
Davana ether 1	-	21.48	1.00	-	-	-	-	-	-	-	-	-	-
Davana ether 3	-	21.96	0.45	-	-	-	-	-	-	-	-	-	-
Davanone	-	22.67	1.19	-	-	-	-	-	-	-	-	-	-
Nerolidol	1566	2298	4.65	1566	22.96	0.87	0.72	1566	22.88	0.29	1566	23.01	7.73
Germacrene D-4-ol	-	-	-	1574	23.39	3.68	4.15	-	-	-	-	-	-
(+)-Spathulenol	1578	23.42	3.07	-	-	-	-	-	-	-	1578	23.42	3.59
Caryophyllene oxide	1582	23.52	3.77	1582	23.51	2.61	1.68	-	-	-	1582	23.54	6.22
Epiglobulol	-	-	-	-	-	-	-	-	-	-	1583	24.62	0.48
Humulene oxide II	-	-	-	1606	24.19	0.78	0.53	-	-	-	-	-	-
<i>cis</i> - $\alpha$ -Bisabolene epoxide	-	24.2	2.41	-	-	-	-	-	-	-	-	-	-
$\tau$ -Muurolol	-	-	-	1645	25.01	0.48	0.42	-	-	-	-	-	-
Eudesm-11-en-1-ol	-	-	-	-	-	-	-	-	-	-	-	25.38	1.21
Vulgarone B	-	-	-	-	-	-	-	-	-	-	1647	25.72	1.04
$\alpha$ -Cadinol	-	-	-	1653	25.30	2.10	1.31	-	-	-	-	-	-
$\alpha$ -Bisabolol	-	-	-	-	-	-	-	-	-	-	1680	25.99	0.91
Farnesol	-	-	-	-	-	-	-	-	-	-	1697	26.69	1.61
8- $\alpha$ -Acetoxyelemol	1740	24.91	0.57	-	-	-	-	-	-	-	-	-	-

◇ - Flower    ▼ - Leaves

Table 5: Other compounds

Compounds	<i>Lantana camara</i>			<i>Lantana trifolia</i>				<i>Ocimum americanum</i>			<i>Tephrosia vogelii</i>		
	KI	RT	% RA ▼	KI	RT	% RA ◇	% RA ▼	KI	RT	% RA ▼	KI	RT	% RA ▼
1-Octen-3-ol	-	-	-	980	6.75	0.32	0.53	-	-	-	-	-	-
Pentyl-3-methylbutanoate	-	-	-	1104	10.22	0.46	-	-	-	-	-	-	-
Oct-1-en-3-ylacetate	-	-	-	-	-	-	-	1114	10.33	0.53	-	-	-
6-Demethoxyageratochromene	-	-	-	-	-	-	-	-	-	-	-	20.43	1.09
Pentadecane	-	-	-	-	-	-	-	1498	20.46	0.28	-	-	-
Heptadecane	-	-	-	-	-	-	-	1700	23.85	0.33	-	-	-
2-Hydroxy-4,6-cyclooctadien-1-one	-	-	-	-	-	-	-	-	-	-	-	24.19	3.53
Squalene	-	24.74	5.60	-	-	-	-	-	-	-	-	-	-
Nonadecane	-	-	-	-	-	-	-	1900	26.22	0.51	-	-	-
Phytol	1949	34.26	0.84	1949	38.28	0.48	1.08	-	-	-	1949	34.27	0.75
10-Methyleicosane	-	-	-	-	-	-	-	2000	28.48	0.31	-	-	-
2-Pentadecanone	-	-	-	-	-	-	-	-	-	-	-	29.44	0.42
Docosane	-	-	-	-	-	-	-	2200	30.63	0.31	-	-	-
Octacosane	-	-	-	-	-	-	-	2800	32.32	0.30	-	-	-
Dotriacontane	-	-	-	-	-	-	-	3200	33.43	0.32	-	-	-
Tetatriacontane	-	-	-	-	-	-	-	3400	35.65	0.40	-	-	-
Pentatriacontane	-	-	-	-	-	-	-	3500	36.31	0.31	-	-	-
Hexatriacontane	-	-	-	-	-	-	-	3600	37.03	0.31	-	-	-

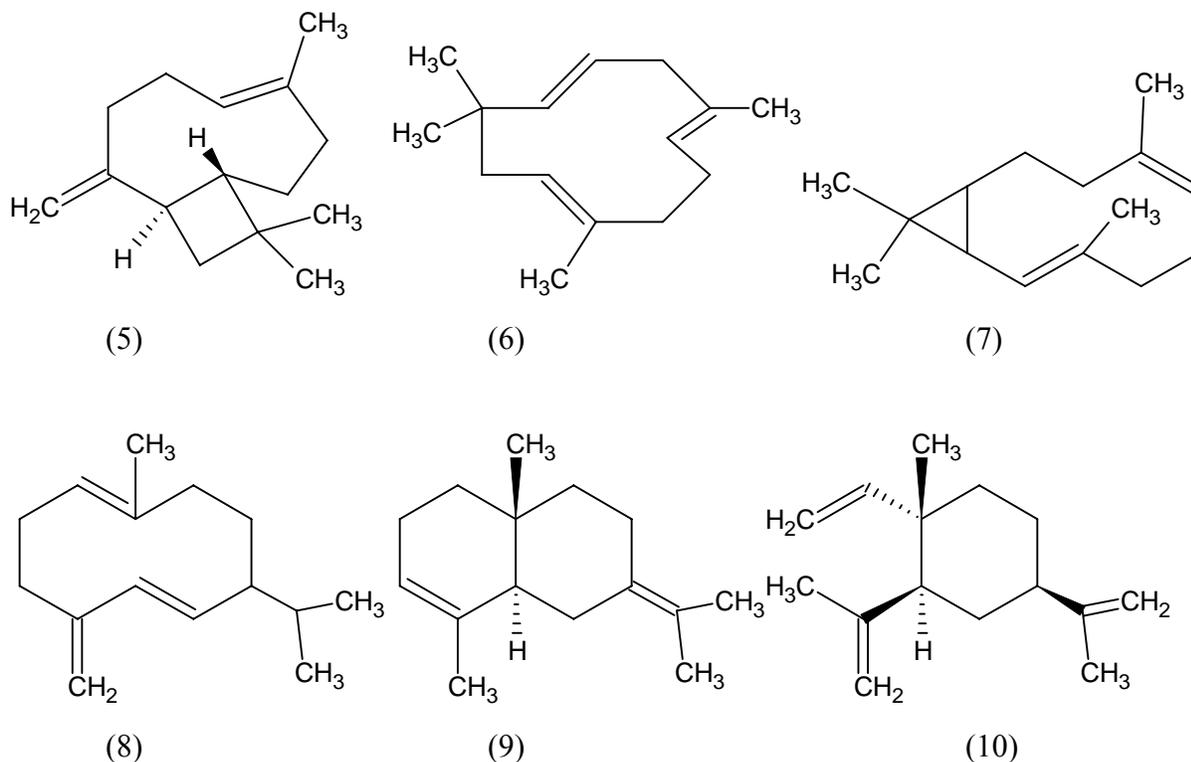
◇ - Flower    ▼ - Leaves

## CHAPTER FIVE: DISCUSSION AND CONCLUSION

### 5.1: Comparing the major compound identified with literature

#### 5.1.1: *Lantana camara*

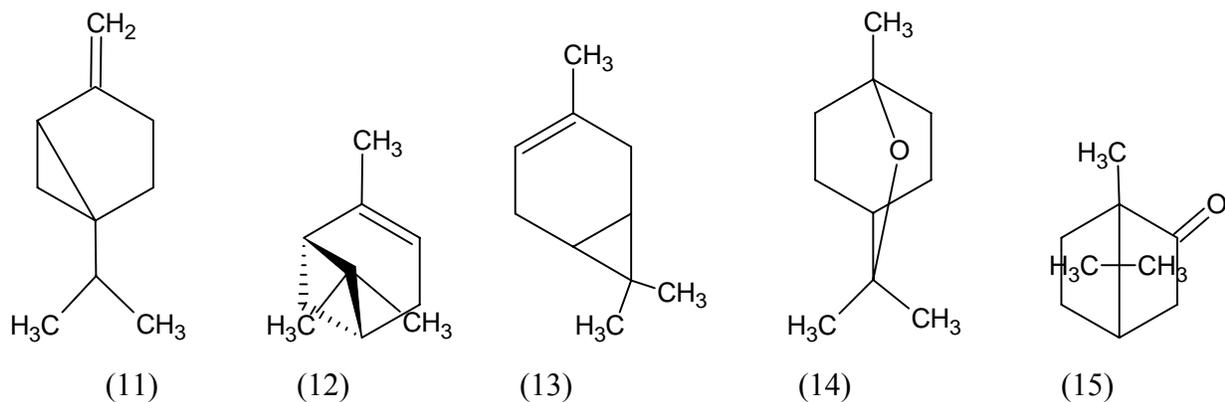
Among 41 compounds identified in the oils of *L. camara* from Western Kenya, sesquiterpene hydrocarbons are found in higher amounts. Considering only components present in >1%, the major sesquiterpenes were  $\beta$ -caryophyllene (5) (21.35%),  $\alpha$ -humulene (6) (8.73%), bicyclogermacrene (7) (4.86%), germacrene D (8) (1.97%), seline-3, 7(1)-diene (9) (1.38%) and  $\beta$ -elemene (10) (1.07%). These compounds have been reported as present in the oils of *L. camara* from different regions.  $\beta$ -Caryophyllene (5) was reported to be present in high concentrations in oils of *L. camara* from Nigeria (24.6%) (Oyedeki *et al.*, 2003), Madagascar (18.8%) (Mollenbeck *et al.*, 1997), Pakistan (19.8%) (Khan *et al.*, 2002), Iran (22.5%) (Fatemeh, 2002), India (48.8-66.5%) and Japan (43.0%) (Da Silva *et al.*, 1999).



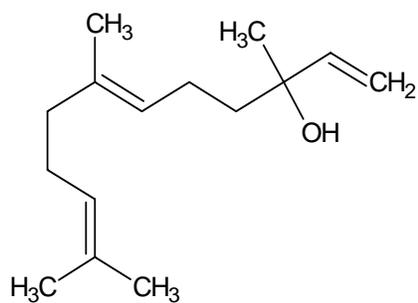
The sesquiterpenes were found to be the second most abundant in the essential oils from Cameroon (13%) and Madagascar (12%) (Ngassoum *et al.*, 1999), and South China (12.35%) (Sundufu & Shoushan, 2004). There has been no detection of  $\alpha$ -humulene in the oils of *L.*

*camara* from Japan, India and south-east Brazil (Khan *et al.*, 2002; Da Silva *et al.*, 1999). However, it is present in the *L. camara* oil from Ajouan (21.8%) (Sundufu & Shoushan, 2004), Comoro Islands (21.8%) (Peyron *et al.*, 1972), Egypt (11%) (Saleh, 1974), Nigeria (19.5%), Iran (6-10.8%) and South China (9.31%) (Sundufu & Shoushan, 2004).

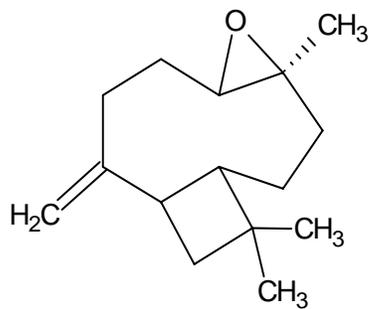
The dominant monoterpene hydrocarbons from the oil under study are sabinene (11) (9.15%),  $\alpha$ -pinene (12) (1.3%) and  $\delta$ -3-carene (13) (1.17%). Sabinene (11) has also been reported in high amounts in the oils from Nigeria (8.8%), Cameroon and Madagascar (1-9%) and Iran (7.3-16.5%).  $\delta$ -3-Carene (13) was also found in the essential oil of leaves, flowers and stem (1.5, 1.8 and 0.1% respectively) from India (Khan *et al.*, 2002; Khan *et al.*, 2003) and Madagascar (10%). Oxygenated monoterpenes represented by 1, 8-cineole (14) (5.54%) and camphor (15) (1.3%) are also commonly reported in the literature.



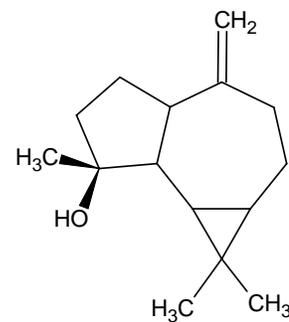
The oxygenated sesquiterpenes in the oil analyzed here were mainly dominated by nerolidol (16) (4.65%), caryophyllene oxide (17) (3.77%), spathulenol (18) (3.07%), *cis*- $\alpha$ -bisabolene epoxide (19) (2.41%) and davanone (20) (1.19%). Nerolidol (16) was reported in small amounts in Indian *L. camara* oil and Brazilian commercial oil (Weyerstahl *et al.*, 1999). In contrast, davanone has only been reported as a major component in *L. camara* essential oil from Madagascar (15%).



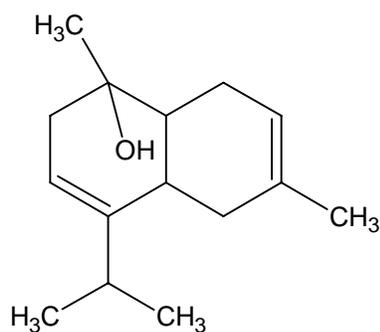
(16)



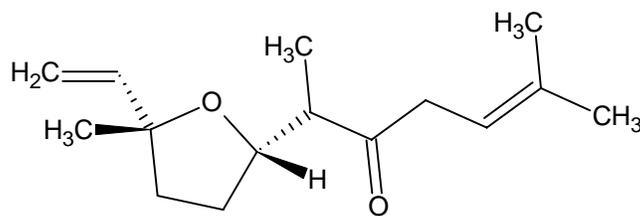
(17)



(18)



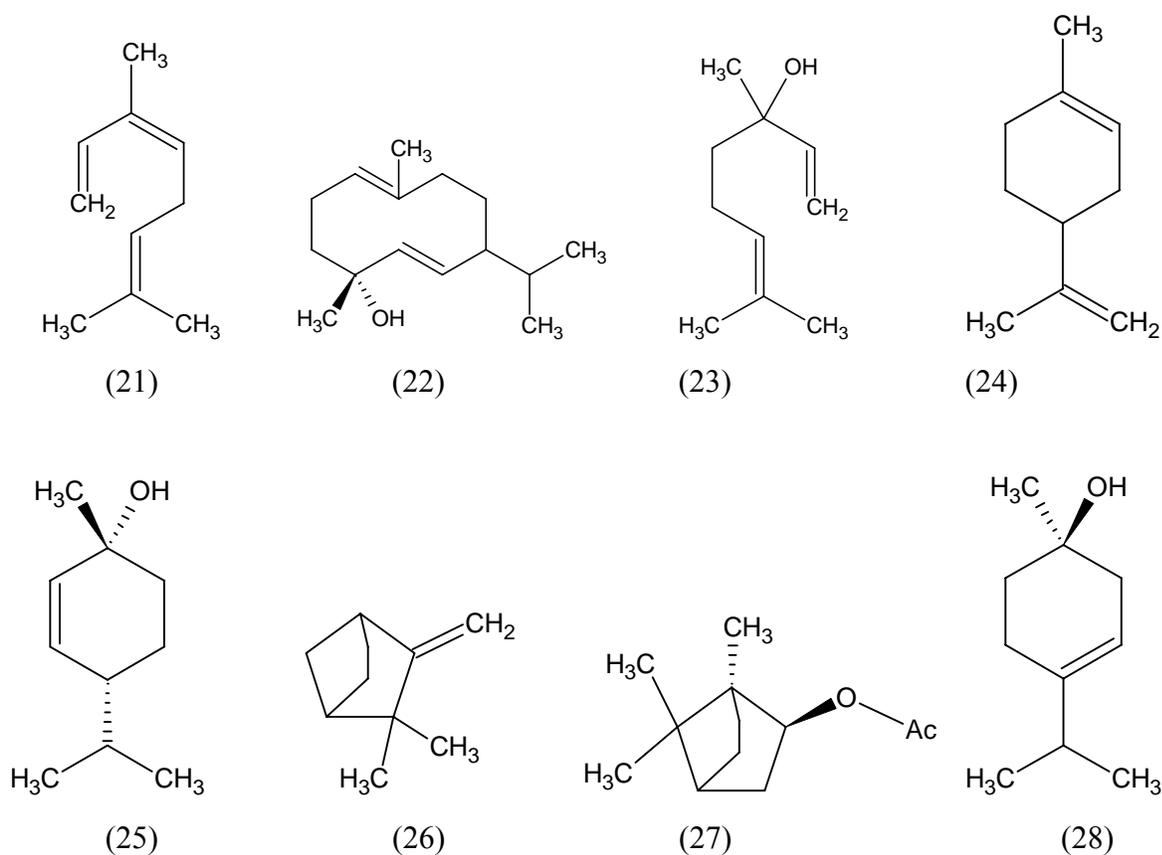
(19)



(20)

### 5.1.2: *Lantana trifolia*

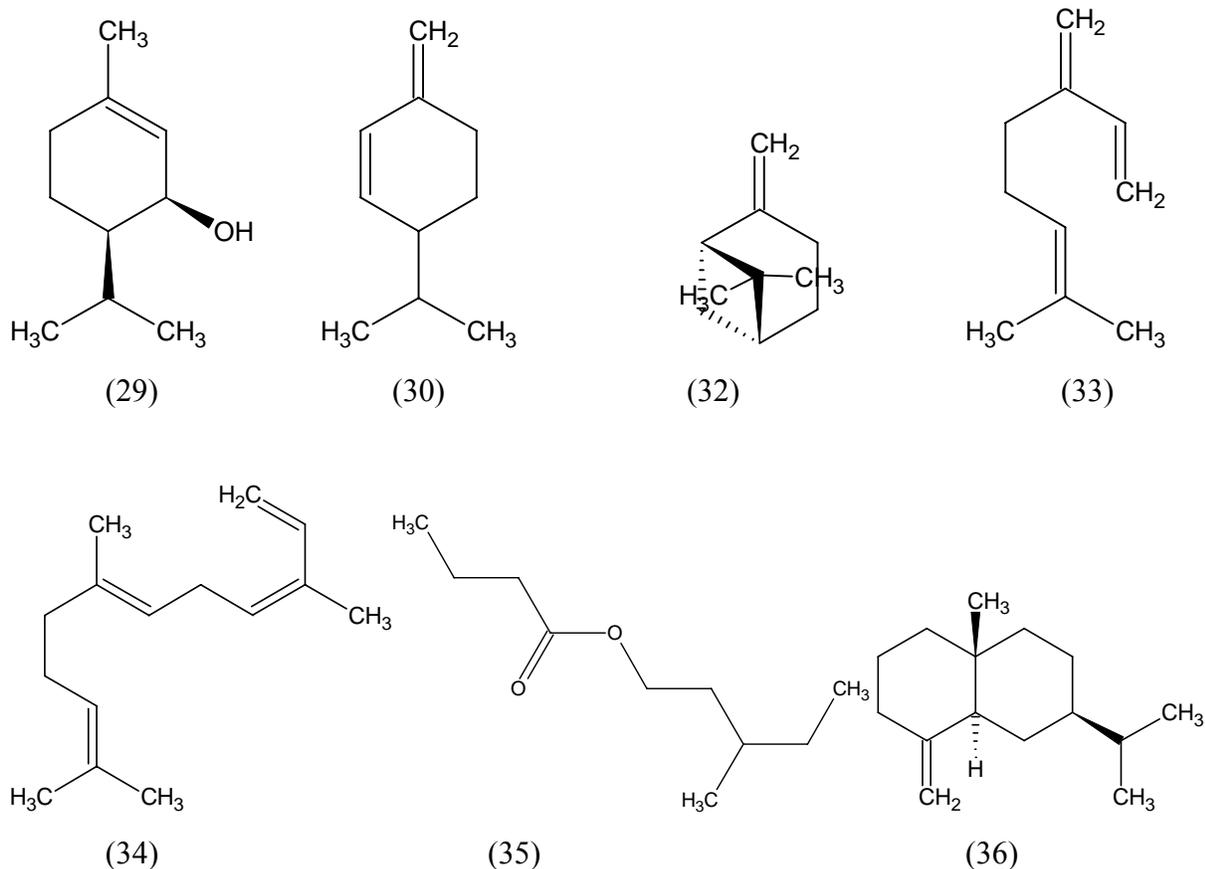
The chemical composition of the leaves' and flower oils of *L. trifolia* from Kenya is reported here for the first time. The 47 compounds identified have been grouped in classes so as to make the comparison of the oils easier. The analysis showed that germacrene D (8) was the main component for both leaves (23.14%) and flowers (24.88%). Comparing the relative amounts of each compound separately and considering only components at  $\geq 3\%$ , other major components in the essential oil of leaves were *cis*-ocimene (21) (7.27%)  $\beta$ -caryophyllene (5) (6.32%), germacrene D-4-ol (22) (4.15%), linalool (23) (3.88%) and limonene (24) (3.41%). The other major components in the flower oil are  $\beta$ -caryophyllene (5) (11.93%),  $\alpha$ -humulene (6) (4.65%), *trans*-p-menth-2-en-1-ol (25) (3.77%), germacrene D-4-ol (22) (3.68%) and *cis*-ocimene (21) (3.29%).



These compounds were also reported in the essential oil of *L. trifolia* from Rwanda. Germacrene D (8) (25 % and 33% for leaves and flowers respectively) was also reported as the main sesquiterpene.  $\beta$ -caryophyllene (5) was also identified as the second most abundant sesquiterpene. Otherwise, there were significant quantitative differences in the other major components.

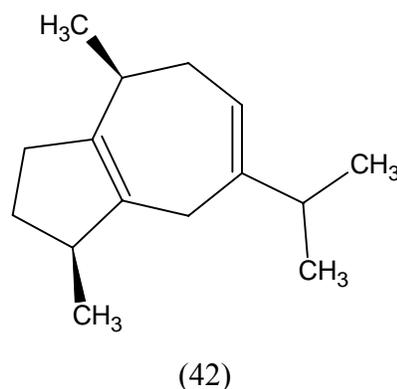
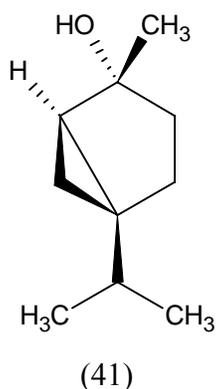
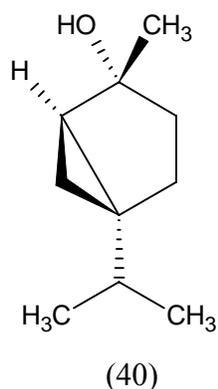
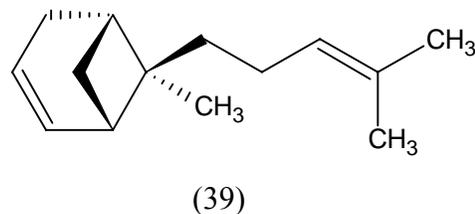
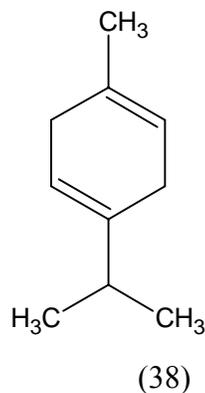
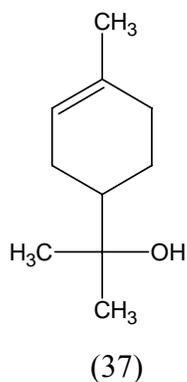
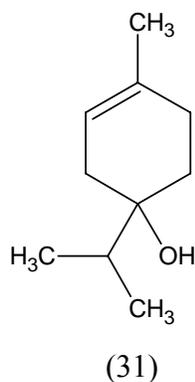
On comparing some of the components present in the flowers and leaves, the amount of camphene (26), *dl*-limonene (24), *cis*-ocimene (21), linalool (23) and bornyl acetate (27) in flowers were found to be half as much as in leaves where as 1-terpineol (28), *cis*-piperitol (29),  $\beta$ -caryophyllene (5),  $\alpha$ -humulene (6) and caryophyllene oxide (17) were found to be half as much in leaves compared to flowers. However, some components were found to be present in one part and absent in the other. Those only present in the flowers are  $\beta$ -phellandrene (30), 1, 8-cineole (14), camphor (15), 4-terpineol (31),  $\alpha$ -farnesene (34) and pentyl-3-methylbutanoate (35) while 2-  $\beta$ -pinene (32),  $\beta$ -myrcene (33) and  $\beta$ -selinene (36) were only present in the leaves. The

difference in composition might be due to different bioynthetic pathway that synthesis the compounds for specisic fuction or purposes.



### 5.1.3: *Ocimum americanum*

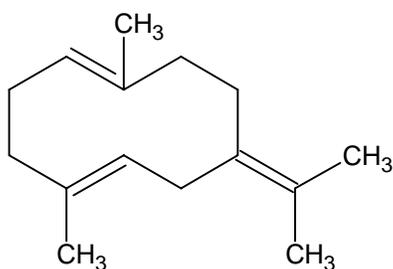
In the essential oil of *O. americanum* analysed in this study, 36 components, which represent 88.51% of the whole oil were identified. Of the 36 compounds identified, 10 were aliphatic hydrocarbons. The main component was terpinen-4-ol (31) (43.21%), followed by 1, 8-cineole (14) (16.13%) and (+) -  $\alpha$ -terpineol (37) (4.01%). Other constituents present in appreciable amounts are  $\gamma$ -terpinene (38) (2.59%), *trans*- $\alpha$ -bergamotene (39) (2.68%) and  $\beta$ -caryophyllene (5) (3.06%), *trans*-sabinene hydrate (40) (1.48%), *cis*-sabinene hydrate (41) (1.94%), linalool (23) (1.42%) and  $\delta$ -guaiene (42) (1.43%).



The oil contains mainly oxygenated monoterpenes, which accounting for 71.24% with 18 components identified. The non-oxygenated ones represent 5.76% of the oil. The oil presented a very complex monoterpene fraction. The sesquiterpene and aliphatic hydrocarbons represent 8.13 and 3.58% of the total oil, respectively. Within this sesquiterpene fraction, only 1.1 % was oxygenated. However, *O. americanum* oil with 10 components is qualitatively rich in aliphatic hydrocarbons although the relative amounts are low.

#### 5.1.4: *Tephrosia vogelii*

The essential oil of the *T. vogelii* leaves had 34 compounds representing 73.09% of the oil. It contained very few monoterpenes, all in trace amounts with an exception of  $\alpha$ -pinene (12) (2.74%) and *dl*-limonene (24) (1.58%). Both oxygenated and non-oxygenated sesquiterpenes are present in higher amounts accounting for 62.55% of the total oil. Considering components of >3%, the dominant ones are nerolidol (16) (7.73%),  $\beta$ -caryophyllene (5) (6.89%), caryophyllene oxide (17) (6.22%), germacrene D (8) (6.20%),  $\beta$ -selinene (36) (3.73%), germacrene B (43) (3.72%) and spathulenol (18) (3.59%).



(43)

Besides terpenoids, the oil also contained ketones and alcohols, 2-Hydroxy-4, 6-cyclooctadien-1-one (3.53%) being the major one. Two isomeric sesquiterpenes, *cis*- and *trans*- $\beta$ -farnesene (0.39 and 1.18%, respectively) were also identified.  $\beta$ -ionone (0.37%) was found as the only oxygenated monoterpene in trace amounts.

## 5.2: Comparing the major compounds in the essential oil samples

In discussing the essential oil components identified in the plants under study, only components with concentration >3% are considered. For easy comparison, the components have been classified into monoterpenes and sesquiterpenes.

### 5.2.1 Monoterpenes

*L. camara* leaves oil was found to contain sabinene (9.15%) and 1, 8-cineole (5.54%). Only trace amounts of sabinene were found in *T. vogelii* but absent in the other samples. 1,8-cineole appeared as a second major product in *O. americanum*, small amounts in *L. trifolia* flower oil but absent in the leaves and *T. vogelii* oil. The main components of *L. trifolia* leaves oil were *cis*-ocimene (7.27%), linalool (3.88%) and limonene (3.41%) while the flower oil had *trans*-*para*-menth-2-en-1-ol (3.77%) and *cis*-ocimene (3.29%). Interestingly, the amount of limonene, *cis*-ocimene and linalool in the flowers was found to be almost half that of the leaves. Limonene was observed to be in small amounts in all other plant samples. *Cis*-ocimene was in small amount in *L. camara* but absent in the other two oil samples while linalool was in reasonable amount in *O. americanum* but also absent in the other two oil samples. *O. americanum* was found to be dominated by mainly oxygenated monoterpenes; 4-terpineol (43.21%), 1, 8-cineole (16.13%) and  $\alpha$ -terpineol (4.01%). Except for the 1, 8-cineole, the other two components were found in small amounts in both *L. camara* and *trifolia* but 4-terpineol was absent in *L. trifolia* leaves oil. Monoterpenes in *T. vogelii* leaves oil were poorly represented with the major components being

$\alpha$ -pinene (2.74%) and limonene (1.58%). Some monoterpenes were found to be a characteristic of all oil samples analysed. These were  $\alpha$ -pinene, 2- $\beta$ -pinene,  $\beta$ -myrcene and limonene.

### 5.2.2 Sesquiterpenes

In *L. camara*,  $\beta$ -caryophyllene (21.35%),  $\alpha$ -Humulene (8.73%), Bicyclogermacrene (4.86%), nerolidol (4.65%), caryophyllene oxide (3.77%) and spathulenol (3.01%) were identified as the major compounds. *Trans*-caryophyllene was found to be dominant in all oil samples.  $\alpha$ -Humulene and Bicyclogermacrene were in reasonable amount in *L. trifolia* but absent in *O. americanum*. The Humulene was also present in *T. vogelii*. Nerolidol appears as a major component in *T. vogelii* but in small amount in *L. trifolia* and *O. americanum*. Caryophyllene oxide and spathulenol are among the dominant components in *T. vogelii* but absent in *O. americanum*. Only caryophyllene oxide was present in *L. trifolia*.

The dominant sesquiterpenes in the leaves and flowers of *L. trifolia* were germacrene D (23.14% and 24.88%),  $\beta$ -caryophyllene (6.32% and 11.93%), germacrene D-4-ol (4.15% and 3.68%) respectively, and  $\alpha$ -Humulene (4.65%) present in the flowers only. Germacrene D was among the major components of *T. vogelii*, in small amounts in *L. camara* but absent in *O. americanum*.  $\beta$ -caryophyllene is the major component in *L. camara*, second in *T. vogelii* and among the dominant components in *O. americanum*.  $\alpha$ -Humulene is a second major component in *L. camara*, in small amount in *T. vogelii* and absent in *O. americanum*. Germacrene D-4-ol was found to be absent in all other oil samples.

In *O. americanum* oil, the only dominant component above 3% was  $\beta$ -caryophyllene (3.06%) and was present in all oil sample analysed. Lastly, nerolidol (7.73%),  $\beta$ -caryophyllene (6.89%), caryophyllene oxide (6.22%), germacrene D (6.20%),  $\beta$ -selinene (3.735), germacrene B (3.72%) and 2-Hydroxy-4, 6-cyclooctadien-1-one (3.53%) were found to be the main components of *T. vogelii*. All other components have been discussed except for  $\beta$ -selinene and germacrene B, which are also appearing in trace amounts in *L. trifolia* but absent in *L. camara* and *O. americanum*.

### 5.3: Insecticidal activity of the major compounds

#### 5.3.1 Monoterpenes

The most detailed studies of the effects on insect pests have been those using terpenes. Some monoterpenes like *d*-limonene (Karr *et al.*, 1990) have been reported to possess insecticidal activities and can inhibit reproduction in several species of insects. 1, 8-cineole and R-(+)-limonene has been shown to have insecticidal activity against *Rhyzopertha dominica* F. and *Tribolium castaneum* Herbst (Prates *et al.*, 1998). These two monocyclic monoterpenoids are natural products from plants with low toxicity to mammals. Limonene is a major component of *Citrus* spp. essential oils and also used as an ingredient of soaps, perfumes and food additives. Cineole is a constituent occurring in variable amounts in the essential oils of *Eucalyptus* spp. leave (Prates *et al.*, 1998). Fumigation screening test on *Tribolium castaneum*, *Sitophilus oryzae*, *Oryzaephilus surinaniensis*, *Musca domestica* and *Blattella germanica* revealed cineole to produce 100% mortality to all tested species and limonene with 100% mortality to four of the five species tested (Lee *et al.*, 2003). 1, 8-cineole has also been reported in allelopathic effect as toxic deterrent or inhibitory compounds, repellency and toxicity against three stored product coleopteras: *Callosobruchus maculatus* F., *Rhyzopertha dominica* F. and *Sitophilus oryzae* L. (Fischer, 1991; Aggarwal, 2001).

The common monoterpene, 1,8-Cineole, which is also a major constituent of *Ocimum kenyese* (Obeng-ofori *et al.*, 1997) was also found to be highly toxic and repellent to *S. granaries* L., *S. zeamais*, *T. confusum* and *P. truncates* (Horn), where as, 1,8-cineole and  $\delta$ -3-carene have been shown to be involved in cases of both direct plant defence and pollination (Ratta & Klepzip, 1992; Reichardt *et al.*, 1990a; Knudson *et al.*, 1993).

Linalool was found in the oil of *O. americanum* under study is known to have a toxic effect to the bruchids, *Zabrotes subfasciatus* and other storage pests (Weaver *et al.*, 1991). Pine oil, a by-product of the sulphate wood pulping industry, has  $\alpha$ -terpineol among its major constituent and has been known to repel tree bark beetle species. Ojimekwe & Adler (1999) found  $\alpha$ -pinene and terpineol to possess potent repellent and toxic effects to *T. confusum*.  $\alpha$ -terpineol also showed significant activity on the growth of anastomosis groups of *Rhizoctonia solani*, however it was not active against *Fusarium* species except for *Fusarium sambucinum* (Cakir *et al.*, 2004).

The minor monoterpene hydrocarbons myrcene,  $\beta$ -phellandrene and *trans*-ocimene elicited electrophysiological responses of the antennae of female *Traumatopoea pityocampa*. Weaker, but repeatable responses were also found to two other components: limonene, *cis*- $\beta$ -ocimene (Zhang *et al.*, 2003). The terpenoids bornyl acetate and terpinolene revealed good adulticidal activity against *Callosobruchus chinensis* L. and *Sitophilus orzae* L. (Park *et al.*, 2003) possess antifeeding activity against *Hylobius pales* Herbst. (Salom *et al.*, 1994), larval growth inhibition effect against *Chonstoneura occidentalis* Freeman (Zou & Cates, 1997) and pheromonal effect against *Periplaneta Americana* L. (Manabe & Nishino, 1983). Bornyl acetate and camphor present in Tanacetum oils were found to be the most active repellents against potato beetles (Scheerer, 1984).

### 5.3.2 Sesquiterpenes

Terpenes, the main constituents of essential oils, play an important role in plant defence mechanisms. Plants have the ability to produce volatiles under different conditions, thereby influencing the behaviour of both prey and the prey's predator. Amongst those reported in the sample under study, evidence of specific info-chemicals produced by infested or healthy host plants suggest the role of germacrene D in recruiting the predatory mite *Amblyseius cucumeris* as a natural defence against herbivores (Manjunatha *et al.*, 1998). This compound has also been found to be an important signal for *H. virescens* in the interaction with host plants (Rosterlien *et al.*, 2000). Recently, it has been shown that (-) - germacrene D acts as a masking substance of attractants for the cerambycid beetle, *Monochamus alternatus* (Yamasaki *et al.*, 1997).

$\beta$ -caryophyllene has showed a highly significant effect on mortality *Spodoptera exigua* (Langenheim *et al.*, 1980). This correlates with reduction in the level of lepidopteran herbivory in several populations of the tropical leguminous tree *Hymenaea stigocarpa* (Langenheim & Hall, 1983). Literature (Weissbecker *et al.*, 2000) shows that the two-spotted predaceous stinbug, *Perillus bioculatus*, was attracted towards the sesquiterpenes  $\beta$ -caryophyllene, germacrene D, bicyclogermacrene and  $\alpha$ -humulene in volatiles released from damaged potato by Colorado potato beetle. All these compounds are significant components of the essential oils under discussion. *E*- $\beta$ -farnesene has been reported to be attractive to western flower thrips but the attraction was only detected in intermediate concentrations and not at higher or lower concentrations. The presence of germacrene D was found to have a masking effect on the attractiveness of the terpenes was inhibited (Koschier *et al.*, 2000). Germacrene D is an

important compound in the biological control of this cerambycid beetle, which is a vector of the pinewood nematode *Bursaphelenchus xylophilus*, a forest disease agent (Carrubba *et al.*, 2002).

Volatile oil of *Streblus asper* of which its major constituents were phytol 45.1%,  $\alpha$ -farnesene 6.4%, *trans*-farnesyl acetate 5.8%, caryophyllene 4.9% and *trans-trans*- $\alpha$ -farnesene 2.0%, showed significant anticancer activity ( $ED_{50} < 30 \mu\text{g/ml}$ ) from cytotoxicity primary screening tests with P388 (mouse lymphocytic leukemia) cells (Phutdhawong *et al.*, 2004).  $\beta$ -caryophyllene, a common sesquiterpene widely distributed in plants possess anti-inflammatory and anti-carcinogenic activities while also playing a role in plant defense (Tellez *et al.*, 1999).  $\beta$ -caryophyllene had a highly significant effect on mortality correlates with reduction in the level of lepidopteran herbivory in several populations of the leguminous tree *Hymenaea stigonocarpa* and attracts adult predatory green lacewings, *Chrysopa carnea* (Langenhein *et al.*, 1980; langenhein & Hall, 1983; Hedin *et al.*, 1978).  $\beta$ -caryophyllene oxide was reported to exhibit a pronounced inhibition effect on the growth of agricultural pathogenic fungi with maximum activity against *Fusarium culmorum* among other *fusarium* species (Cakir *et al.*, 2004). Nerolidol has a woody floral flavour and is an extremely useful chemical in the perfume industry (Choi *et al.*, 2002).

#### 5.4: Conclusion

The main constituents of essential oils, terpenes, play an important role in insect communication systems offering prospects of opportunities for manipulating pests. In the light of this, investigations on essential oils and their isolates have revealed their great potential as insect pest control agents.

The essential oil of *L. camara* is classified as  $\beta$ -caryophyllene chemotype and *L. trifolia* classified as germacrene D chemotype. Due to the high amounts of sesquiterpene hydrocarbons and their important role in insect communication systems, this plant should be tested in further research. According to the terpinen-4-ol content, essential oil of *O. americanum* could be classified as terpinen-4-ol chemotype, which has not yet been reported from the East African region, but literature (Sanda *et al.*, 1998) shows that this chemotype has been observed in Togo. The essential oil of *T. vogelii* has shown high amounts of sesquiterpenes, most of which are present in many insecticidal plants. Therefore, the plant could have a great usage in the field of pest control.

Despite the plants coming from different families, most compounds were found to be present in all the oil samples.  $\beta$ - Caryophyllene has been identified in all the samples. These could be contributed to its functions to the plant. Although *L. camara* and *L. trifolia* are from the same genus and family, they differ in their major components. This might be due to the fact that they are coming from different geographical conditions or the different biosynthetic pathways in which the plant synthesizes its components. Similarly, some compounds in *L. trifolia* are present in the leaves but absent in the flowers or vice versa. Each part of the plants has its own functions hence the difference in chemical composition.

Moreover as people search for alternatives and provided that there are no suitable synthetic substitutes for many of the compounds or difficulty in profiling and mimicking complex compound mixtures in the volatile oils, the original plant extracts will continue to be used long into the future.

#### **5.5: Recommendation**

1. The effects of crude volatile oils are not particularly dangerous to consumers since they are commonly used in many pharmaceutical preparations and they should also easily evaporate during cooking of the foodstuff. Therefore more investigations are necessary in this field as the study has given very promising results.
2. Although the use of pure essential oils for insect and pest management may not be an economically sound proposition under field condition, cost benefit relationships and persistence need to be investigated in detail to make these readily available agents for insect and pest management.
3. More work is to be carried out in comparing the methods of extraction for the same plants, elucidating the chemical composition of the oil from the same plant but different geographical regions and seasons of the year.

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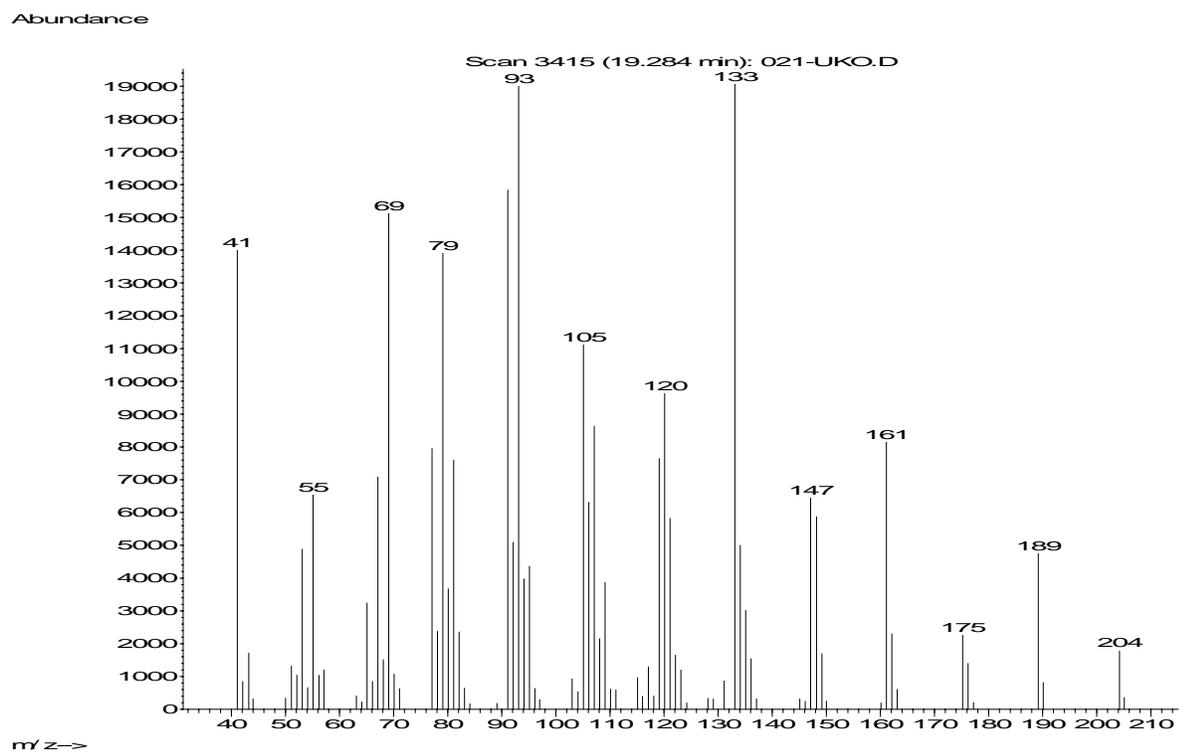
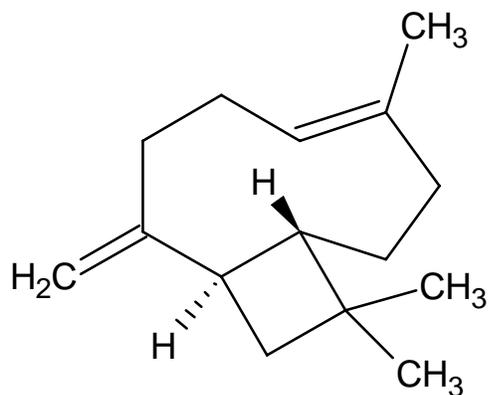
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## APPENDIX I: $\beta$ - Caryophyllene Spectrum

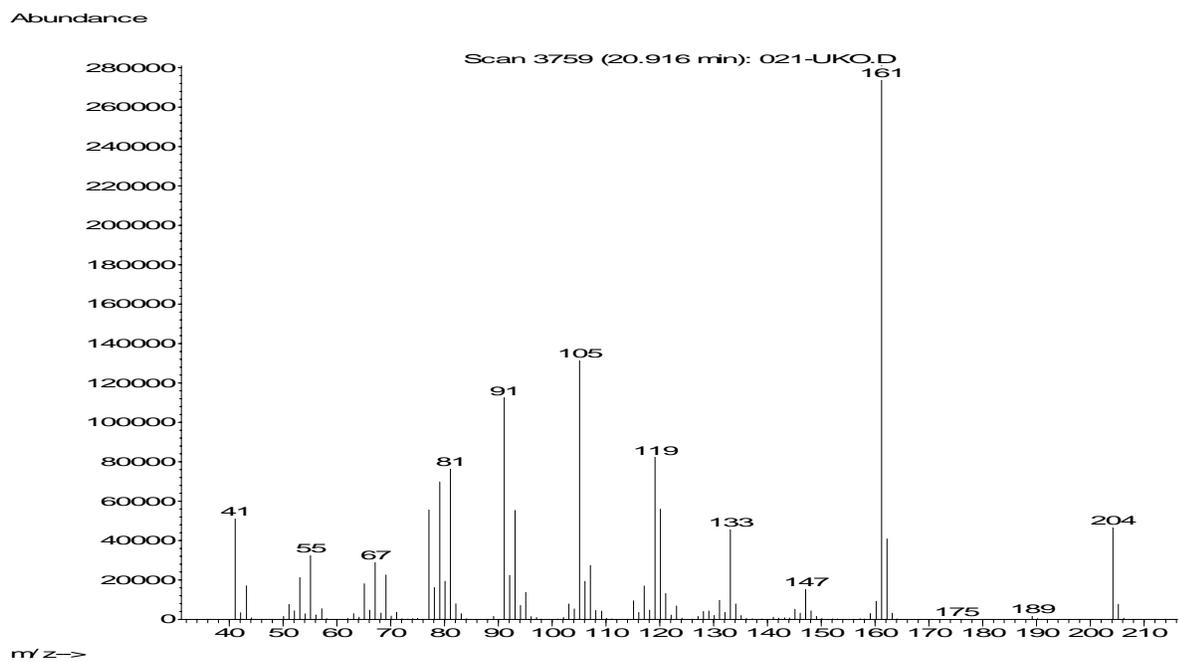
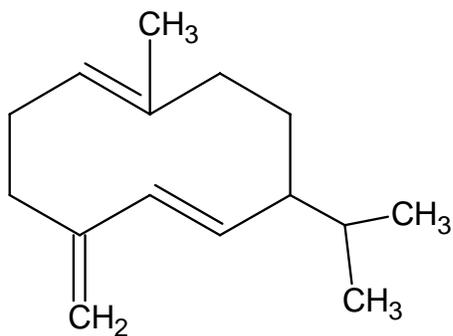
$\beta$ - Caryophyllene (21.35%): The most abundant component of *Lantana camara*



## APPENDIX II: Germacrene D Spectrum

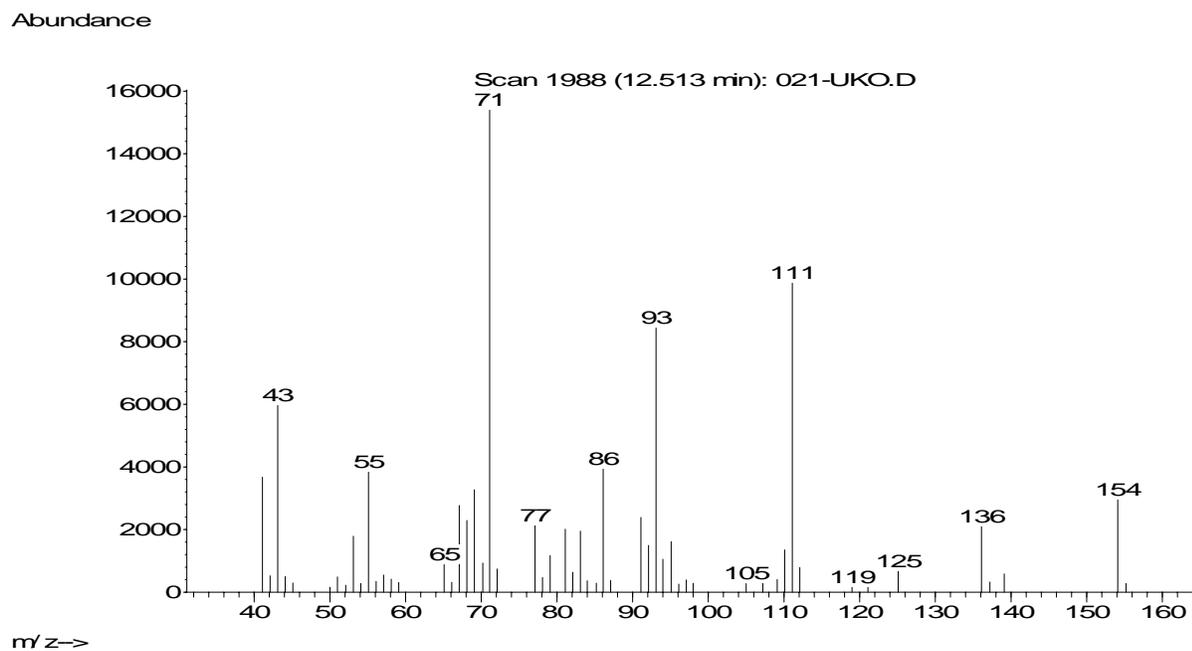
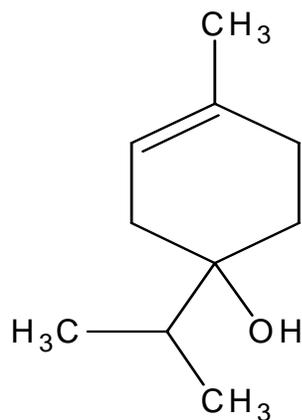
**Germacrene D (25 & 33% flowers and leaves respectively):**

The most abundant component of *Lantana trifolia*



### APPENDIX III: Terpinen-4-ol Spectrum

Terpinen-4-ol (43.21%): The most abundant component of *Ocimum americanum*



## APPENDIX IV: Nerolidol Spectrum

Nerolidol (7.73%): The most abundant component of *Tephrosia vogelii*

