"PHARMACOGNOSTICAL AND BIOLOGICAL STUDIES OF KENYAN *LIPPIA* SPECIES WITH SPECIAL REFERENCE TO THEIR ESSENTIAL OIL CONTENT"

BY

JULIUS WANJOHI MWANGI

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College of Health Sciences

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DECLARATION

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

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J. W. MWANGI

This thesis has been submitted for examination with our approval as University Supervisors.

.....

Prof. I. Addae-Mensah
Dept. of Pharmacy
University of Nairobi

.....

Prof. G. Muriuki
Dept. of Pharmacy
University of Nairobi

.....

Prof. R.M. Munavu
Dept. of Chemistry
University of Nairobi

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D E D I C A T I O N

To my family

A C K N O W L E D G E M E N T S

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ABSTRACT

The study of essential oils of all 8 Lippia species (Verbenaceae) naturally occuring in Kenya was carried out. The essential oils were obtained by hydrodistillation and analysis was mainly carried out by GLC and GC/MS.

Two chemical varieties (chvar) of Lippia ukambensis were found on the basis of their camphor and 1,8-cineole content. These were thus designated Lippia ukambensis chvar camphor and Lippia ukambensis chvar cineole. The essential oil yield from L. ukambensis chvar camphor averaged 1.8% while it was 1.6% from L. ukambensis chvar cineole. L. ukambensis chvar camphor oil contained an average of 37.3% camphor with only traces of 1,8-cineole. L. ukambensis chvar cineole on the other hand had an average of 23.7% 1,8-cineole while camphor accounted for an average of only 1.1%. The other difference in chemical composition of these varieties was the presence of borneol in L. ukambensis chvar camphor oil and its absence in L. ukambensis chvar cineole oil in which α-terpineol was present instead. Other significantly different compounds between the chemovarieties were camphene, limonene, p-cymene which were more in L. ukambensis chvar camphor oil and, 3-carene, trans-sabinene hydrate, β-cubenene and terpinen-4-ol which were more in L. ukambensis chvar cineole oil.

Trans-sabinene hydrate was one of the major compounds in both chemovarieties with an average of 18.9% in the essential oil of L. ukambensis chvar camphor and 24.7% in that of L. ukambensis chvar cineole.

The major compound in Lippia somalensis oil (0.7%) was 1,8-cineole (average 31.9%). This essential oil had a lot of qualitative chemical similarities and quantitative differences with those from L. ukambensis chemical varieties. These differences and similarities were compared by using 15 compounds found in the oils of these Lippia species. It was found for example, that while no camphor, trans-sabinene hydrate and borneol were detected in L. somalensis oil, this oil had more 3-carene, p-cymene, myrcene, limonene, β-ocimene and γ-terpinene than both essential oils of L. ukambensis chemical varieties. Many other quantitative differences were noted.

The essential oil of Lippia dauensis (2.4%) contained ocimene (24.7%), 2-methyl-6-methylene-7-octen-4-one (15.7%) myrcene (12.9%), cis-tagetone (11.0%) and 2-methyl-6-methylene-2, 7-octadien-4-ol (9.4%) as the major components The other significant compounds in this oil were p-cymene, dihydrotagetone, trans-tagetone and α-terpinene.

The essential oil of Lippia javanica (average 1.6%) contained myrcenone (average 32.9%), cis-ocimenone (average 31.9%), trans-ocimenone (average 15.8%) and myrcene (average 7.5%). Cis-ocimenone changed into a very polar reddish-brown compound unless the oil was stored in the deep freezer

D-Carvone (average 60.0%) was the major compound in the essential oil of Lippia carviodora (average 3.0%). Limonene and carvinyl acetate were the other major compounds in this oil. Lippia carviodora var minor oil (0.2%) on the other hand contained mainly sesquiterpene hydrocarbons with β-cubenene (32.0%) and β-elemene (13.7%) being the major components. It was therefore clear that the composition of essential oil of L. carviodora and L. carviodora var minor were very different. A substantial amount of salicylic acid was isolated from the diethylether fraction of methanol soxhlet extraction of L. carviodora var minor leaves.

Limonene (average 36.2%), piperitone (average 27.2%) and piperitenone (average 9.4%) were the major compounds in the essential oil of L. wilmsii (1.1-2.2%). Other notable compounds in this oil were linalool, 1,8-cineole and γ-terpinene. The essential oil of Lippia grandifolia (0.7%) contained linalool (46.1%), thymol (15.2%), β-cubenene (11.7%)and p-cymene (10.4%) as the major components.

The antimicrobial tests of the essential oils showed that L. grandifolia oil and L. javanica oil were the most active. Indeed, L. grandifolia oil was fungicidal to Colletotrichum coffeanum at a minimum inhibitory concentration (MIC) of 50 µg/ml. This is the causitive agent

of Coffee Berry Disease which is one of the most prevalent and feared diseases in coffee farming in Kenya. The oil was also fungicidal to Microsporum audouinii at its MIC 100 µg/ml, M. canis and Candida albicans at 500 µg/ml.

The antibacterial activity of L. grandifolia oil on a number of common bacteria was also carried out. This essential oil was active against Staphylococcus aureus, S. albus, Bacillus cereus and Escherichia coli at MIC 500 µg/ml. The other essential oil with notable antimicrobial activity was fresh Lippia javanica oil which exhibited MIC 500 µg/ml for Candida albicans and Colletotrichum coffeanum and 1000 µg/ml for most of the other microorganisms tested.

The essential oils of L. dauensis and L. javanica were the most active oils as larvicidal agents, having LD_{50} of 66.1 ppm and 74.1 ppm respectively. The larvicidal activity of some of the oxygenated and hydrocarbon monoterpene ingredients of essential oils of Lippia species is also reported. Generally, hydrocarbon monoterpenes were the most active.

The bioassay of the repellant activity of essential oils of Lippia species to maize weevils (Sitophilus zeamais) showed that most of the oils were more active than the synthetic standard, DEET (N,N-diethyltoluamide) at

various dose levels. L. ukambensis chvar cineole had the lowest activity in comparison with the other oils of Lippia species or the standard. The essential oil of L. ukambensis chvar camphor was the most active at the lowest dose level tested (0.625 µl) being 1.5 times more active than DEET.

All the essential oils of Lippia species had a marked spasmolytic effect on the isolated ileum and trachea. The oils also reduced the force of contraction of the heart.

In view of the ease of cultivation of these plants, agreeable aroma of their oils due to the favourable constituents and the biological activities of the essential oils of Lippia species in Kenya, it is suggested that these plants are suitable for possible commercial exploitation.

CHAPTER 1

INTRODUCTION

1.1. Historical background

The Egyptians were among the first people to show an appreciation for perfumes, using them for sacrificial religious rites, for their toilets and as fragrant oils for massaging their bodies. As far back as 4,000 B.C., the Egyptians were using cedar oil in embalming of the dead. Fragrant woods were first used as offerings to the gods and burnt before altars in temples. The Egyptians believed that prayers would reach the gods more quickly, wafted by the blue smoke which slowly ascended to heaven [1].

At the opening of the tomb of Tutankhamun in 1922 some vases were found containing ointments which when opened released a distinct odour of spikenard (from roots of Nardostrachys fatamansii - Verbenaceae). Also present in the tomb was gum-resin olibanum. The scent had been retained since 1350 B.C. that is, for more than 3,000 years [1,2].

Myrrh and frankincense, by far the most prized possession of the civilized world then was used extensively in religious rites. Perfumes were also very popular with ancient Moslems, Hindus and Greeks and many other communities [3].

1.2 OCCURENCE OF ESSENTIAL OILS

Essential oils are odorous principles found in a large number of plants. They are known as "Volatile oils" because they readily volatilize at room temperature when exposed to the air. The term "essential oils" is applied since these oils were regarded as representing the "essence" or the main physiologically active ingredient of aromatic plants.

Essential oils are very common in the plant kingdom. They occur in a number of unrelated plant families such as Compositae and Myrtaceae. Depending on the plant family, essential oils occur in specialized secretory structures such as glandular trichomes (Labiatae), oil tubes known as vittae (Umbelliferae), in oil glands (Myrtaceae) or in oil cells (Lauraceae) [4]. The essential oil may remain in these structures for a very long time. For example in 1967 a weak essential oil profile was obtained from a piece of mentha leaf taken from the herbarium sheet prepared in 1810 [5] Apparently the persistence of an essential oil depends not only on the volatility of the constituents but also on the location of these oils.

Essential oils normally accumulate in a specific part of the plant e.g seed (nutmeg) in fruits (fennel) leaves (bay, geranium, eucalyptus, peppermint), flowers

and leaves (lavender, rosemary), flowers alone (cassia, cinnamon), roots (sassafras, angelica from Angelica species) and woods (camphor, cedar, sandalwood, pine) etc. The oils may however also occur in two or more parts of the same plant. For example, in cinnamon essential oil is found in roots, leaves and bark. The oils which occur in different parts of the same plant may have similar or entirely different composition. For example, cinnamon bark oil contains chiefly cinnamaldelyde (1), while the oil from the roots contains mainly camphor (2) and that from the leaves mainly eugenol (3). The essential oil from Eucalyptus citriodora leaves contains mainly oxygenated terpenes while the other plant parts such as flowers and fruits contain mainly monoterpene hydrocarbons [6].

Recent work has indicated that monoterpenes are produced in submerged cultures of Ceratocystis variopora and a few other fungi [7]. Some insects, [8], algae [5] and even scent glands of alligators [9] are known to produce small quantities of essential oil components,

1. 3 EXTRACTION OF ESSENTIAL OILS

Essential oils may be extracted from plant materials in different ways. Four basic methods are in use:- distillation, extraction with fat, extraction with organic solvents and expression [10, 11, 12]. The following is a brief summary of the methods.

1.3.1. Distillation

Two types of distillation are used: Water and steam distillation.

(i) Water distillation [10, 11, 12]

This method, also sometimes referred to as "direct distillation", is applied to plant material whose essential oil is not likely to be decomposed by boiling. In this method, plant material is introduced into the still (distillation chamber) mixed with water and subjected to heat until it starts boiling. Vapour mixture consisting of water and essential oil passes through a connecting tube into a condenser where it is condensed by external cooling (usually water). The distillate flows into a receiver where the oil separates automatically and may be collected. This method sometimes gives poor quality essential oil as a result of chemical degradation during distillation due to some of the oil coming into contact with highly heated walls.

(ii) Steam distillation [10, 11, 12]

In this method, the plant material is supported on a grid within the still. Saturated or supersaturated steam is injected into the still (distillation chamber) from an external boiler and rises through the charge. If the material is semi-dried, a small amount of water in the charge is necessary to enable the essential oil to diffuse out of the plant tissue. A better quality

of essential oil is obtained in this method. Cooling and collection follows the same procedure as in water distillation.

In view of the location of essential oils, the plant material must be disintegrated prior to distillation. For example, in seeds, roots or barks the essential oil is often found in deeper tissues which must be ruptured to enable the penetration of steam. After comminuting the material, the distillation should be performed immediately to avoid loss due to evaporation or chemical changes due to exposure of the oil. Where the oil is found in glandular trichomes or locations which are easily accessible to distillation, the material does not require comminution.

Distillation time depends on the location of the oil in the plant tissue and on the boiling points of the oil components. Generally sesquiterpene hydrocarbons and their derivatives are less volatile than monoterpenes. It usually takes 3 - 4 hours for a complete or thorough distillation, but some plant materials (eg. vetiver roots) require about 36 hours to isolate the oil completely due to the presence of oxygenated sesquiterpenes. The optimum time of distillation is usually determined by preliminary tests.

1.3.2. Extraction with Fat

The essential oil content in some flowers or flower petals is so small that they yield no oil at all on distillation. The oil may also be destroyed by the action of steam, or the minute quantities of oil are lost in the large volume of distillation water. In such cases extraction with fat is employed. Where cold fat is employed, the process is referred to as enfleurage. The principle of this method is simple. Batches of freshly picked flowers are strewn over the surface of fat (e.g. lard) which is spread as a thin layer on glass plates. Fat has the ability of absorbing fragrance. After a few hours a new layer of flowers is introduced until the fat bacomes saturated with the flower fragrance. There are many modifications of fat extraction. The essential oil may be separated from the fat by extraction with alcohol. The process was formerly used extensively in the production of perfumes and pomades /10,11,12/.

1.3.3. Extraction with Organic Solvents [10,11/12]

The modern essential oil isolation is accomplished by extraction using organic solvents such as petroleum ether and benzene. Since temperature during this process is low (about 50°C) it does not exert its action on delicate constituents of the flower oil. Compared with distilled oils, the extracted oils retain natural odours originally present in flowers.

Despite this advantage offered by this method and enfleurage, these cannot replace distillation which remains the principal method of extraction essential oils. By using portable direct stills, distillation can be carried out even in remote areas whereas organic solvent extraction requires complicated and expensive apparatus while enfleurage is also labour intensive.

1.3.4. Expression

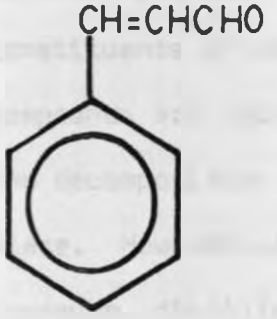
This procedure is applied only to citrus oils. The outer peel containing essential oil is squeezed in presses and the oil decanted or centrifuged to separate water and cell debris. Much essential oil from these fruits is produced as by-product of the concentrated citrus juice industry. The method is used for oils of orange, lemon tangerin, grapefruit and others [11].

1.4. CONSTITUENTS OF ESSENTIAL OILS

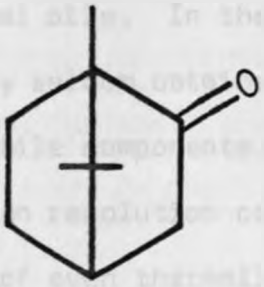
The main constituents of essential oils are terpenes and benzene derivatives. Most essential oils consist largely of monoterpene hydrocarbons ($C_{10}H_{16}$) and their derivatives. Closely related are sesquiterpene hydrocarbons ($C_{15}H_{26}$) and their derivatives which may also be present. Oxygenated derivatives include alcohols, aldehydes, ethers, epoxides, esters etc.

Only in a few cases does an essential oil possess a single component in a high percentage. They usually contain complex mixture of many compounds sometimes as many as 250 components such as those found in the essential oil of Passiflora edulis [5]. However some essential oils such as clove oil (over 85% eugenol (3)) and eucalyptus oil (over 70% 1,8-cineole (4)) contain very high amounts of the major components [11]. The characteristic odour of essential oils is determined mainly by the oxygenated derivatives and their stereochemical arrangements may also be important for the odour as in the case of geraniol (5) and nerol (6).

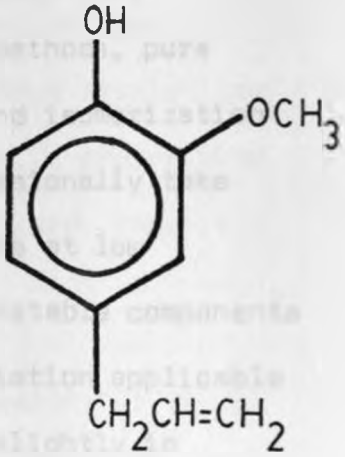
Some essential oils occur as glycosides in the cells and they are hydrolysed under the influence of enzymes in order to liberate the essential oil which may then be isolated by steam distillation (e.g Black mustard seed) [11].



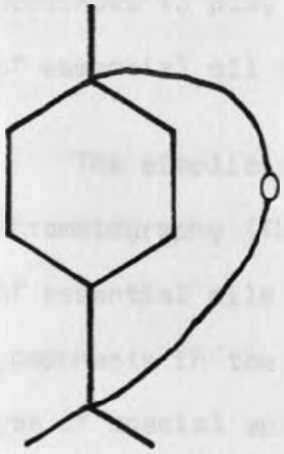
Cinnamaldehyde (1)



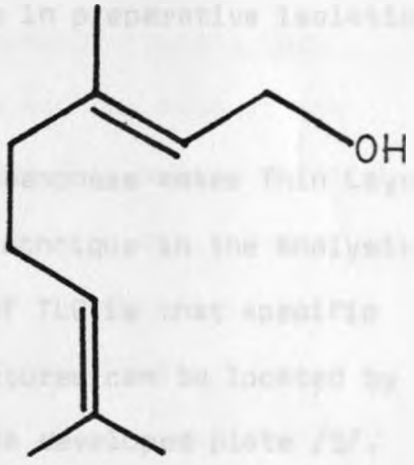
Camphor (2)



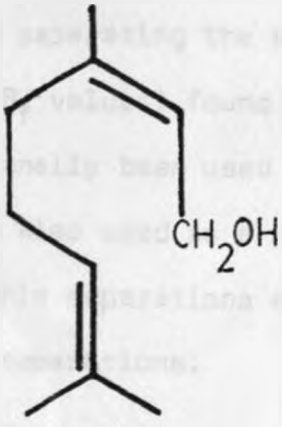
Eugenol (3)



1,8-Cineole (4)



Geraniol (5)



Nerol (6)

1.5 SEPARATION AND ANALYSIS OF ESSENTIAL OIL CONSTITUENTS

Vacuum fractional distillation as well as chemical methods have been applied for the separation of the constituents of essential oils. In these methods, pure compounds are relatively seldom obtained and isomerization and decomposition of labile components occasionally take place. However with high resolution columns at low pressure, distillation of even thermally unstable components has been possible making fractional distillation applicable in separating even isomers differing only slightly in their boiling points. Fractional distillation therefore continues to play an important role in preparative isolation of essential oil constituents.

The simplicity, rapidity and cheapness makes Thin Layer Chromatography (TLC) an important technique in the analysis of essential oils. The advantage of TLC is that specific components in the essential oil mixtures can be located by use of special spray reagents on the developed plate [5]. The low sensitivity and difficulty of accurate quantification makes TLC difficult for separating the multi-component mixtures (with similar R_f values) found in essential oils. However TLC has occassionally been used to obtain fairly pure compounds. TLC is also used as a pilot technique for column chromatographic separations and to gauge the column chromatographic separations.

Gas liquid Chromatography (GLC) has now become one of the most important tools in the analysis of essential oils and plays a central role in the study of volatile oils today. With the right columns the essential oil can be separated into many distinct components. Good resolutions and reasonable speed call for small samples in preparative GLC while large samples,even when well resolved, require long separation times [13].

To reduce artifact production during essential oil distillation and to reduce the labour, "direct injection" of plant material has been adopted [5]. In this method samples of dry leaf material are placed directly into the inlet port of the GLC apparatus and the heat of the inlet oven volatilizes the oil, which then passes directly on the column into the flow of the carrier gas. Automation of the GLC analysis is also possible thereby facilitating analysis of many samples. By coupling GLC with a mass spectrometer (GC/MS), using suitable library searches, Kovats indices and retention times of authentic standards, many essential oils with complex chemical composition have been analysed [15].

Quantitative analysis in GLC is carried out by calculation of the area under the peak either by using an electronic integrator or manually by triangulation or any other method. Each component is usually expressed as a percentage of the total (normalization method). This method is suitable for essential oils since all the components are volatile within GLC operating conditions.

Column chromatography has been applied for essential oil analysis especially in prefractionation of components in essential oil (e.g hydrocarbon and oxygenated terpenes) [16]. Active sites on dried silica and impurities of metals may cause isomerization in a number of oil constituents. However, by use of pure silica gel followed by deactivation of the dried silica gel by addition of 5 - 7% water, the isomerization may be avoided [13].

High Pressure Liquid Chromatography (HPLC) has been applied recently for the analysis of essential oils. Many essential oils components cannot be analysed by HPLC with UV detection at 254nm because they lack chromophoric groups. For this reason either refractive index or low UV (200 - 210nm) detectors have to be used. For example, reversed phase HPLC has been applied for the separation of essential oils of Citrus ladanifer leaves. H_2O-MeCN elution on octyl and

octadecylsilane - bonded silica was applied to resolve complex mixture of sesquiterpenes and oxygenated volatile constituents comparable to the quality of GLC analysis [17]. A general method for HPLC prefractionation of essential oils and flavour mixtures for GC/MS analysis has also been applied for separation of monoterpenes and sesquiterpenes. 8% Ethylacetate in hexane-dichloromethane (1:1) was used as the solvent system, using silica columns and refractive index detection [18].

Instead of HPLC, the relatively newly developed techniques of Droplet Counter - current Chromatography (DCCC) and Rotational Locular Counter-current Chromatography (RLCC) can be used for the separation of essential oils into fractions or in the ideal case into individual pure compounds [13]. These methods, based on the partition of oil constituents in a biphasic solvent system, allow above all the concentration of minor constituents, since relatively large samples can be separated in one analytical run.

The infrared spectra and NMR data of the isolated compounds may be obtained in order to help in identification of the compounds.

1.6 CHEMOTAXONOMIC SIGNIFICANCE OF ESSENTIAL OIL CONSTITUENTS

Essential oil studies have been useful in taxonomy (a) as an aid in defining the species, (b) for detecting hybridization in natural population, (c) in confirming the presence of geographical races and (d) in confirming genetic and tribal limits /5/. For example analysis of turpentine from oleoresins of various Pinus species in Eastern Mediterranean has been applied to separate P. halepensis from other Pinus species. Likewise the analysis of leaf-oil of Myrica species has been employed in resolving the taxonomical confusion among these species. Again the hybrids between Pinus backsiana and P. contorta have been recognized only through the analysis of their essential oils. Reports are also available on the use of the essential oils in confirming the genetic and tribal limits/5/.

1.7 BIOSYNTHESIS OF MONOTERPENES AND SESQUITERPENES

In this section, the biosynthesis of monoterpenes and sesquiterpenes which are the chemical classes to which essential oils belong is discussed.

The structural unit in the biosynthesis of terpenes is isopentenyl pyrophosphate (IPP) (active-isoprene)

from mevalonic acid (MVA). This undergoes a head-to-tail condensation with dimethylallyl pyrophosphate (DMAPP) (formed by isomerization of IPP) to give geranyl pyrophosphate (GPP). The open chain monoterpenes are thought to arise from GPP by hydrolysis, isomerization, rearrangement, reduction, dehydration etc. The addition of another IPP unit to GPP gives farnesyl pyrophosphate (FPP) which also leads to many sesquiterpenes [9, 19, 20, 21]. The stereochemical details and mechanism of conversion of MVA into IPP, DMAPP to GPP are well described in the literature [11, 19].

Cyclohexane monoterpenes are considered to be biosynthesised from neryl pyrophosphate (NPP) (7) or linaloyl pyrophosphate (LPP) (8). GPP might be isomerized enzymatically to NPP or to its biochemical equivalent LPP by an enzyme-bound intermediate [9].Fig 1 shows the proposed biogenesis of cyclohexyl monoterpenes [19].

The cation(9) derived from GPP cannot cyclize for stereochemical reasons but can form acyclic monoterpenes, e.g geraniol (5) and nerol (6). These may then cyclize into (10) through (7) or (8). The biogenetic route shown in Fig 1 although biogenetically and chemically plausible, needs more experimental data to place it on a solid foundation [9].

Biosynthesis of monoterpenes and sesquiterpenes

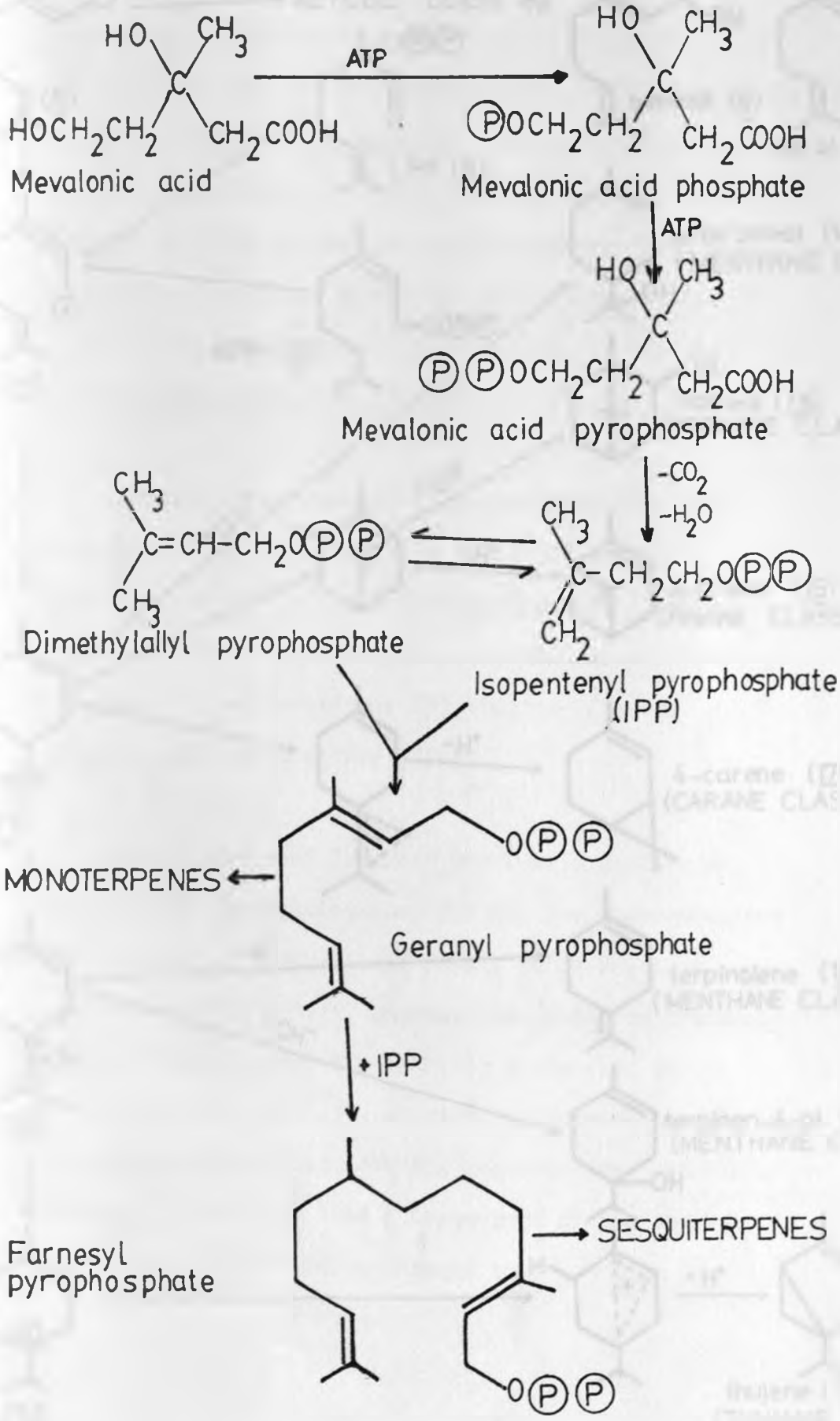
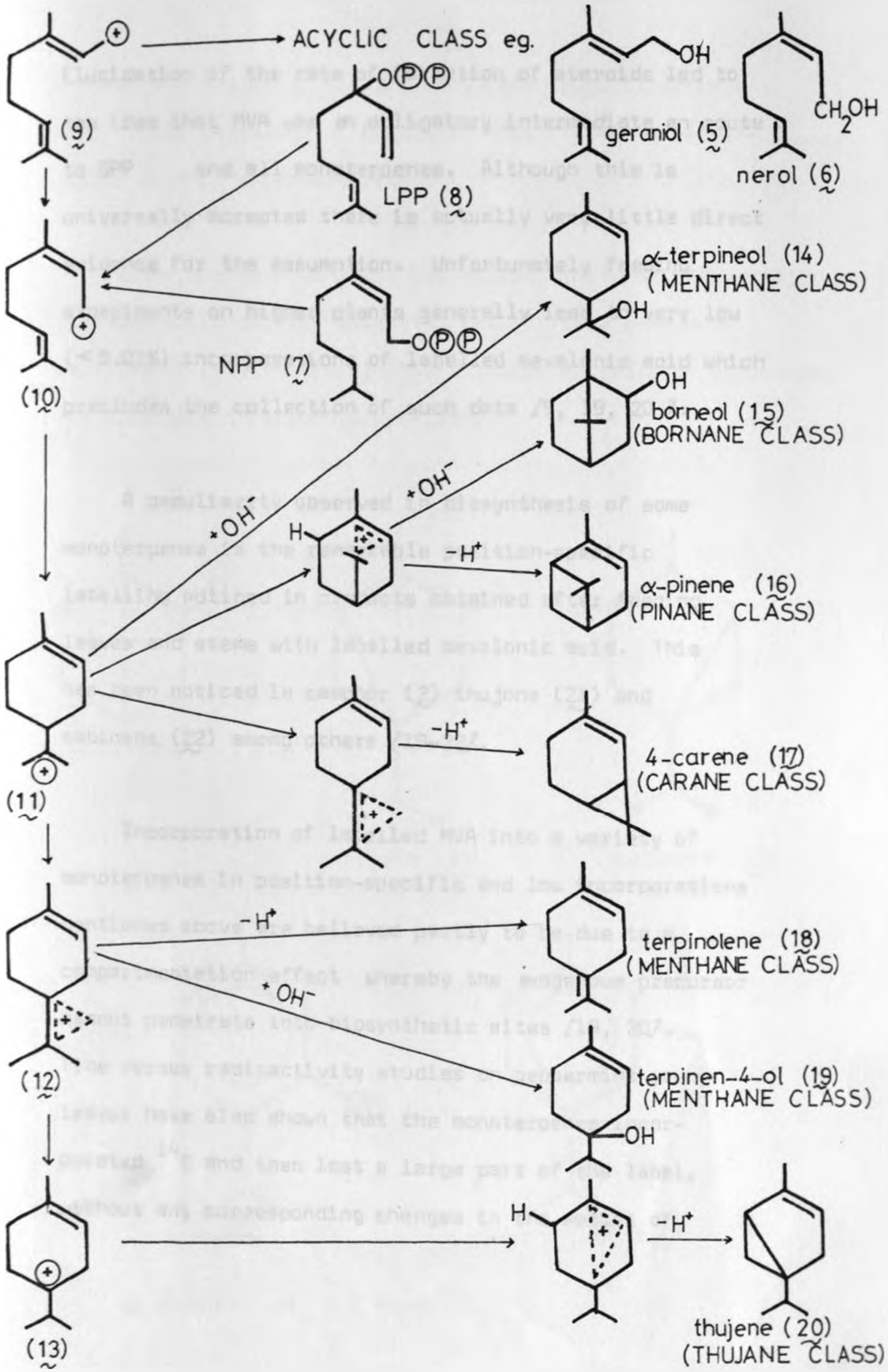


FIG 1 Proposed biogenesis of cyclohexyl monoterpenes



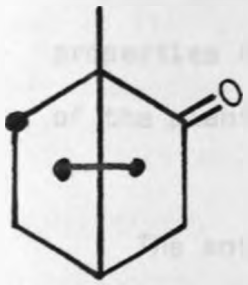
Elucidation of the rate of formation of steroids led to the idea that MVA was an obligatory intermediate en route to GPP and all monoterpenes. Although this is universally accepted there is actually very little direct evidence for the assumption. Unfortunately feeding experiments on higher plants generally lead to very low (< 0.01%) incorporations of labelled mevalonic acid which precludes the collection of such data [9, 19, 20].

A peculiarity observed in biosynthesis of some monoterpenes is the remarkable position-specific labelling noticed in products obtained after feeding leaves and stems with labelled mevalonic acid. This has been noticed in camphor (2) thujone (21) and sabinene (22) among others [19-22].

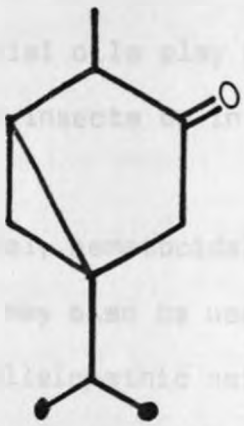
Incorporation of labelled MVA into a variety of monoterpenes in position-specific and low incorporations mentioned above are believed partly to be due to a compartmentation effect whereby the exogenous precursor cannot penetrate into biosynthetic sites [19, 20]. Time versus radioactivity studies on peppermint young leaves have also shown that the monoterpenes incorporated ^{14}C and then lost a large part of the label, without any corresponding changes in the amount of

monoterpene present [9, 22]. All these facts illustrate some of the labelling difficulties encountered in biosynthetic studies of monoterpenes.

Among the various plant terpenoids, there exist phenolic derivatives in various essential oils. These compounds (e.g thymol (23) carvacrol (24)) although aromatic in structure, are terpenoid in origin [11,12,19].

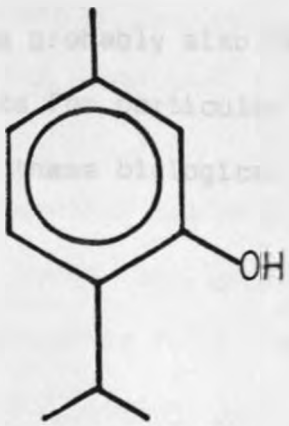


(-)-camphor
(2)

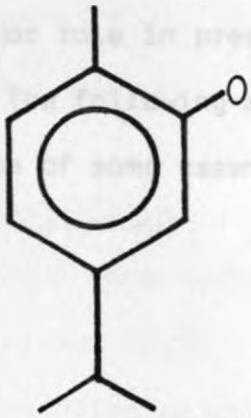


thujone (21)

sabinene (22)



thymol (23)



carvacrol (24)

● position of the label

1.8 BIOLOGICAL ACTIVITIES OF ESSENTIAL OILS

The biological activities of essential oils are important to plants. Several essential oils and their monoterpenoids possess insect repelling properties eg citronellal (25) [23].

A number of monoterpenes posses a pronounced attraction for certain insects, and it is probable that the combination of attraction and repellent properties of essential oils play a role in protection of the plant against insects or in pollination.

The antimicrobial, nematocidal and insect antifeeding effects may also be useful in the protection of the plant. The allelopathic nature (influence or effect of one living plant upon another) of certain essential oils or their constituents especially on germination of seeds probably also plays a major role in preserving nutrients for particular plants. The following are some of these biological activities of some essential oils.

1.8.1 Antimicrobial activity

The antifungal activity of some essential oils has been highlighted. Among the various cultivars of Piper betle L. only "Kapoori" vines proved resistant to various fungal diseases. The essential oil (yield 0.1%) was found to be the factor responsible for the tolerance exhibited by this plant against leaf spot and sclerotial wilt diseases. Tolerance to these fungi appeared partly due to its fungitoxic essential oil [24]. Apparently the same plant (Piper betle) could also be protected from pathogenic fungi by the essential oil of Ocimum gratissimum. The major fungitoxic compound in O. gratissimum oil was eugenol (3). The oil was either equally effective or superior to synthetic commercial fungicides and was non-phytotoxic to host plants. Thus, it was concluded that the oil could be used as a reliable indigenous and biodegradable agent against fungi that cause losses to the betelvine industry [25].

The essential oil of Ageratum houstonianum posesses a broad fungitoxic spectrum against a number of phytopathogenic fungi [26]. The oil was most effective to Fusarium lateritium subspecies cajani being fungicidal at a minimum inhibition concentration

(MIC) of 0.3%.Cedarwood oil has been shown to exhibit fungicidal activity against Epidermophyton floccosum and Trichophyton rubrum at concentrations of 6000 ppm and 4000 ppm respectively [27]. Thymol (23) from Trachyspermum ammi has been shown to be fungitoxic at 1000 ppm against Epidermophyton floccosum, Microsporum canis and Trichophyton mentagrophytes. The essential oil from the same plant was effective at 900 ppm [28]. Keratinophilic fungi such as Verticillium tenuipes, Malbranchea pulchella, Keratinophyton terreum and Chrysosporium tropicum have been shown to be affected by essential oils [29]. Other reports indicate that not only are essential oils active against human pathogenic fungi but some oils are more effective than the commercial fungicides on plant pathogenic fungi [30, 31]. For example Trachyspermum ammi oil (dethymolysed oil) were 20 times more active than Bavistin, 16 times Blistol-50 and 5.3 times Brassicol and Dithane Z-78. Cymbopogon oliveri oil was 30 times more active than Bavistin and 8 times Brassicol and Dithane Z-78. These are some of the prevalent synthetic fungicides in India [30].

Microscopic lesions caused by essential oils of Thymus vulgaris, T. serpyllum, Mentha arvensis and Eucalyptus citriodora on Aspergillus flavus and A. fumigatus have been studied [32]. Signs of severe fatty acid degeneration were always present with numerous basophylic or acidophilic granules. The basophilic nuclei were less, often vesicular or pyknotic, with 1-3 nucleoli. Chlamidospores were often produced and colonies whitened quickly. All these changes indicate unhealthy features in the fungi and are usually followed by death.

Several workers have reported on the antibacterial activity of several essential oils [33 - 36]. As an antiseptic, thymol is known to be 20 times more active than phenol [23]. In certain cases some bacteria have been reported to be very susceptible to the essential oils compared with the control antibiotics [37,38]. Essential oils of Ocimum gratissimum for example inhibited Salmonella species at minimum inhibitory concentration (MIC) of 6.25 µg/ml which was less than that of ampicillin (8.0 µg/ml) [39].

1.8.2. Larvicidal, Insecticidal and Anthelmintic Activity

Some essential oils have been studied for their larvicidal activity [39]. Ocimum sanctum oil was found to produce 16% mortality at 0.01% concentration, 16% at 0.002% concentration for Eucalyptus globulus oil and 8% at 0.002% concentration for Ocimum basilicum oil. (5E) - Ocimenone isolated from Tagetes minuta [40] caused 100% mortality of mosquito larvae at 40 ppm. In spraying experiments in USSR for mites and aphids, 1% essential oil of Lippia citriodora killed over 67% of the mites and nearly 93% aphids tested [41].

The anthelmintic activity of the essential oil of Callistemon viminalis against earthworms and tapeworms was better than that of piperazine citrate and was comparable to hexylresorcinol against hookworms [42]. The nematicidal activity of the essential oils of various Cymbopogon species and their major constituents geraniol, citronellol has been determined with 4 plant nematodes, seed-gall nematode (Anguina tritici), citrus nematodes, (Tylernchulus semipenetrans), root-knot nematode (Meloidogyne javanica) and cereal cyst-nematode (Heterodera avenae) [43]. These essential oils were found to be nematicidal and their activities correlated with their chemical constitution. The residual water from steam distillation of a number of Lippia species (L. aristata and L. sidoides) have been shown to exhibit high molluscicidal activity against Biomphalaria glabrata, the most important host of Schistosoma

mansonii in Brazil [44]. Ascaridole (26) from Chenopodium ambrosioides var anthelminticum essential oil is a powerful anthelmintic and serves in many medicinal and veterinary preparations [45].

1.8.3. Pheromones, Repellent and Antifeeding Effects of Essential Oils

Pheromones are compounds produced by an organism for the purpose of communicating with other organisms of the same species to attract members of the opposite or the same sex, to spread an alarm or to mark the trail to food. The sex attractants of insects have been studied extensively [8,46], with an aim (already realized in some cases) of synthesizing them to serve as bait with which to lure and entrap the female-seeking males of a species before they can mate, to confuse them, and distrupt their search, or to lure them to areas that are treated with pesticides or pathogens which can be spread by infected individuals to the rest of the population.

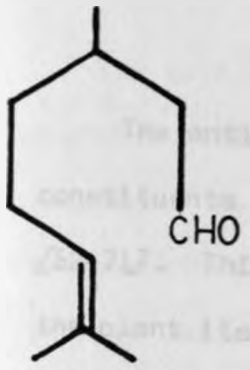
The essential oil constituents released as pheromones by insects such as aphids when attacked by predators may serve in plants as false alarms, thereby protecting the plants from insect attacks. (E)-β- Farnesene (27) and (-)-

germacrene (28) have been identified as alarm pheromones for many aphids and when released from aphids, cause dispersal of others feeding nearby [7, 48]. Citral (29) has also been shown to be a minor alarm pheromone in some mites [49].

Presence of several sex and aggregation terpene based pheromones has been reported severally. The presence of these compounds probably plays a role in the selection of hosts and oviposition sites. For example males of Podisus fretus (Hemiptera, Pentatomidae) release a long range attractant pheromone containing 49% linalool (30), nerolidol (31) α-terpineol (14) and other non-terpenoid compounds [50]. Among the volatile constituents that emanate from alfalfa seeds, females of alfalfa seed chalcid (Bruchophagus roddi) flew to among others, (E)-farnesene (27), α-copaene (32), γ-muurolene (33) at a concentration of 0.01% [51]. Host plant attractants for the carrot fly, Psila rosae [52] and European elm bark beetles have also been identified [53]. The attraction of various insects to the plant may sometimes be harmful to plant. The male beetles (Ips confusus) for example initiates the attack on ponderosa pine (Pinus ponderosa) and produces a pheromone attractive to both sexes but more so to the female. The massive invasion of beetles attracted by this pheromone frequently kills the tree [54].

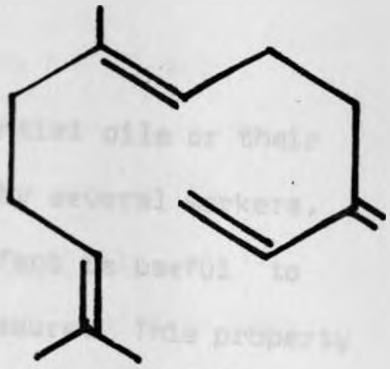
This attraction has been exploited in baited traps. Beetle (Polygraphus poligraphus) responded to traps baited with (-)-terpinen-4-ol while (+)-terpinen -4-ol (19) inhibited the response [55]. Some of these attractants such as eugenol (3) have a low order of toxicity[56].

Some essential oil constituents are known to have insect repellent activity. This activity could be useful for the protection of plants against insects. Herbs have traditionally been used as intercrops with crop plants on the assumption that their odour repels pest species. Among alcohol extracts and essential oils of Labiatae herbs tested in the laboratory for deterrent/repellent responses to ovipositing Plutella xylostella (L.) and feeding larvae of P. xylostella and Pieris brassicae L., essential oils of sage and thyme reduced oviposition on pieces of brassica leaf [57]. Inclusion of essential oil principles in the well known repellent, N,N-diethylphenylacetamide significantly enhances the protection time against biting insects [58]. Other essential oils or their components known to have repellant activity include camphor (2), citronellal (25), citronella oil, essential oils of Ocimum suave, lemongrass oil, Tagetes minuta oil and many other essential oil constituents [36,56,59,60,61].

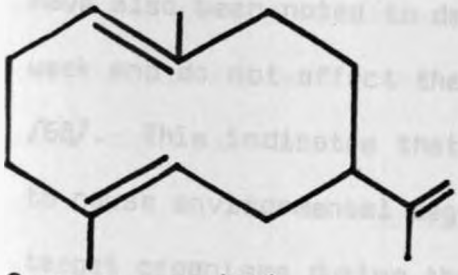


Citronellal (25)

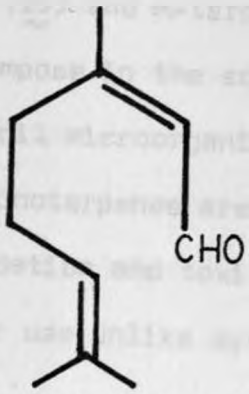
Ascaridole (26)



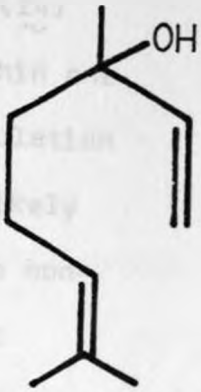
β-Farnesene (27)



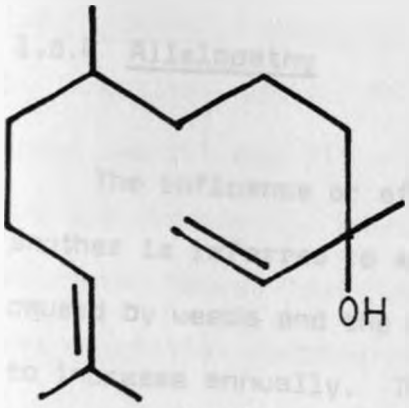
Germacrene (28)



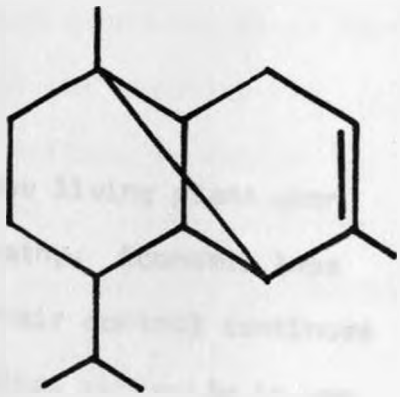
Citral (29)



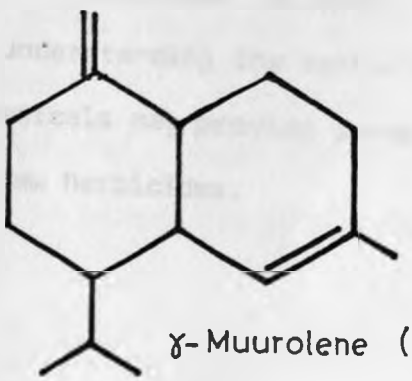
Linalool (30)



Nerolidol (31)



α-Copaene (32)



γ-Muurolene (33)

The antifeeding effect of essential oils or their constituents has been investigated by several workers. [62-71]. This feeding deterrent effect is useful to the plant itself as a protective measure. This property may also be exploited in crop protection against insects or worms (by intercropping or spraying). At least p-cymene (34), terpinen-4-ol (19) and α-terpineol (14) have also been noted to decompose in the soil within one week and do not affect the soil microorganism population [68]. This indicates that monoterpenes are not likely to cause environmental degradation and toxicity to non-target organisms during their use unlike synthetic pesticides [61].

1.8.4 Allelopathy

The influence or effect of one living plant upon another is referred to as allelopathy. Economic loss caused by weeds and the cost of their control continues to increase annually. The herbicides currently in use are known to have undesirable effects. It has been suggested that understanding the mechanism of actions of allelopathic chemicals may provide leads to the development of new herbicides.

The allelopathic nature of plants containing essential oils has been known for sometime now. The presence of 1,8-cineole (4) and camphor (2) in the atmosphere and soil around Salvia leucophylla was reported as early as 1972 [72]. These compounds were very toxic to seed germination and seedling growth. The S. leucophylla plants developed zones around them which were devoid of other herbs or shrubs. The weed status of plants in the Labiatae family depends primarily on competition for environmental resources and on characteristics dependent on the phytochemical content (essential oils) of the plants. Salvia is notable for its allelopathic activity [73].

Vapours from the leaves and extracted oil from Trichostemma lanceolatum inhibited growth of other plants in laboratory tests. The inhibitor was terpinen-4-ol (19) which was 0.3 and 1.9 times as inhibitory as camphor (2) and 1,8-cineole (4) respectively [74]. These compounds as stated above, have previously been shown to influence the vegetation pattern near Salvia leucophylla

Linalool (30) has been shown to be inhibitory to germinating seedlings[75]. This observation has been recently confirmed [76]. These workers have shown that linalool at 600 ppm causes 80% growth reduction in the

radicle of lettuce seedlings. Using the same bioassay, fruit essential oil of Piper guineense and others from different sources have been assayed [77]. Above 100ppm there was significant inhibition by P. guineense oil Chromolaena odorata leaf oil showed mild stimulation at 25 ppm and little or no effect at higher concentrations. Eugenia uniflora caused a maximum increase in seeding root of 39% at 200 ppm. Above 200 ppm the root length decreased. This pattern is characteristic of auxins. Auxins in general stimulate growth of particular plant organs at certain low concentrations. At higher doses they inhibit this growth. For example, there is growth promotion of stems by auxins (eg indoleacetic acid) from about 10^{-9}M to 10^{-5}M. This is followed by growth inhibition at higher doses. The same observation is made for buds and roots [78].

1.8.5 Pharmacology

Apart from the biological activities of essential oils, some of their pharmacological effects have also been investigated. Stitcher [23] lists some of these pharmacological activities. These include sedative (Melissa oil), antiseptic (thymol in Thyme oil), expectorant (1,8-cineole in Eucalyptus oil) diuretic (terpinen-4-ol in Juniper oil) and, central

nervous system stimulant and skin irritant (camphor in various oils).

Rosemary oil, 1,8-cineole(4) and bornyl acetate (35) have been shown to depress contractility of cardiac muscle and inhibit acetylcholine-induced contraction of guinea pig ileum [79]. The essential oil of Plectranthus incanus has shown multiple pharmacological actions including bronchodilation, spasmolytic effect on smooth muscle and inhibition of rate of contraction in the isolated heart[80]. The essential oil extracted from Tagetes minuta has been shown to have tranquillizing, hypotensive, bronchodilatory, spasmolytic and antiinflammatory properties [81]. The essential oil of Zanthoxylum budrunga has also been shown to exhibit local anaesthetic activity, a dose-dependent hypotension and spasmolytic action on isolated guinea pig ileum [82].

The antiinflammatory activity of essential oils has also been investigated. The essential oil extracted from Zanthoxylum budrunga has been studied for antiinflammatory activity against exudative phase of inflammation by formalin-induced rat hind paw oedema and against the proliferative phase of inflammation by the cotton pellet-induced granuloma in rats. Betamethasone and indomethacin

were used as standard drugs in comparison. The essential oils at 0.1 and 0.2% suppressed both phases of inflammatory reaction significantly and compared very well to the standards [83]. Essential oils of Artemesia sieversiana, A.pontica, A. macrocephalla, A. jacutica, Achillea asiatica and their fractions have also been found to produce pronounced antiinflammatory effect with low toxicity[84].

The antiinflammatory effect of the essential oil (0.1mg/kg) of Curcuma longa was significantly more marked than that observed with cortisone acetate (10mg/kg). This early (3rd day) highly significant antiinflammatory effect of essential oil of C. longa seems to be related to its inhibition of histamine release from tissues and mast cells. The protective effect of the essential oil for the late inflammatory arthritic changes (10-13th day) has been considered to be mediated through hypophyseal adrenal axis. This is likely since the essential oil did not inhibit the late secondary lesions in adrenalectomized animals [85].

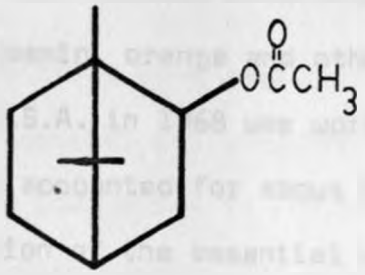
Effects of essential oils on cholesterol in serum has also been investigated. Terpin (36) treatment to cholesterol-fed rabbits neither impaired the absorption

or storage of cholesterol nor did it inhibit the increase of cholesterol plasma level but remarkably prevented the development of atherosclerotic plaque [86]. It was concluded that this resulted either from some competitive inhibition, or perhaps the essential oil acts by blocking mediatory factors promoting the penetration of lipoids into the arterial wall.

Atherosclerosis is the commonest disorder of blood vessels. The disorder is characterized by atheromas, which are plaques in the intima and inner part of the media containing lipid material, mainly cholesterol and cholesterol esters. Large arteries and some medium-sized arteries are affected: the principal sites of atheromatous lesions are in the aorta, the femoral, renal, coronary, carotid and vertebral arteries and the circle of Willis [87]. Atherosclerotic plaques encroach on the lumen and also provide sites for formation of thrombi which may completely occlude the vessel or from which emboli may be detached and block a more peripheral artery. Complications associated with atherosclerosis are many and varied and the treatment is also equally complex [87]. This effect of terpin may therefore

be very important in the control of atherosclerosis in hypertension especially where there are high levels of cholesterol. It could therefore be formulated even in form of pills /87/.

p-Cymene (34)



Bornyl acetate (35)

Terpin (36)

1.9 PRODUCTION AND COMMERICAL USES OF ESSENTIAL OILS AND SPICES

1.9.1 Essential Oils

Today, France leads the world in perfume production followed by England, India and U.S.A. France manufactures perfumes from flowers of roses, jasmin, orange and others[1]. Production of essential oils in U.S.A. in 1968 was worth US $ 57.5 million. Peppermint oil accounted for about ½ of the total value. The consumption of the essential oils during the same year was about US $ 53.2 million [88].

From Turkey and Bulgaria come the famous essential oils of roses, while Italy produces essences of bergamot and citrus plants. The only two perfumery ingredients for which Britain is famous are lavender and peppermint oils [1].

The annual production of Rosemary oil which is mainly produced in Tunisia and Morocco had reached 375-425 tonnes worth Sterlin £ 2.5-2.8 million in 1979. Most of this oil was exported to U.S.A. and EEC countries. Dill oil produced mainly in North America, Europe, India and China had also reached an annual production of 50 - 100 tonnes by 1979. Basil oils produced mainly in Reunion had an annual total world value of Sterlin £ 400,000-450,000 at the same period [89].

Other essential oils are produced in many other countries. These include citronella oil(Ceylon) lemongrass oil (India), cinnamon oil (Sri Lanka), pepper oil (Brazil, India, Madagasca), nutmeg oil (Indonesia, Grenada),Cardamon oil (India, Guatemala) and ginger oil which is produced in India and China [90].

Many independent African countries have been experimenting with a number of essential oil yielding plants. Some of these plants are indigenous to African countries while others have been introduced. For example, in the Limpopo valley, Zimbabwe and in Shire valley of southern Malawi, marjoram, lavender and thyme have been grown commercially. Nigeria is a world exporter of ginger. In Tanzania, eucalyptus, rosemary and dill have been cultivated commerically. On Pemba and Zanzibar islands large scale distillation of oil from clove buds, is performed for export. The main income of these islands is derived from this aromatic tree [1].

In spite of great efforts by the chemical industry to produce perfumes the worldwide demandfor natural flavours and fragrances is steadily increasing. Indeed, it was estimated that the consumption, in 1979, of terpenes such as, for example, citral, citronellol, geraniol and

linalool which are particularly important to fragrance and flavour industry was worth more than 200 million US dollars. It must be much more today since the forecast of an annual growth rate of more than 10% for the period of up to 1985 was predicted [91]. Synthetic perfumes always have a kind of "Chemical note" and never have the "freshness" of the natural perfumes.

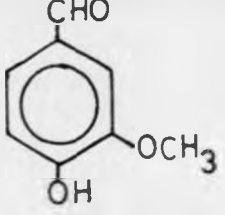
Many monoterpenes are used as ingredients of flavour and fragrance components or as a source of raw materials for chemical modification to provide valuable flavour and perfume materials. For example citral (29) which may be isolated from various essential oils can be converted into ionones which have a "violet" odour which is the odour of essential oil from leaves and flowers of Viola odorata. Similarly citronellal mostly found in Eucalyptus citriodora oil may be converted into hydroxycitronellal which has a "convallaria" odour which is the odour of essential oils from flowers of Convallaria majalis. Both of these derivatives have a delightful fragrance. From cheaper oils containing eugenol (3), vanillin (37) may be obtained which is in great demand in making certain products e.g ice-creams, fruit essences etc. The cheap pine oil containing high amounts of pinene may be used as a starting material for synthesis of oxygenated

terpenes such as α-terpineol (14) which is an important component of perfumes [88].

Among the cosmetics, the soap industry is the main consumer of essential oils. In soap industry the essential oils utilized are usually the cheap ones due to their less strong odour. Various essential oils are used as fixatives (e.g cedar oil, sandalwood oil). These oils have high - boiling point components (usually oxygenated sesquiterpenes) which lower the volatility of other essential oil components thereby making the perfume available over a longer period of time. In some cases these fixants also have a sweet odour.

Essential oils are used on a large scale in the food industry. They are used in alcoholic drinks such as rum, in carbonated beverages, soft drinks, powders etc. Spices which contain essential oils as their flavouring principles, have been used since time immemorial for flavouring various foods.

In Medicine volatile oils are either used as flavouring agents to mask disagreeable tastes of certain medicines or as medicines themselves. They are used as sedatives (eg. Mellisa oil), antiseptics (eg. Thyme oil), expectorant (eg Eucalyptus oil), diuretics (eg. Juniper oil) [37].



Vanillin (37)

In dental practice some essential oils and their constituents (eg.eugenol (3), thymol (23) are used as antiseptics or anaesthetic agents. Table 1 shows some of the commonly used oils.

1.9.2. Spices

Spices which are strongly flavoured aromatic vegetable products due to the presence of essential oils are mainly used as food flavours , fragrances or preservatives. They are considered as suitable secondary or tertiary crops in many countries. Due to this diversification of agricultural activity into the spice sector nearly all the countries of the world grow some spices. As a result of this, the world spice trade has grown from US $ 150 million between 1950-1952 to over US $ 1000 million in early 1980s [92]. Other countries such as Federal Republic of Germany, France, Japan, Indonesia imported spices worth US $ 71.2, 51.4, 59.3, 74.7 million respectively in 1982. India which exported spices worth US $ 154 million in 1982 is one of the major spice exporting countries. Others include Malaysia, Spain, Jamaica, Madagascar, Indonesia, Singapore and Brazil [92]. Brazil is a world producer of pepper (Piper nigrum) but also imported spices worth US $ 5.2 million while Mexico and Argentina although world producers imported in 1980, spices valued

Table 1. COMMONLY USED ESSENTIAL OILS

Plant Species	Type of oil	Uses
Pipenta racemosa	Bay oil	Much used in perfumes, toilet preparations and bay rum.
Lavendula officinalis	Lavender oil	Used to scent baths.
Cymbopogon citratus	Lemmongrass oil	Used to adulterate lemon oil and for its own fragrance
Cymbopogon nardus	Citronella oil	Repellent to mosquito and other insects.
Citrus aurantium	Neroli oil	Cologne and liqueurs
Cinnamomum cassia, C. zeylanicum	Cinnamon oil Cinnamon leaf oil	Perfumes, medicines and flavourings.
	Cassia oil	An expensive oil used in perfumes and medicines
Cananga odorata	Ylang ylang oil	One of the most important perfume raw materials.
Iris florentina and other I. species	Iris root oil	Powdered used in dusting powders, and oil used in perfumery.
Mentha arvensis	Mentha oil	Flavouring chewing gums, tooth paste, cigarettes, medicines, as an inhalant and perfume

Table 1 continued

Eugenia caryophyllus	Clove oil	Medicines, flavouring and perfumery.
Pimpinella anisum	Anise oil	Perfumes, as a medicine and in liqueurs
Juniperus communis	Juniper oil	Used in medicine and in varnish making.
Eucalyptus globulus	Eucalyptus oil	Perfumes, antiseptics, scented soaps and toilet preparations.
Pinus palustris	Turpentine oil	Solvent for thinning paints and varnishes. Starting raw material for synthesis of other terpenes e.g camphor

at over US $ 8.5 and 5.3 million respectively. USSR, Poland, Yugoslavia, Hungary, Czechoslovakia and many Middle East countries are also involved in the spice trade /93, 94/. An interesting observation is that many developed countries import the essential oils or spices from developing countries only to re-export them later, sometimes paradoxically to developing countries.

In Commonwealth Africa spice trade has a very important place. Mauritius has the biggest import market (with over 1000 tonnes of different spices annually) followed by Nigeria, Tanzania and Kenya. These are mostly from Asia and minimal from Commonwealth Africa. Also a significant feature of the spice economy of Commonwealth Africa is that several countries, including Zambia, Zimbabwe, Malawi, Nigeria and Mauritius are net importers, indicating the scope for expanding regional trade /92/.

Spices sector is important in Tanzania's economy and crucial to islands of Zanzibar and Pemba where cloves and Cardamon are cultivated. For example in 1982 export earnings mainly from cloves was US $ 50.5 million forming 11% of the total merchandise. Seychelles exported over 837 tonnes of spices mainly Cardamon which was 13% of the Island's domestic merchandise in 1981. Nigeria exports mainly ginger and chillies while Sierra Leone

exported 372 tonnes of ginger in 1982 [92]. Egypt in 1979 exported spices worth US $ 3.1 million but also imported spices at the value of US $ 3.0 million in the same year [95].

The unit cost of the essential oils and spices vary widely. In 1975 for example, the market value of Rose oil was 4320 US $ /Kg while peppermint oil was 26 US $/Kg in USA [88]. The synthetic imitations of the essential oils usually are much cheaper than the natural products. For example, while the cost of Cinnamon bark oil was US $ 20-30/kg in 1972, the synthetic Cinnamon oil from Japan was valued at 2-3 US $/Kg [90].

To be noted is that although the figures given in the discussion on essential oils or spice trade are not as recent as one would have liked, they nevertheless give a reasonable impression on the impact of this trade worldwide.

1.10 PRODUCTION AND RESEARCH ON ESSENTIAL OILS IN KENYA

The Commercial production of essential oils in Kenya started with Cedarwood oil after the First World War (1914-1918). Kenyan Geranium oil popularly called "Mawal oil" was also known the world over for its quality. A few other plants were also cultivated for the commercial exploitation of their essential oils even during the Second World War (1939 - 1945) [96]. In 1960 for example about 9.0 tonnes of geranium oil worth Sterling £ 10,231 was exported from Kenya [97]. Since then commercial production of essential oils has dwindled to almost zero. No records are available to indicate how and why this state of affairs came about. Cedarwood oil from *Juniperus procera* Hochst obtained from waste wood shavings in the pencil industry in Nyahururu is the only oil being produced commercially. With the government control of felling trees, the production of this essential oil is bound to be reduced. However, in Naivasha some attempts have been made to distil geranium oil privately. A private firm (Bees Company) is also cultivating some herbs containing essential oils at Naivasha. These herbs are used as flavouring agents without extracting the oils.

Kenya therefore imports all the essential oils or their constituents for perfumery, cosmetic, food products and pharmaceutical applications. In 1987 for example the essential oils or the essential oil based products (eg concentrates of essential oils in fats, in fixed oils, in waxes, mixtures of essential oils, aqueous distillates etc) import into Kenya amounted to over 402.7 tonnes at the cost of Kshs. 80.0 million. Most of these were imported from European countries. During the same year about 265 tonnes of the same products worth Kshs. 48 million were exported from Kenya /98/. These figures excluded the imported finished goods containing essential oils or essential oil-based products such as cosmetics and pharmaceuticals. The exported essential oils or related products recorded arose from either re-exports of the same or in formulated products.

Apart from chillies, most of the spices are also imported into Kenya. Some amounts of ginger and coriander are grown for domestic use. In fact Kenya exported 57 tonnes of ginger in 1973 but only 7.0 tonnes in 1978 /94/. During 1987 for example about 175.5 tonnes of various spices worth over kshs. 2.4 million were imported into the country while more than 203 tonnes was exported at the cost of Ksh. 3.4 million /98/. Kenya also exported about 189 tonnes of spices comprising mainly chillies in 1982 while it imported about

171 tonnes of spices in the same year [98]. Most of the spices were imported from India, Malaysia, United Kingdom, Switzerland, Singapore, Uganda, Tanzania and Ethiopia.

It can therefore be seen from the foregoing that both essential oils or essential oil-based products and spices have an important place in the economy of this country. Table 2 shows some of the most commonly available medicinal products in Kenya containing essential oils or their ingredients.

Research on essential oil-containing plants started during the early 1930s. Some plants analysed for their oils included Plectranthus species, peppermint, Cymbopogon nardus, C. citratus and Brachylaena hutchinsii. This last plant yielded an essential oil with a persistent odour which offered possibilities for use in soap, perfumery and as a perfume fixative [99].

The general screening of the Kenyan plants for their essential oil has been in progress since 1978 in the Department of Pharmacy, University of Nairobi. Out of more than 200 plants belonging to more than 38 families screened for essential oils, several families were selected

which were particularly rich in essential oils. These were Pinaceae, Burceracea, Labiatae, Verbenaceae and Umbelliferae. Further research was considered important to establish the practical application of these oils and the viability of a commerical production of essential oil containing plants /1/. Table 3 shows some of the plants which were screened for their essential oils.

Kenya is endowed with diverse climatic and physical conditions. A number of plants which are sources of common spices (e.g fennel, anise, and dill) are imported into this country. Preliminary results obtained so far reveal that such plants could be grown in Kenya, and yield of essential oil is usually much higher than that found from the imported material. Some of these plants are grown in other parts of the world where the climate is less favourable compared with that in Kenya. Kenya has therefore a unique opportunity in creating such an industry.

The success of pyrethrum introduction and cultivation in Kenya bears testimony to this. Cultivation of essential oil-bearing plants on commercial scale in rural areas would earn the local farmer a source of extra income, a buffer against cashcrop market fluctuations and would also be useful in primary health care due to their medicinal properties.

Table 2. Some Products Containing Essential Oils or their Components available in Kenyan Market

Product	Ingredients
Ambikof	Anise oil, Peppermint oil
Baby Chest Rub	Camphor, oil of Turpentine, Menthol, Eucalyptus oil, Cedarwood oil, Nutmeg oil, Oil of Thyme.
Benylin expectorant	Menthol
Boots Vapour Rub	Menthol, camphor, Eucalyptus oil, Turpentine oil, thymol
Caladryl cream	Camphor
Capsoline	Camphor, Turpentine oil, Eucalyptus oil
Cofta tablets	Menthol, oil of anise, oil of Mentha piperita, oil of Pinus pumil, Eucalyptus oil.
Deep Heat	Menthol, Eucalyptus oil, Turpentine oil.
Halls Mentholyptus	Menthol, Eucalyptus oil
Kavrol capsules	Menthol, Cinnamon oil, Pine oil, terpineol, thymol.
Nurse Harvey Gripe mixture	Dill oil, Caraway, Weak Ginger tincture
Rivolyn expectorant	Menthol
Robb	Menthol, Camphor, Oil of Eucalyptus, oil of Pini pumil

Table 2 continued

"Sabanga ya Pateli" (Kiswahili)	Menthol, thymol, camphor, Eucalyptus oil, peppermint oil.
Skores	Menthol, Eucalyptus oil
Sloans liniment	Camphor, Anise oil.
Sting Relief	Eucalyptus oil, menthol, camphor
Tiger Balm	Menthol, camphor, Peppermint oil, clove oil, Cajuput oil, Cassia oil.
Vaseline Constant Care (Lip balm)	Camphor, fragrance
Vicks Inhaler	Menthol, camphor, Pine needle oil, methylsalicylate
Vicks pastilles	Menthol
Vicks vapour rub	Menthol, camphor
Mentholatum	Menthol, camphor, oil of Eucalyptus oil of Pini pumil

Table 3. Some Indigenous and Introduced Plants Containing Essential Oils in Kenya

Plant	Yield and Possible Uses
Pinus patula ***	Resin (8%) - source of turpentine of great economic value in various industries.
Eucalyptus citriodora **	Leaves (5%)-source of citronellal
E. globulu citratus **	Leaves (2%)-source of 1,8-cineole
Cymbopogon citratus **	Leaves (1%)-source of citral.
Ocimum kilimandscharicum *	Herb (4-8%)-source of camphor
O. suave*	Leaves (2%)-source of eugenol
Rosmarinus officinalis**	Leaves (2.5%)-oil used for the scenting of soaps and room sprays. Also used for flavouring all kinds of foods.
Thymus vulgaris **	Herb (3%)-source of thymol
Origanum majorana **	Herb (2.5%)-dried herb is also a common spice for the seasoning of food products in general
Salvia officinalis **	Herb (2.5%)-oil used for flavouring of table sauces of canned and packed foods, soups, meats, and especially sausages.
Satureia biflora *	Herb (1-2%)-source of citral
Commiphora spp. *	Oleo-gum-resin (7-10%)-application in various perfume industries.
Boswellia spp *	Oleo-gum-resin (7%)-utilization in perfumery

Table 3 continued

Matricaria chamomilla**	Flowers (1%)-there is a great demand for this plant as a source of extracts used in pharmaceutical and domestic industries.
Tagetes minuta *	Herb (2%)-utilized in perfumery and other industries.
Brachylaena hutchinsii	Wood (2%)-application of oil in perfumery.
Peucedanum elgonense *	Fruits (4%)-investigation not completed
Heteromorpha trifoliata *	Fruits (3%)-as above
Coriandrum sativum **	Give good yield of volatile oils and could be exploited commerically as a source of volatile oils in Kenya. Also used as spices.
Foeniculum vulgare	
Carum carvi	
Pimpinella anisum	
Anethum graveolens	
Clausena anisata *	Leaves (1-2%) - investigation not completed.
Citrus spp. **	Potential source of essential oils of economic value.
Osyris abyssinica *	Wood (4%)-may be used as a substitute for Sandalwood oil
Pelargonium spp *	Herb (0.1%)-oil of commerical value.

* Indigenous plants

** Introduced plants

The antimicrobial and insecticidal properties if found significant would lead to the utilization of essential oils as insecticides and fungicides in agriculture. Since the odour of these oils repelspest species, the plants could be intercropped with crop plants [100]. The finding that wild and domestic animals prefer to feed on plants containing essential oils with least antimicrobial properties (sesquiterpenes) while rejecting feed containing oils with strong antimicrobial properties (oxygenated monoterpenes) suggests that the volatile oils could be used to advantage in forest and wildlife management [101, 102].

AIM OF THE PRESENT WORK

During the general screening of the Kenyan plants for their essential oils, some plants in the genus Lippia were found to contain essential oils. The aim of the present work was therefore:-

(a) To determine the essential oil content and chemical composition of all indigenous Lippia species in Kenya.

(b) To explore the possibility of occurence of chemical varieties of Lippia species based on essential oil composition and to note the effect of geographical factors such as seasonal variation and altitude on essential oil content and composition.

(c) To evaluate biological activities of the essential oils isolated.

(d) To determine the scope or extent of any possible economic potential of any of these species.

CHAPTER 2

LITERATURE SURVEY

2.1 Description of Lippia Species

The genus *Lippia* belongs to the family Verbenaceae which has about 75 genera and 3,000 species [103]. The name *Lippia* is dedicated to Agostino Lippia, an Italian naturalist who first described the genus. There are about 220 species of *Lippia* distributed mostly in tropical America and Africa [104].

Lippia species are shrubswith simple pubescent leaves and flowers in pedunculate crowded spikes with small calyx, 2 or 4 lobed and two-keeled. Their corolla is obscurely bilabiate with 4 rounded lobes, white or cream in colour. The stamens are four ircluded in the corolla tube. The fruits contain two hard mericarps, each one-seeded [105,106].

2.2. Uses of Lippia Species

Lippia species have been used in traditional medicine in several countries especially in South America. Both *Lippia alba* and *Lippia citriodora* are well known all over the world. *L. alba* is used in seasoning foods. Herbal

"tea " made from this plant is also taken for colds and also considered as a nervine and stomachic /107, 108/. L. citriodora which is indigenous to S. America is naturalized in all parts of the world. The lemon-scented leaves are used in herbal teas, for flavouring beverages, desserts, fruit salad and jellies. Decoction of leaves and flowers is given as a febrifuge, sedative and antiflatulence. Its oil imparts a refreshing odour to toilet waters and perfumes and is also used for scenting bath salts. The oil blends with various perfumes and can be used for flavouring liqueurs and non-alcoholic beverages/107, 109/.

L. berlandieri Schauer is mostly found in Mexico and a preparation from its leaves is traditionally used to flavour food, as a stimulant and to control menstruation /109,110/. The dried leaves of L. dulcis Tev . which is found in Tropical America are used for their expectorant and demulcent effect in traditional cough preparations. L. germinata HBK is spread from Mexico through South America and in West Indies. It is locally used for its relaxant, sudorific and antispasmodic effect. It is also used to control menstruation and treat stomach problems. L. graveolens HBK which is found in Mexico and Guatemala is used to flavour food, as a tonic and also as an expectorant. L. linguistrina (Lag.) Britt. is found in U.S.A and Mexico. In Mexico its leaves

are used as relaxant, to control menstruation and to treat bladder complaints. The essential oil from the plant is used to make perfumes in Southern Europe /109,110/. L. lyciodes Steud. is found in both South and North America. The infusion of the flowers is locally used to treat catarrh and colds. L. pseudo-thea Schau. found in South America is used to make tea in parts of Brazil while leaves of L. saberrima Sond. which is found in South Africa are used for their haemostatic effect. L. umbellata Cav (L. chiapensis, L. Pringlei, L.substrigosa) is used traditionally to treat colic. It is indigenous to Mexico /109, 110/. In Guyana Lippia micromera is used as culinary herb /111/. L. oatensii Rolfe found in South Africa and Zimbabwe is used as a mosquito repellent while L. rehmanii HHW Pears also found in the same place has been used as diuretic /112/. L. helleri is locally used as condiment and for treatment of colds. Its essential oil is suitable for shaving and hair lotions, soaps and possibly in candies and liqueurs. The aroma from this oil resembles that of origanum and marjoram oils / 112/.

2.3. Pharmacology of Lippia species

A few Lippia species have been investigated pharmacologically. For example, the pharmacological effects of several fractions (hydrolate, pseudohydrolate, monoterpenes, thymol (23),carvacrol (24) and sesquiterpenes) obtained from essential oils of leaves of L. grata were compared with those produced

by commerical thymol and carvacrol, the main constituents of this plant [113]. The most characteristic effects seen due in part to thymol and carvacrol content of the essential oil were: ataxia, decreased spontaneous activity and somnolence in mice, a contraction of the toad rectus abdominis muscle, and increase in the amplitude of muscle contraction and contracture in the indirectly stimulated toad gastrocnemius-sciatic and rat phrenic-nerve preparations, an increase in amplitude and a decrease in frequency of toad heart, and a smooth muscle relaxant effect on the rabbit uterus and rat uterus. While monoterpenes presented a depressant effect on isolated toad heart, sesquiterpenes had no effect. Both of these fractions were ineffective on the rat phrenic-nerve-diaphragm preparation indirectly stimulated. These fractions also produced a spasmolytic effect on rabbit duodenum.

From root essential oil of L. triphylla, verbenone (38) has been isolated. This compound possesses cardiotonic and respiratory stimulant properties [114]. In rats, administration of essential oil from L. triphylla leaves 10mg/kg) resulted in decreased conditioned avoidance response. Dopamine levels in the encephalon and corpus striatum were found to increase while the telencephalon cortex levels were not altered. Noradrenaline, serotonin

and 5-hydroxy indoleacetic acid levels were also altered [115]. It was concluded that the learning impairment caused by the oil resulted from altered neurotransmitters metabolism. The essential oil of L. citriodora has also been shown to have intestinal spasmolytic action in guinea pigs [116].

Verbenone (38)

2.4. ESSENTIAL OIL COMPOSITION OF VARIOUS LIPPIA SPECIES

The chemical composition of essential oils of various Lippia species has been reported by several workers. The following is the reported composition of essential oils from Lippia species world-wide.

Essential oils of Lippia species found in other parts of the world

1. Lippia affinis aristata Schau

The essential oil (0.4%) from leaves collected in Brazil had 15 identified compounds. The main ingredients included β-caryophyllene (39) (32.5%), γ-cadinene (40) (15.8%), γ-elemene (41) (12.4%), sabinene (22) (11.0%) and limonene (42) (6.3%) [15].

2. L. affinis sidoides Cham

Essential oil (4.0%) isolated from leaves of this plant in Brazil has been shown to contain 12 compounds. Thymol (23) (43.5%), α-phellandrene(43) (22.4%), β - caryophyllene (39) (9.7%) and p-cymene (34) (8.6%) were the major constituents [15].

3. L. alba (Miller) N.E. Brown

The essential oil of L. alba has been extensively studied and its chemical composition from different locations of Argentina reported [117 - 123]. While dihydrocarvone (4), citral (29), 1,8-cinole (45), piperitone (45), α-pinene (16) and limonene (42) were found to be the major compounds in different samples, some of the essential oil samples lacked these components. Analysis of essential oil of L. alba from Tucuman province Argentina has revealed that piperitone (45) (36.7%) and limonene (42) (34.2%) were the major constituents from expressed leaves while compound (45) (85.0%) and limonene (5.2%) were found in steam distilled leaves [124]. This suggested that some changes may have occurred during steam distillation. The essential oil (0.1%) obtained from L. alba leaves in Brazil contained 14 compounds of which β- caryophyllene (39) (24.3%), geranial (46) (12.9%) and 2 - undecanoate (47) (9%) were the main components [15].

4. L. alnifolia Schau

Of the compounds identified in the essential oil (0.1%) of L. alnifolia from Brazil, β- caryophyllene (39) (37.4%), carvacrol (24) (27.3%) and p-cymene (34) (13.7%) were the main constituents [15].

5. L. americana

The essential oil from this Lippia species has been shown to contain cadin-4-ene-1-ol (48) [125].

6. L. aristata Schau

The essential oil of this plant has been shown to have 11 components. The major compounds included β- caryrophyllene (39) (23.3%) sabinene (22) (21.1%) limonene (42)(16.8%) γ-elemene (41) (12.4%) and γ-cadinene (40) (8.4%) [15].

7. L. citriodora H.B. & K

The essential oil of L. citriodora (True verbena oil) (0.1-0.7%) was reported to contain citral (29) (26.39%) and 1,8-cineole (4) (4%) [107]. Other components were methylheptanone (49) carvone (50) linalool (30), α-terpineol (14), boneol (15), nerol (6), nerolidol (31), geraniol (5), citronellol (51) cedrol (52) and β- caryophyllene (39). Assis et al [126] have also reported that the essential oil of L. citriodora from Brazil contains α-pinene (16), limonene (42), 1,8-cineole (4), linalool (30), citral (29), and geraniol (5).

8. L. dulcis

The essential oil of this plant contained about 86% mono- and 13% sesquiterpenes. Camphor (2) was the major constituent. A sweet principle, hernandulcin (53) which

was previously reported as the sweet principle of L. dulcis was not detected, 6- Methyl-5-hepten-2-one (49) was detected as decomposition product of compound (53) [127].

9. L. fissicalyx

The essential oil of L. fissicalyx has been shown to have limonene (42), dihydrolippione (54), menthone (55), pulegone (56) and α- and β-pinene (57), camphene (58), 1,8-cineole (4) and p-cymene (34) [128]. However it has also been reported [129] that this essential oil contains α-pinene (16), camphene, β-pinene, limonene (30%), 1,8-cineole, linalool (33), menthone isomenthone (59), camphor (2), pulegone, piperitone (45), carvone (50) lippione (60) and dihydrolippione.

10. L. germinata

From leaves of L. germinata in India, the essential oil (0.5%) contained 50% lippione (64) [130, 131].

11. L. grata Schau

Craveiro et al [15] have reported that the essential oil of this plant contains 16 compounds of which carvacrol (24) (20%), p-cymene (34) (22.2%) thymol (23) (18.8%) and

γ-terpinene (61) (14.4%) are the major constituents. The same essential oil has been reported [113] to contain carvacrol (24) and thymol (23) as the main constituents.

12. L. grisebachiana

The essential oil content of this plant was 1.7%. Out of 23 compounds identified in this essential oil, linalyl acetate (62) was the major compound (23.4%) [132].

13. L. helleri Britton

The essential oil (0.4 - 0.5%) of L. helleri has been reported to contain carvacrol (24) and thymol (23) [131, 133].

14. L. integrifolia

The essential oil from this plant has been shown to contain α-pinene (16), limonene (42), 1,8-cineole (4), and camphor (2) [134]. From the same oil presence of some sesquiterpenes such as α-humulene (63) β-caryophyllene (39), spathulenol (64), bicyclohumulendione (65) and africanone (66) has been demonstrated [135].

15. L. junelliana

The essential oil (2.3%) from this plant was reported to contain phenols (6%), α-pinene (16), phellandrene (43) and borneol (15) [136].

16. L. lycioides

The essential oil (0.4%) of this plant was analysed in 1950 [137] and 1,8-cineole (4) and limonene (42) identified. Later 1,8-cineole (3%), cedryl acetate (67) (9.69%) α,β-unsaturated ketones, limonene and sesquiterpene alcohol presence were reported [138].

17. L. origanoides

The essential oil obtained from L. origanoides from the Amazon region of Brazil contained thymol (23) (20.6%), p-cymene (34) (8.5%) and γ-terpinene (61) (22.4%) [139].

18. L. polystachya Griseb

Essential oil from this plant (1.75%) has thujone (68) (35%) as the major compound while other constituents such as sabinene (22), limonene (42) and α-pinene (16) appear in lower amounts [131, 140].

19. L. seriphioides Gay and L. trifida Gay

The essential oil from these two species contained thymol (23) and carvacrol (24) as the main components [131].

20. L. triphylla

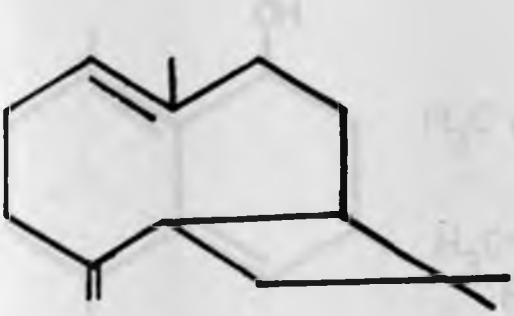
GLC analysis of the leaf oil showed the presence of 68 compounds, a number of which were identified. Geranial (46) and neral (69) were in the greatest amounts which together accounted for 38% in leaf oil. The twig oil showed the same composition as the leaf oil, but compounds were present in smaller amounts and some only in traces. Limonene and neral accounted for 45% of this oil. A number of compounds were absent from root oil [114].

21. L. wrightii

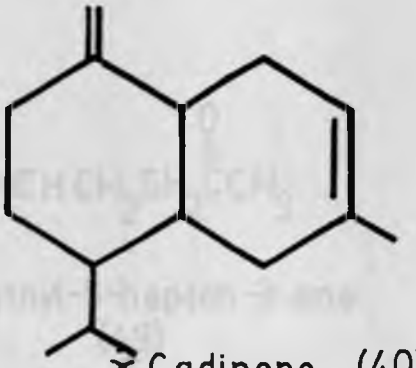
The essential oil from this plant contains about 80% hydrocarbon terpenes [14].

2.5 NON-VOLATILE CONSTITUENTS OF LIPPIA SPECIES

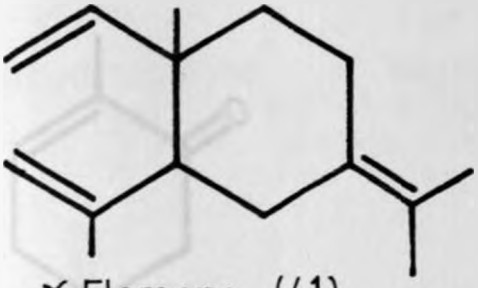
Research work on the non-volatile components of some Lippia species has been reported [125, 142, 143, 144]. The non-volatile portion of these Lippia species has been shown to contain mostly common amino acids, fatty acids, long chain hydrocarbons and their alcohols. The common ubiquitous sterols such as β-sitosterol (70) and stigmasterol (71) have also been found.



β-Caryophyllene (39)



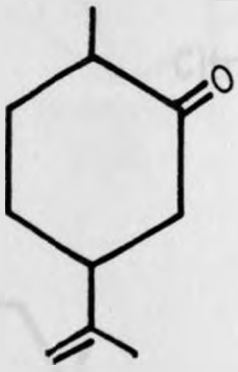
δ-Cadinene (40)



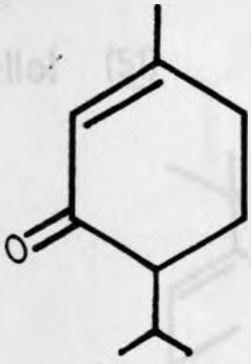
γ-Elemene (41)

Limonene (42)

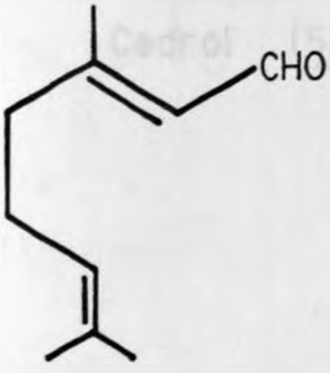
α-Phellandrene (43)



Dihydrocarvone (44)



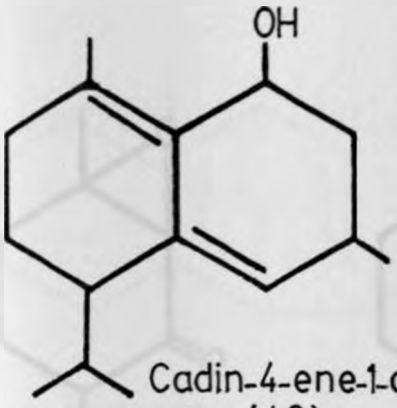
Piperitone (45)



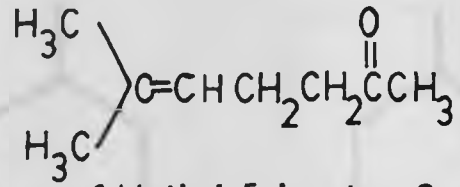
Geranial (46)

$CH_3(CH_2)_8COOH$

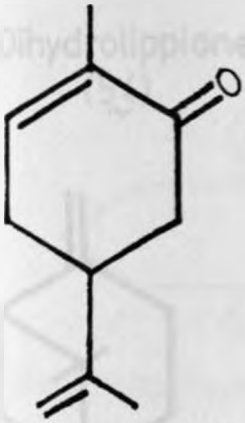
2-Undecanoate (47)



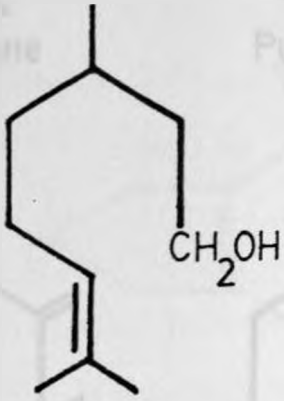
Cadin-4-ene-1-ol
(48)



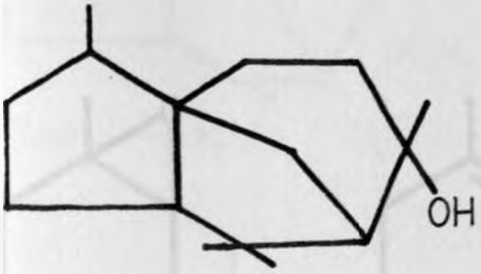
6-Methyl-5-hepten-2-one
(49)



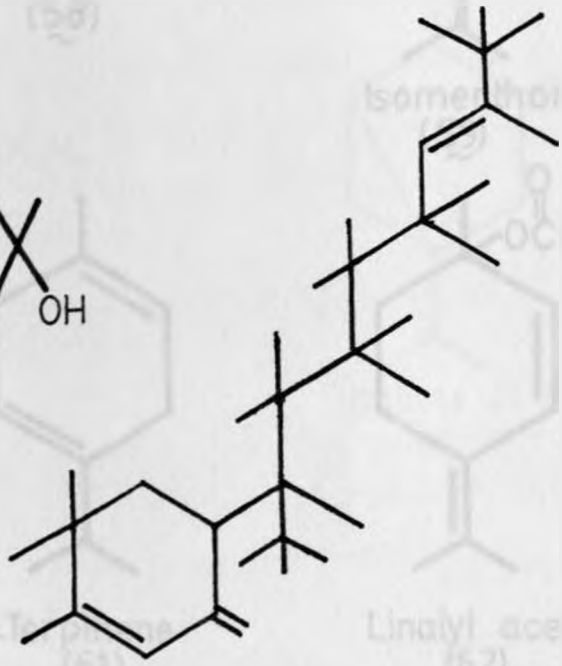
Carvone (50)



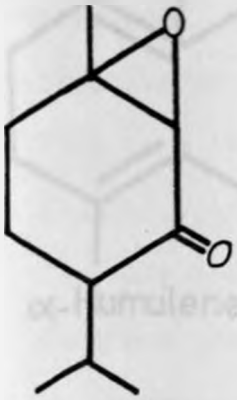
Citronellol (51)



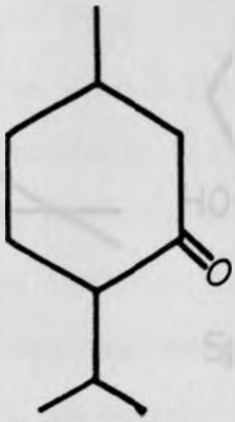
Cedrol (52)



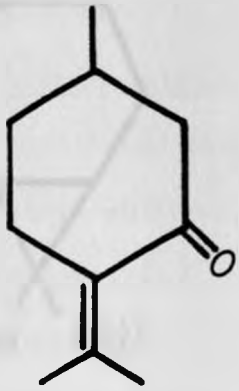
Hernandulcin (53)



Dihydrolippione
(54)



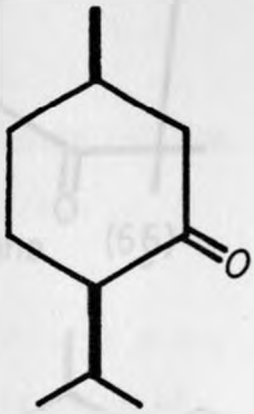
Menthone
(55)



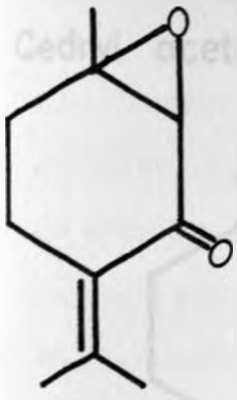
Pulegone
(56)

β-Pinene
(57)

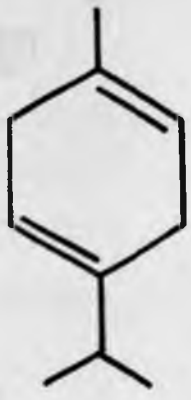
Camphene
(58)



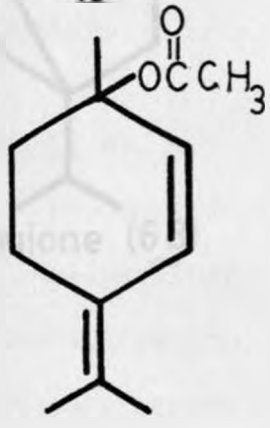
Isomenthone
(59)



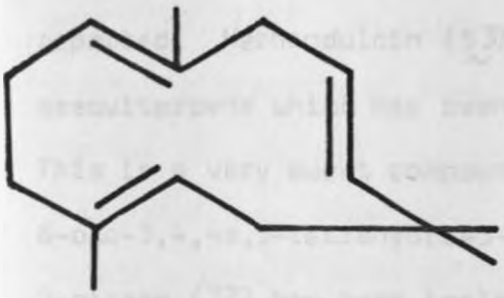
Lippione
(60)



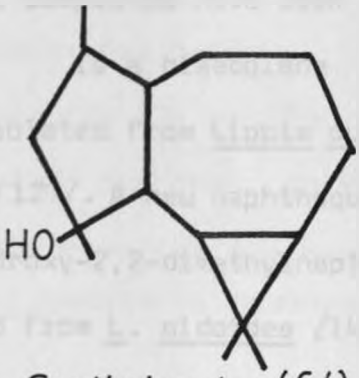
γ-Terpinene
(61)



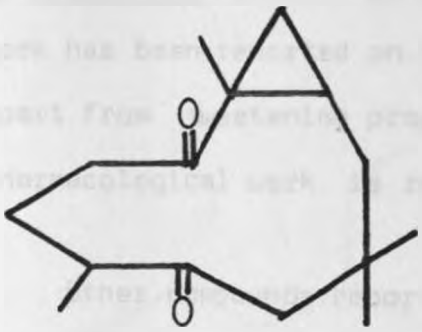
Linalyl acetate
(62)



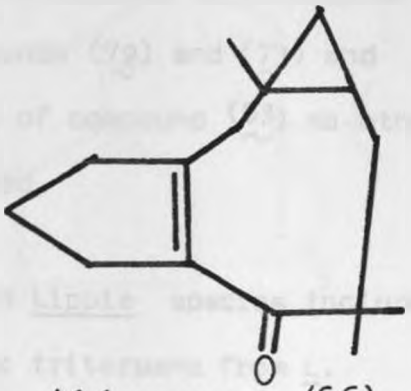
α-Humulene (63)



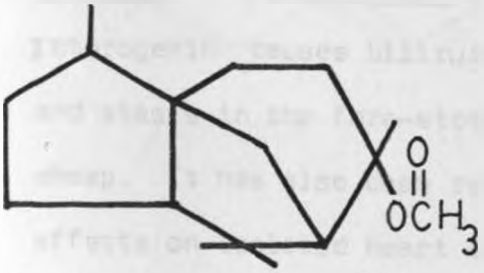
Spathulenol (64)



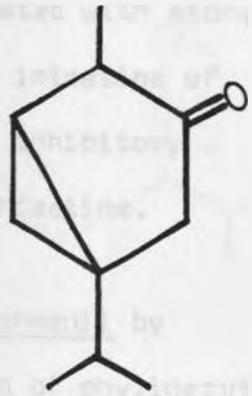
Bicyclohumulendione (65)



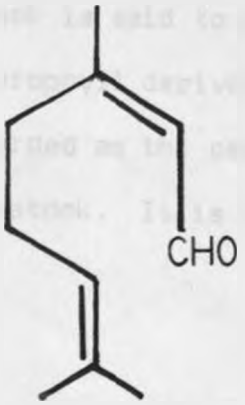
Africanone (66)



Cedryl acetate (67)



Thujone (68)



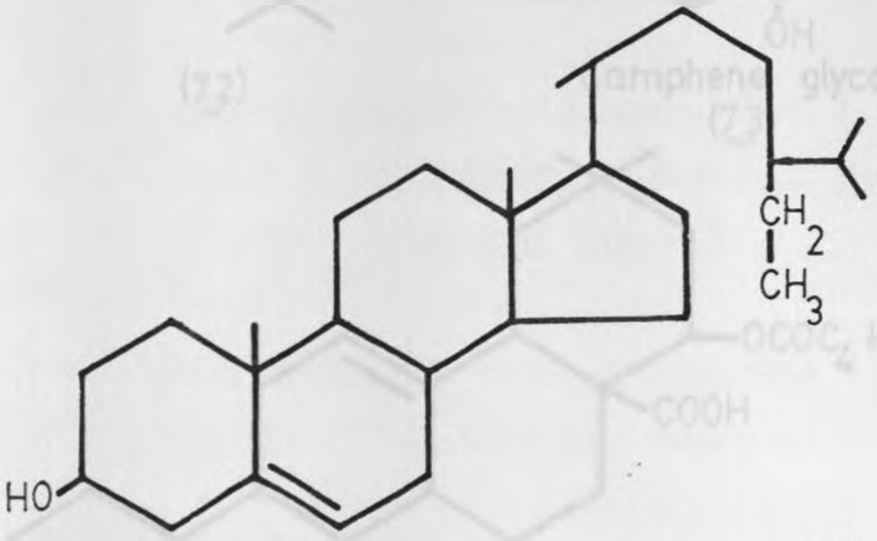
Neral (69)

However in a few cases new compounds have been reported. Hernandulcin (53) is a bisabolane sesquiterpene which has been isolated from Lippia dulcis. This is a very sweet compound /127/. A new naphthoquinone, 6-oxo-3,4,4a,5-tetrahydro-3-hydroxy-2,2-dimethylnaptho-1, 2-pirane (72) has been isolated from L. sidoides /144/. Camphene glycol (73) has also been isolated from L. ukambensis /143/. No pharmacological or biological work has been reported on compounds (72) and (73) and apart from sweetening property of compound (53) no other pharmacological work is reported.

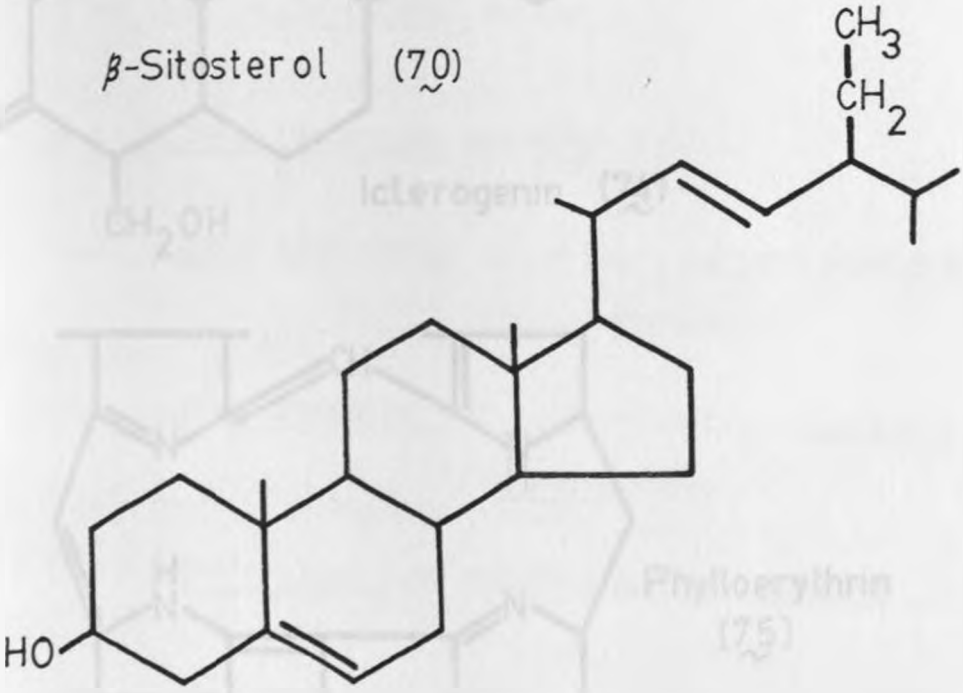
Other compounds reported in Lippia species include icterogenin (74) - a pentacyclic triterpene from L. javanica and L.rehmanii /112/.
Icterogenin causes bilirubinaemia associated with atony and stasis in the fore-stomachs and large intestine of sheep. It has also been reported to have inhibitory effects on isolated heart and mammalian intestine.

Consumption of L. javanica and L. rehmanii by livestock is said to induce the production of phylloerythrin(75) (a chlorophyll derivative) in blood /112/. Phylloerythrin is regarded as the causative agent for photosensitization in livestock. It is interesting to note that more recent

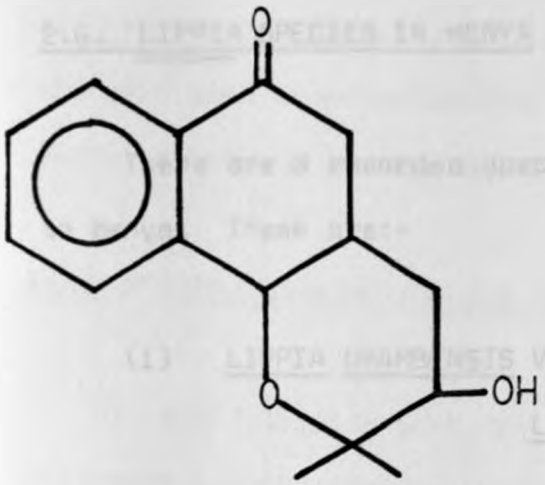
work [142, 145] on Lippia species has neither shown the presence of these compounds (74 and 75) nor has any photosensitization been reported. This therefore shows that more research work on the non-volatile constituents of Lippia species is necessary.



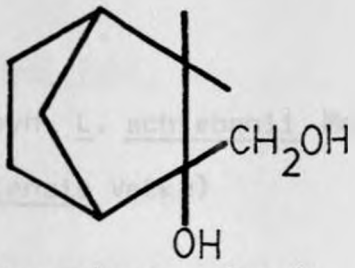
β-Sitosterol (70)



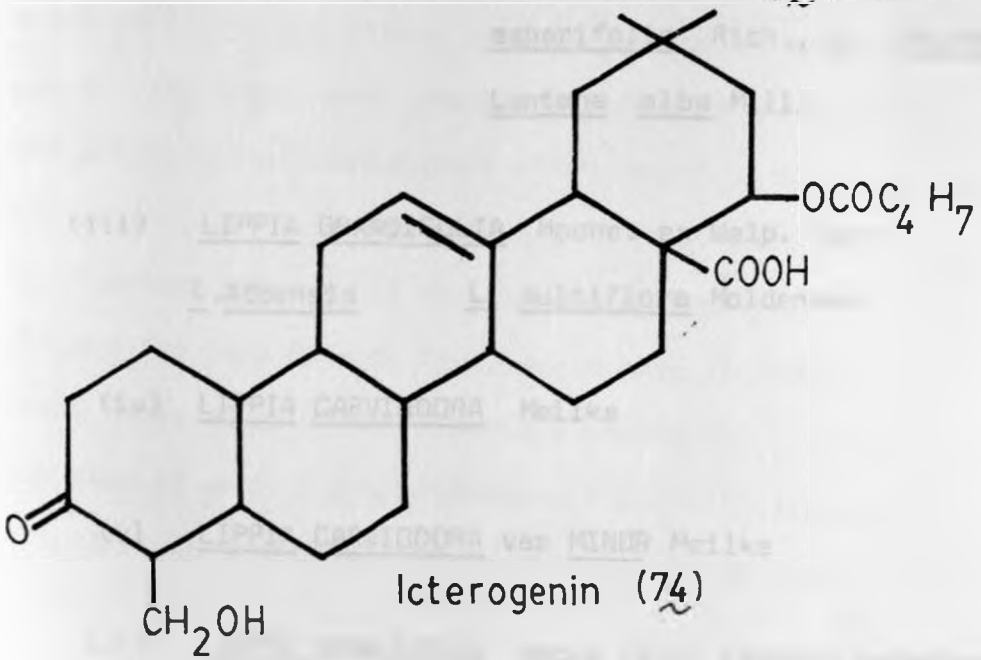
Stigmasterol (71)



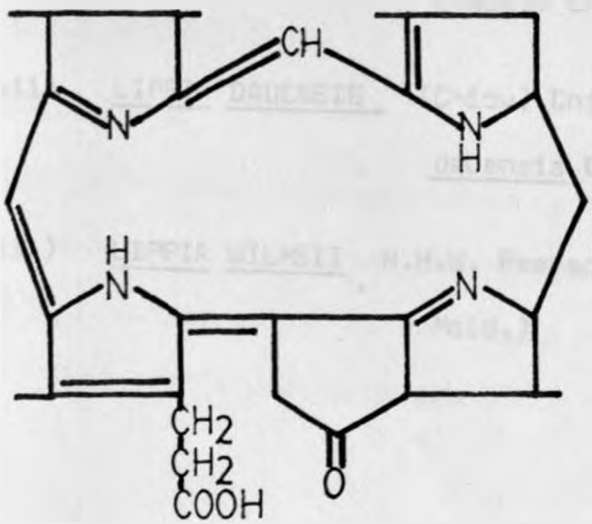
(72)



Camphene glycol
(73)



Icterogenin (74)



Phylloerythrin
(75)

2.6. LIPPIA SPECIES IN KENYA

There are 8 recorded species of Lippia indigenous to Kenya. These are:-

(i) LIPPIA UKAMBENSIS Vatke (syn. L. schiebenii Mold. L. kituiensis Vatke)

(ii) LIPPIA JAVANICA (Burn f.) Spreng. (syn. L. asperifolia Rich., L. Whytei Mold. Lantana alba Mill).

(iii) LIPPIA GRANDIFOLIA Hochst ex Walp. (syn. L.Adoensis L. multiflora Moldenken)

(iv) LIPPIA CARVIODORA Meilke

(v) LIPPIA CARVIODORA var MINOR Meilke

(vi) LIPPIA SOMALENSIS Vatke (syn. Lantana somalensis (Valke) Engler.

(vii) LIPPIA DAUENSIS (Chiov) Chiov (syn. Lantana dauensis Chiov)

(viii) LIPPIA WILMSII H.H.W. Pearson (L. africana Mold.)

NB Lippia citriodora Kunth is an introduced plant and only used as an ornamental flower due to its characteristic fragrance especially at night.

DESCRIPTION, DISTRIBUTION AND TRADITIONAL USES

In the following text, it will be noted that different Lippia species sometimes bear similar local names. Generally, local names or common names in most cases do not differentiate between species in the same genus. The local names are associated with the use of the plant, certain properties of the plant such as bitterness, sweetness, production of latex etc. It is therefore not unusual for plants belonging to different genera or even families to bear the same local name. This can be a serious problem if plants are studied without proper taxonomic identification. Medicinal uses can also become problematic in this regard.

(i) LIPPIA UKAMBENSIS

Description

This is an erect pubescent shrub up to 3 m with opposite subsessile lanceolate oblong leaves and 2 (uncommonly 4) long pedunculate spikes at each upper node. The bracts are acuminate, the lowest enlarged while the upper ones

are lanceolate and spreading in fruit [105, 146, 147] (Plate 1).

Distribution

This is one of the most widely distributed Lippia species in Kenya. In Machakos, the plant is found about 6 km north of Nunguni at the Western side of mountain slopes of Kithembe hill. It is also found near Kitui town and Migwani in Kitui district. L. ukambensis is abundant on the Nyeri-Kiganjo and Nyeri-Mweiga roads. In Nairobi, the plant is common on the slopes of Ngong hills, Nairobi National Park, Karura forest and at Muguga area. The plant is also found along Kajiado-Namanga road. In Meru, L. ukambensis is found near Kangeta School, Meru museum compound, North-east Nyambene hills and on the Meru-Nanyuki road around the Isiolo road junction. The plant is also found in Embu around the eastern side of Rubingazi river. L. ukambensis is abundant in Nakuru National Park, slopes of Menengai crater and along Nakuru-Njoro road, Kendong range, West Longonot and Ol Doinyo. The plant is found in many other places in Kenya such as Kirinyaga district, Thika and Marsabit near the Forest station [105, 148].

L. ukambensis is reported in Zambia, Malawi, Tanzania, Mozambique and Zimbabwe [148].

Traditional Uses

Lippia ukambensis is known as Muthiriti by Meru and Kikuyu [148].

The aroma from the boiled leaves of this plant is inhaled as a remedy for colds and fever [148].

(ii) LIPPIA JAVANICA

Description

This is a much branched shrub reaching a height of 4 m. Stems are woody and clothed with short whitish stiff hairs. The leaves have a velvety texture. They are opposite or alternate shortly petioled, oblong in shape, crenate margin and rugose (4cm - 5cm long). The leaves are also very pubescent beneath and veins are raised. Heads are from the axis of many of the leaves and they are usually not more than 4 - 6 to a node. These heads (1cm diameter) which are at first globose and finally oblong do not form terminal corymbs above the leaves. The bracts are broad, ovate in shape, cuspidate apex and very hairy. The outer bracts are 2mm broad. The corolla are scarcely longer than the bracts, the limb being cream - white. The

fruits are very small and light-brown in colour [105, 106, 146-149] (Plate 2).

Distribution

This is the other Lippia species which is widely spread. In Narok district L. javanica is found in Masai Mara game reserve, Keekorok plains, Loita hills, Marandana hills S. of Morijo Ol Kiloriti in grassland and 16 km North of Narok in Mau escarpment. L. javanica is a very common feature in Narok district. The plant is also common in Nairobi area at Kasarani, Nairobi dam, Langata road, Nairobi-Kangundo road, Muguga forest North of Karura forest, Kabete campus and at Muthangari near the police station. L. javanica has also been noticed at Songor in Kisumu and at Keumbu in Kisii. The plant is found in Nakuru town and the surrounding areas, on Naivasha-Nairobi road, at Kendong valley near Naivasha and a number of areas in Kericho and Kitale districts. L.javanica is also common in many other places such as Sagana near the railway line, areas around Machakos town, Mutituni, in Mbiuni Location and Chyulu hills in Machakos district. The plant is also found in Uaso-Nyiro area and Baringo district and on Mt. Elgon slopes [105, 148].

L. javanica is found in other African countries such as Tanzania, Uganda, Ethiopia, S. Africa, Malawi, Mozambique and Zimbabwe [148].

Traditional Uses

L. javanica has several local names. It is called Osononi or Olsinoni (Masai), Mogandu, Kyulu, Muthiiti (Kamba), Onyinkwa (Kisii), Mpambake (Kiswahili), Mathiriti (Kikuyu). It is also known as Chemosoriot in Nandi and Mende or Ang'we-rao (Luo). The Kipsigis generally call it Mukyot or Muokiot (singular) Mukiniko (plural) and is also known as Sulasula in Kabras and Kakamega areas [148, 149, 150, 151].

The twigs and leaves are used for brooms as they leave a delicious scent. They are especially used to scent a new habitat. The plant is also used for closing up holes in huts before putting mud on top of "Manyattas" (Masai houses). The Masai also stew up leaves to make a brew for fever and the odour of crushed leaves when inhaled clear the nose in case of headcolds. The plant has also been used to treat certain types of insanity (not specified) [148].

L. javanica leaves are boiled either with milk or water for coughs, colds and bronchial troubles in general. Smoke from the burning plant is sometimes inhaled for respiratory diseases. The leaves are variously described as having the odour of vanilla or mint and has been used as a tea substitute /112, 152/. It has also been reported that an infusion of the leaf is drunk for "gangrenous rectitis". The plant is also traditionally used to treat measles, urticaria, other rashes and as a remedy for malaria and dysentry /112/.

An infusion of the leaves is given to patients with fever. In treatment of malaria a decoction of the boiled leaves is taken and the whole body bathed with the same fluid. Pounded leaves are also applied on cut wounds, or soaked in water and the juice drank for treatment of tapeworms and indigestion /151/.

(iii) LIPPIA GRANDIFOLIA

Description

This is an erect undershrub reaching 3 m with short, pubescent branchlets. The leaves are 2 - 4 or sometimes more in a whorl. These leaves are sub-sessile or sessile, oblong or oblanceolate-oblong in shape, subcoriaceous,

obsecurely crenate, but little rugose, rather scabrous on the upper surface, shiny pubescent beneath. The lower leaves on the stem sometimes reach 10 - 12.5cm long. The inflorescence is very variable, the very numerous heads sometimes forming a dense terminal panicle, the upper internodes sometimes long with several heads from each node on short peduncles. The heads are permanently globose, about 0.5cm diameter. The bracts are hairy ovate in shape with a cuspidate apex, the outer bracts being 3mm broad. The calyx are villous 2mm long. The corolla whitish and not longer than the bract /105, 106/ (Plate 3).

Distribution

L. grandifolia is reported or observed in only a few areas. It is reported to be at Kakamega Forest, Ngoina Forest in Sotik, Mt. Elgon and Cherangani forest station as a roadside weed in Acacia savanna. A small quantity has been observed at Bandasa near maize plantations around Marsabit forest /148/,

Traditional Uses

Lippia grandifolia is known as Bawaptarit by the Nandi, Puriamauwa by the people of Elgeyo Marakwet and as Gambia tea bush in West Africa. The plant is used as a tea

substitute which is much appreciated by people of West Africa [41, 109, 110]. The plant is claimed to be sudorific, a febrifuge and a laxative; it is also taken as an after-birth beverage [153]. In west Africa beehives are smoked with this fragrant plant before being placed in trees to enhance settling of bees [41].

Recently, the decoction of the processed leaves taken in the form of tea and claimed to control high blood pressure and to induce tranquilizing effect in man has been investigated [154]. Pharmacological investigations of the aqueous extract of the leaf have demonstrated a muscle relaxant property bordering on tranquillizing action and an antihypertensive effect in both man and animals.

(iv) LIPPIA CARVIODORA

Description

This is a shrub up to 1 m in height but mostly 0.5m with wiry stems and small aromatic leaves. The flower heads are small with corolla on short pedicels. The bracts are pale-green and enlarged greatly in fruit [147,148] (Plate 4).

Distribution

This Lippia species is found in the Samburu-Isiolo game reserve and at Archers post in Buffalo Springs. In this area, the plant is found in dry scrub in Commiphora species, Acacia species and Grewia species bushland on gravelly soil with some common distinctive shrubs being Dirichleta glaucences, Sericocomopsis pallida, Caucanthus albidus, Combretum aculeatum and Turrea parviflora. L. carviodora is found at Moile Hill 11km off Laisamis (on Isiolo-Marsabit road) at a round base of hill with sandy gravelly soil with granite rocks. The plant is also reported at Mt. Kulal (Marsabit) and has also been observed on Turbi-Forole road (Marsabit) in dense bush.

The presence of L. carviodora has been reported in several places. Collections have been reported from Lorin plateau 36^{0} 23'E, 2^{0} 20'N at a lava boulder-strewn hillside, 10 km South of road junction to Kakuma on Lokitaung-Lodwarroad by river Lomunyenakwam (3^{0} 32'N, 35^{0}23E) in sandy pocket amongst lava boulders on steep slope and at Oropoi river 3^{0} 50'N, 34^{0} 20' E under riverside woodland or Lawsonia, Maytenus, Terminalia and Acacia species. Some L. carviodora has also been reported at Meru National Park in Acacia-Commiphora

bushland on red soil 3 km South of Rujewero river ($0^{o}8'N$, 38^{o} 15'E).

Traditional Uses

Lippia carviodora is referred to as Uuru, Eomorsin or Esrilipong by the Turkana [148,155], Urgo or Lominyani by the Samburu [148]. The plant is mixed with ghee to preserve it. The leaves of the plant are used as a tea substitute by Turkana and Samburu. The leaves smell of sage and an infusion is said to produce a stimulating beverage. Some Samburu women are said to like L. carviodora leaves in their tea so much that they cannot take tea without these leaves [148]. Roots are used for medicinal purposes (not specified) by Turkana [148].

(v) LIPPIA CARVIODORA VAR MINOR

Description

The shrub is up to 1 m. The stems are also wiry with the same features as L. carviodora but the bracts never enlarge greatly in fruit [148]. (Plate 5).

Distribution

There are very few reports on the distribution of this plant in Kenya. The plant is reported at Galana Ranch (Tsavo East National Park) on plains and foot of hills. It is also reported to be present at Manda hill (Tsavo West National Park) /148/. However a very high concentration of this plant especially during rains has been observed along Tsavo-Ngulia Road /148/.

Local Uses

No local names or traditional uses were found for L. carviodora var minor in the literature.

(vi) LIPPIA SOMALENSIS

Description

This is a much branched undershrub 1-2m high, with very scabrous slender woody branchlets. The leaves are small in pairs, shortly petiolated obovate orbicular in shape, obtuse apex and very rigid. These leaves which are under 2.5cm long have crenate margin, scabrous and rugose above, pubescent with much-raised veins beneath. The head is globose in shape (1 cm long), 2 - 4 from the upper nodes on long stiff ascending peduncles. The bracts are very closely imbricated pubescent, orbicular, with a large cusp. The outer bracts are broad. The

corolla is milk-white and not larger than the bract. The plant may be confused with L. ukambensis /105, 106/ (Plate 6).

Distribution

This plant is reported to be present in several places at Mt. Kulal and Mt. Marsabit both in Marsabit District. The plant is also found at Ndoto mountains (37^{o}E, 1^{o} 15'N) in Samburu district /148/.

Traditional Uses

The local names and traditional uses of this plant were not available.

(vii) LIPPIA DAUENSIS

Description

This is a much branched erect herb/shrub up to 1 m in height. The leaves are pinnatifid, shortly petiolated and the heads are on long stiff ascending peduncles. The flowers are small and whitish in colour /148/. (Plate 7).

Distribution

L. dauensis is reportedly found near a waterhole at Lulis which is 9 km from Banissa on Banissa-Malka Mari road (4^{o}1'N, 40^{o}20'E) in Mandera district.

In Marsabit district L. dauensis has been reported to be located about 20 km South of Maikona in soft brown soil which apparently churns when wet; the plant being found at the base edge of lava. The plant is also reported to be at Forole which is at the Kenya-Ethiopia border ($3^{0}41'N$, $38^{0}0'E$). L. dauensis is also found in Ethiopia and Somalia /148/.

Local Uses

The plant is known as Urgo by the Boran. They use the plant for scenting cooking fat. For this purpose, the fat is boiled together with leaves, strained and cooled /148/.

(viii) LIPPIA WILMSII

Description

This is a perennial herb from a woody rootstalk. The plant creeps along the ground as single shoots in Acacia glassland on the slopes of volcanic hills. The leaves are elliptical to ovate, pubescent but not scabrid, margin dentate, paired spikes at each node with spreading ovate bracts /148/ (Plate 8).

Distribution

This plant is reported to be located at Menengai Crater (Nakuru) on the hillside and in Kilunguni location (Machakos district) 5 km off Nunguni on

mountain slopes in hard stony soil in Acacia woodland. It is also reported in a few other places such as Thika, Kapenguria and Dunduri near Nakuru. L. wilmsii is also found in Zambia, Tanzania, Malawi, Zimbabwe, South Africa, Somalia, Uganda and Zaire [148].

Local Uses

The ripe fruits of this plant are eaten by children.

2.7. PREVIOUS WORK ON KENYAN LIPPIA SPECIES

The only reported analysis of essential oil from a Lippia species collected from Kenya was in 1950 [156] on Lippia carviodora. The rest of the work has been reported on Lippia species collected from other countries, but also occurring in Kenya.

LIPPIA UKAMBENSIS

The essential oil of L. ukambensis has been reported on only once [143]. The analysis of the pale yellow oil obtained from Tanzanian plants (leaves) (0.3%) contained α-pinene (16) (0.3%), camphene (58) (4%), β-pinene (57) (2.1%), limonene (42) (2.2%), 1,8-cineole (4) (11.3%), 4-thujanol (sabinene hydrate) (76) (18.5%), camphor (2) (36.5%), α-terpineol (14) (2.3%) and β-cubenene (77) (6.5%). Five components were not identified.

LIPPIA JAVANICA

Guenther [45] mentioned that the essential oil of L. asperifolia (=L. javanica) contained 80% myrcenone (82) and ocimenone collectively called tagetonones. The individual contribution of each compound was neither reported nor was the type of isomer of ocimenone, cis-ocimenone (79), or trans-ocimenone (80) mentioned.

Leaves and branches of L. asperifolia from Angola have also been studied [157]. The essential oil (1.02%) contained ketones 60.45%, 1, 8-cineole (4) and limonene (42). The nature of the ketones was not reported. The flowering tops were reported to yield 0.4% essential oil rich in ocimene (81) [112]. It also contains p-cymene (34) linalool (30) and caryophyllene (39) [142].

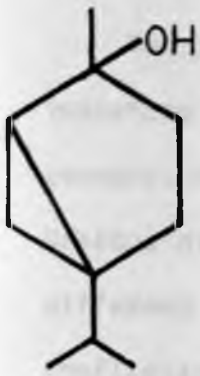
LIPPIA GRANDIFOLIA

The essential oil of this plant has been investigated by several workers under different names (L. adoensis, L. multiflora) in other parts of the world. From leaves, stalks and flowers of L. adoensis collected in Eritrea (Ethiopia) the essential oil distilled (0.7%) contained carvone (50) (72%) and probably limonene (45) besides unidentified hydrocarbon terpenes [158]. It has also been reported that dried flowering tops of L. adoensis yielded an essential oil (1.4%) containing 34% to 43% camphor (2) while the leaves and stalks gave an oil with very little or no camphor [159, 160]. A fairly thick straw coloured bitter oil with a strong odour of camphor and 1,8-cineole (4) was isolated from L. adoensis grown in Senegal [161].

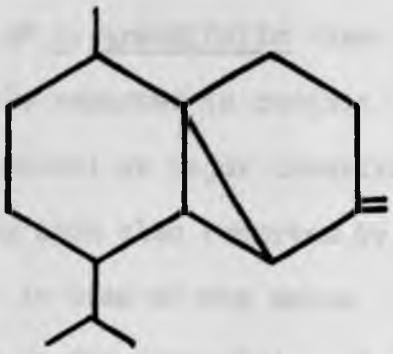
This essential oil contained 29.2% camphor (2) 3.5% borneol (15), α-pinene (16), camphene (58), 1,8-cineole and azulenogenic sesquiterpenes.

The essential oil of L. multiflora in Ghana has been reported to contain camphor (2) as the major component [41]. However Talalaj [162] reported that the essential oil from L. multiflora from Ghana (leaves 0.82%, flowering tops, 1.5% and flower heads, 2.05%) had camphor (2) content of 2.6-3.9%, the latter in leaves.

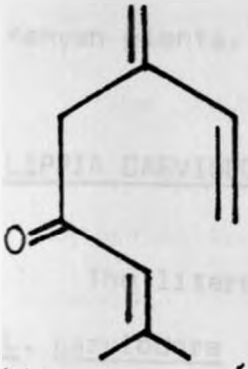
Rovest [163] has reported limonene (42), linalool (30) linalyl acetate (62), carvone (50) and sesquiterpenes in L. adoensis. More recent investigations [76] have shown that the essential oil of L. adoensis leaves from Nigeria contain linalool (30) (81.3%) as the major compound. Other minor component included 1,8-cineole (4) (3.32%) thymol (23) (1.41%), copaene (32) (1.36%, α-terpineol (14) (1.11%), unknown sesquiterpene hydrocarbon (5.66%), α-pinene (16) (0.22%), β-pinene (60) (0.79%) γ-terpinene (61) (0.62%), carvacrol (24) 0.38%) δ-cadinene (82) (0.9%), nerolidol (31) (0.61%) and a number of unknown compounds. The essential oil from the flowers had more or less similar composition, linalool (30) being 94.56%.



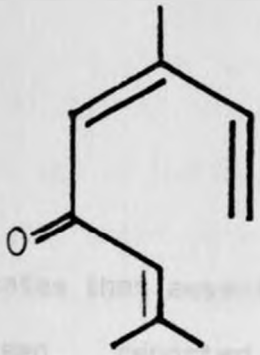
Sabinene hydrate (76)



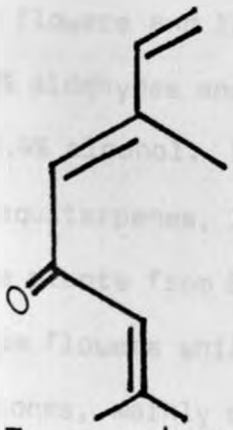
β-Cubenene (77)



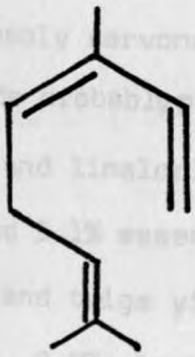
Myrcenone (78)



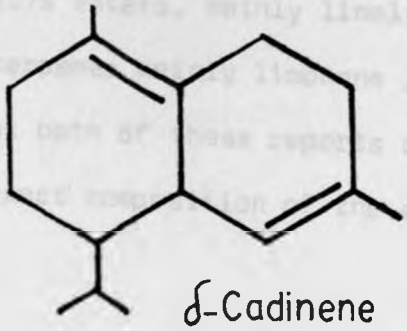
Cis-ocimenone (79)



Trans-ocimenone (80)



β-Ocimene (81)



δ-Cadinene (82)

The essential oil of L. grandifolia has therefore been previously reported to contain carvone, camphor and linalool as major constituents. Various other components were also reported by different researchers. In view of the above conflicting information on the composition of the essential oil of L. grandifolia, it was important to find out the composition of this essential oil from Kenyan plants.

LIPPIA CARVIODORA

The literature indicates that essential oil of L. carviodora has only been reported twice. The essential oil (1%) from Kenyan plants has been reported in flowers and leaves. The oil was reported to have 60% aldehydes and ketones probably carvone (50), 10.4% esters 17.4% alcohol. Other compounds probably present are sesquiterpenes, limonene (42) and linalool (30) [156]. The plants from Somalia yielded 3.1% essential oil from flowers while the leaves and twigs yielded 67.3% ketones, mainly d-carvone, 2.9% alcohols, mainly linalool, 0.7% esters, mainly linalyl acetate (62) and 29.1% monoterpenes mainly limonene [164]. In view of the fact that both of these reports were made in 1950s and the exact composition of the essential oil

from Lippia carviodora from Kenyan species was not mentioned, further work was necessary.

LIPPIA CARVIODORA VAR. MINOR

No scientific work has been reported on this plant.

LIPPIA SOMALENSIS

No report was found on this plant.

LIPPIA DAUENSIS

The leaves and flower tops of the plant from Somalia gave an essential oil of 0.23% which was citrus-yellow in colour with chief constituents as ketones. No individual constituent was identified [165].

LIPPIA WILMSII

No report was found on this plant.

2.8. PRESENT WORK

Lippia species in Kenya (the most common species being L. ukambensis and L. javanica) are considered as troublesome weeds especially in grazing areas [146]. These plants re-establish rapidly after cutting by means of sprouting and germinating of seeds. A number of herbicides have been tried on L. javanica with a lot of success (80-90% kill) when applied as sprays (2,4 - dichlorophenoxy acetic acid) [146,166].

Most farmers in Kenya also equate Lippia species with the much hated Lantana camara (Lantana or Tick-berry) which is an introduced spreading -thick-forming shrub with prickly stems, easily spread by birds through the seeds. Lantana camara is very difficult to control either by herbicides or by biological controls [146].

Some Lippia species such as L. citriodora and L.alba have been exploited commercially [104]. Many other Lippia species are reported to be used in traditional medicine, in herbal "teas" or in perfumery [109, 110].

Considering the above information and in view of the fact that no comprehensive work on Lippia species in Kenya had been reported, the present work was embarked on.

CHAPTER 3

MATERIALS AND METHODS

3.1 COLLECTION OF PLANT MATERIALS

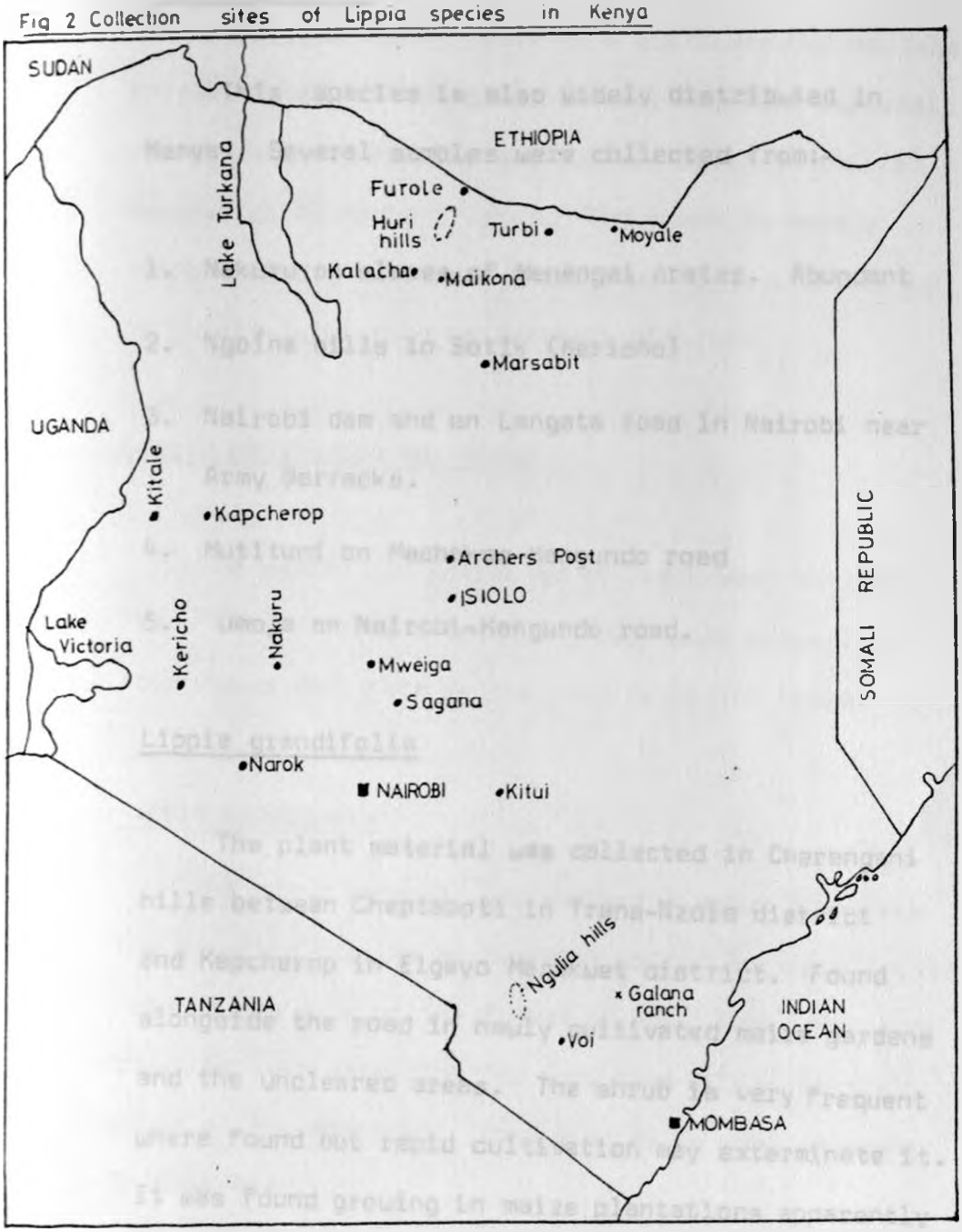
The plant materials were collected both from recorded locations (by the East African Herbarium, Nairobi) and unrecorded places. Depending on the availability of plant material several collections of the same species were made in order to investigate the possibility of a particular essential oil variations due to seasonal or geographical factors and occurence of intraspecific varieties. Leaves and flowering parts were collected. The collection sites are shown in Fig 2, while the morphological features are shown in Plates 1 - 8. Preliminary work had indicated that the twigs yielded only traces of oil.

Lippia ukambensis

Since this plant is widely distributed in Kenya, the plant material was collected from various locations in Kenya. The following are some of the collection sites while the actual collection dates are shown in Tables 4 and 6.

1. Mweiga: Between Mweiga town centre and Nyeri-Kiganjo road junction. Abundant on the roadside.
2. Narok: Between Mau-Narok and Narok town. On the road side at end of Dombeya species ecological zone.
3. Limuru: At Kinungi East and West road junction on Naivasha-Nairobi road. Mixed with Artemisia afra. Settled area.
4. Nairobi (a) Kabete University field station. Introduced into cultivation for experimental purposes.
   (b) At Nairobi dam and on Langata road near Army Barracks.
5. Kitui: About 2 km on road from Kitui town to Machakos town before road junction.
6. Kirinyaga: Between Sagana and Karatina. Also cultivated for experimental work.
7. Nakuru: (a) Nakuru National Park on the eastern side of Lake Nakuru after main gate.
   (b) Near Nakuru Golf club; abundant also on slopes of Menengai crater.
8. Marsabit: Near the District forest office towards the forest gate on road. Morphological features could be confused with those of L. somalensis.

Fig 2 Collection sites of Lippia species in Kenya



Lippia javanica

This species is also widely distributed in Kenya. Several samples were collected from:-

1. Nakuru on slopes of Menengai crater. Abundant
2. Ngoina hills in Sotik (Kericho)
3. Nairobi dam and on Langata road in Nairobi near Army Barracks.
4. Mutituni on Machakos-Kangundo road
5. Umoja on Nairobi-Kangundo road.

Lippia grandifolia

The plant material was collected in Cherangani hills between Cheptaboti in Trans-Nzoia district and Kapcherop in Elgeyo Marakwet district. Found alongside the road in newly cultivated maize gardens and the uncleared areas. The shrub is very frequent where found but rapid cultivation may exterminate it. It was found growing in maize plantations apparently without any harm. A few shrubs were found at Ngoina hills (Kericho) and Badasa near Marsabit forest.

Lippia carviodora

Collected around Ngara Mara dry river bed on Isiolo Marsabit road. The plant is very abundant especially during rains at Samburu and Buffalo Springs National Reserve up to Archers post. The plant is hardly visible in dry season. Small amounts were collected on Furole-Turbi road in Marsabit district.

Lippia carviodora var minor

The sample was collected at Tsavo West National Park. The plant was found in dense bush about 15 km from Tsavo West gate on the road to Ngulia Lodge.

Lippia somalensis

The plant material was collected about 3 km from the security barrier at Marsabit on the Marsabit-Isiolo road on the edge of the forest at Karantina. The plant could be seen extending for more than 200 m. on the slope of Mt. Marsabit. This species is very abundant in this area.

Lippia dauensis

The material was collected about 7 km from Turbi on the Marsabit-Moyale road (about 250 km from Marsabit). The plant was abundant at a seasonal dry river bed after

rains. The plant was also present mixed with acacia along the road towards Turbi settlement.

Lippia wilmsii

The plant material was collected from the slopes of Menengai crater in Nakuru. The plant was very common amongst volcanic small rocks after Nakuru Golf Club and along the roadside towards the top of the crater. It is also quite conspicuous during rains but leaves fall off in dry weather.

Identification and authentication

The authenticity of all the various plant species collected was established by the East African Herbarium (Nairobi), and the voucher specimens deposited at the herbarium, at the Department of Botany herbarium, and the Department of Pharmacy, University of Nairobi, after appropriate codification had been made.

It should be noted that although the literature on distribution of the Lippia species in Kenya served in most cases as a useful guide for possible collection sites, it was found, during the actual visits, that there were certain places where the reported species

were absent. The plants had been eliminated either through cultivation, overgrazing or natural causes. For example, it was necessary to visit Maikona, Kalacha, Hurri Hills and Furole mountains at the Kenya-Ethiopia border (Marsabit district) three times before Lippia dauensis could be located elsewhere (Turbi, on Marsabit-Moyale road). These were the recorded or neighbouring areas where the plant had previously been collected. Again scanning almost the whole of Tsavo East National Park including Galana Ranch (in Malindi district) where L. carviodora var minor had been recorded was not fruitful. However abundant quantities of the plant were accidentally discovered in Tsavo West National Park. The Lippia species were also more easily located and with more leaves and flowers soon after the rains. In dry weather, they shed all the leaves leaving only dry stems or stumps. They were also difficult to recognize in that state.

Herbarium voucher specimens (Department of Botany)

J.W. Mwangi 1 - Lippia wilmsii

" " 2 - Lippia somalensis

" " 3 - L. carviodora

J. W. Mwangi 4. - L. grandifolia

" " 5. - L. carviodora var minor

" " 6. - L. dauensis

" " 7. - L. javanica

" " 8a - L. ukambensis chvar camphor

" " 8b - L. ukambensis chvar cineole

At East African Herbarium (Nairobi);

J. W. Mwangi E.A. 17072 - Lippia carviodora

" " E.A. 17073 - Lippia somalensis

3.2. CULTIVATION OF LIPPIA SPECIES

Lippia species were introduced into cultivation by using cuttings, suckers or by layering method. A rooting hormone, 4-indol-3-ylbutyric acid "Seradix" was used on the stems. Lippia ukambensis chemovariety cineole (explained later in the text) was first introduced into cultivation in August 1983 about 100 m from the wild sample in Kirinyaga district. L. ukambensis chvar. camphor (explained later in the text) was first introduced into cultivation (May 1984) from Kitui at a plot in Kabete Campus, University of Nairobi. Subsequently, L. ukambensis chvar cineole (1/4/85) was introduced into the same plot only 3 m from each other. These two species were also top dressed with CAN (Calcium Ammonium Nitrate) in order to observe the effect on essential oils. Efforts to introduce the other Lippia species into cultivation from seeds or cuttings were also made.

3.3. MICROSCOPIC EXAMINATION OF LEAVES OF LIPPIA SPECIES

Sections which were as thin as possible were cut by use of a dissecting blade. Chloral hydrate solution served as the clearing reagent. Lignification was detected by using phloroglucinol reagent with concentrated

a

b

PLATE 1 Lippia ukambensis (a) flowers and leaves (b) with cabbages

PLATE 2 Lippia javanica

(a)

PLATES 3 Lippia grandifolia (a)

(b)

and (b) Variable inflorescence and leaves

(c)

(d)

PLATES 3 Lippia grandifolia (c) and (d) Variable inflorescence and leaves

(a)

(b)

PLATES 4. Lippia carviodora

(a) Whole plant top view

(b) Pressed specimen

NB Large whitish bracts

PLATE 5 Lippia carviodora variety minor

PLATE 6 Lippia somalensis leaves and flowers

(a)

(b)

PLATES 7 Lippia dauensis (a) Branches (b) Whole plant

C

PLATE 7 Lippia dauensis young plants

PLATE 8 Lippia wilmsii

hydrochloric acid. Observations were made at the highest possible magnification (x 100). Surface preparations for all the species were also made and examined. A Leitz microscope made by Leitz Portugal was used.

3.4. ESSENTIAL OIL DISTILLATION

The essential oils were hydrodistilled using a Clevenger-like apparatus for 3 hours. The material was not ground prior to distillation since the essential oil in *Lippia* species is located in the oil-secreting glandular trichomes on the surface. Preliminary distillations showed that distillation time of 3 hours was adequate in order to exhaust the essential oil from the materials. The essential oil content was expressed on dry basis as an average of three determinations. The oil samples were dried with anhydrous sodium sulphate and stored at low temperature (about $4^{o}C$). *Lippia javanica* oil was however always stored in the deep freeze due to the instability of its contents. This essential oil changed from yellow to reddish-brown on storage in other conditions. The essential oil samples were also stored in sealed amber coloured ampoules and kept at low temperature.

3.5. ANALYSIS OF THE ESSENTIAL OILS

The essential oil samples were analysed by gas liquid chromatography (GLC) and gas chromatography/mass spectrometry (GC/MS). Several GC/MS conditions were used in order to separate and identify as many compounds as possible.

3.5.1 GLC Analysis

GLC analysis of essential oils of *Lippia* species was carried out by modification of the method recommended by the Analytical Methods Committee [167]. Pye Unicam Model 104 instrument fitted with a glass column (2m X 4mm) packed with 12% Carbowax 20M on Chromosorb W HP-DMCS (100-120 mesh) was used. Nitrogen flowed at 30ml/min and temperature programmed at 2^{o}/min from 75^{o} to 220^{o}. A 6% Carbowax 20M mixed with 1% SE 30 column was also used under the above conditions in order to separate the high boiling components.

3.5.2 GC/MS Analysis

Several GC/MS instruments and conditions were used and temperature programmed.

(a) A VG Masslab 12-250 gas chromatograph-mass spectrometer equipped with a Hewlett Packard 5790A GC and a data system was used. A fused silica capillary column (Chrompack 15 m X 0.22mm i.d) coated with methyl silicone film and helium as a carrier gas were used. The injector was used in the splitless mode. Eims spectra was recorded at 70 ev. Temperature was programmed from 45^{0} - 250^{0} at either 5^{0}/min, 9^{0}, 10^{0} or 15^{0}/min. rate of rise in temperature. This analysis was carried out by Dr. Lwande of International Centre of Insect Physiology and Ecology (ICIPE) Nairobi, Kenya.

(b) Identity of the components was also established by using a GC/MS - MOD HP 5995. A fused silica capillary column (50 m) coated with SP-2100, and helium as a carrier gas at 1 ml/min and temperature programmed from 50^{0} to 250^{0} at 4^{0}/min were used. This was carried out by Prof. A.A. Craveiro at Federal University of Ceare (Universidade Federal do Ceara) in Brazil.

(c) GC/MS analysis of the oils was also performed using a 25m - Carbowax capillary column and temperature programmed by Prof. S.A. Matlin, IOCD Analytical Services, City University, London.

The identity of the constituents of the essential oils was established by comparison with retention times of the authentic standards and peak enhancement of the components by co-injection of the essential oil with the standards. Library - MS searches using the databases in the various GC/MS was also carried out in all cases. The mass spectra were also compared with the published data [54, 168, 177]. and or own mass spectra collections (some were donated by M. Humprey of Bush Boake Allen Ltd. London through Dr. W. Evans formerly of Nottingham University London. Others were supplied by Prof. S.A. Matlin of City University, London). In most cases several comparisons, references and GLC runs were necessary in order to identify a single component.

The standard references were obtained from Haarmann and Reimer, Dragoco or Roth (West Germany). The others were donated by PPF (Proprietary Perfumery and Fragrances, U.K). Trans-sabinene hydrate was kindly donated by Dr.

G. Crank of University of New South Wales, Australia.

The quantitative analysis of each essential oil was performed by the normalization method (expressing single components as percentages of the total) after Burchfield and Storrs [16]. Peak area of each component was found by the triangulation method and an average of three runs recorded. The 2% Carbowax or the 6% Carbowax 20M + 1% SE 30 packed columns were used for quantitative analysis.

3.6. TLC AND INFRA-RED SPECTROSCOPY

TLC of the essential oil samples was carried out on large 20 X 20 cm (0.25mm thick) plates in order to find out the constituents. n-Hexane: Ethyl acetate 90:10 was the best solvent system. Preparative TLC on thicker plates (1.0 mm) was also attempted. Infra-red spectra of total oil and a selection of isolated components were recorded. This was performed by using a Perkin-Elmer infrared spectrophotometer model 727B.

3.7 EXTRACTION OF THE NON-VOLATILES FROM LIPPIA CARVIODORA VAR MINOR

Powdered leaves (1128 kg) were soxhlet extracted with petroleum ether (60^{o} - 80^{o}) for 48 hours. The plant material, after separation of the solvent and drying at room temperature was then re-extracted with 70%

methanol for 48 hours. The methanol extract was reduced by vacuum to give a dark resinous brown residue. This residue was extracted with several portions of diethylether and the combined portions reduced. The ether residue on trituration with benzene and cooling in the fridge overnight followed by filtration gave about 13.2g unclean solid. The third recrystallization of the solid with benzene produced white needle-like crystals (about 3 g) which proved pure by TLC in benzene: methanol 15:1 (R_f 0.67) and melting range of 155^{0} - 157^{0} (uncorrected). IR, UV, ^{1}H and ^{13}C-NMR are given in appendices on pages 327 - 330.

3.8. THE ANTIMICROBIAL ACTIVITY OF ESSENTIAL OILS OF LIPPIA SPECIES

3.8.1. FILTER PAPER DISC METHOD

The antimicrobial effect of neat essential oils was carried out by the filter paper disc method. A number of micro-organisms were tested by this method. The modified method of Vincent and Vincent [178] and applied by several workers [135, 179, 180] was used. Filter paper discs (Whatman 1) sterilized by dry heat were separately saturated with the pure essential oils. They were then removed from the oils with sterile forceps and after gently shaking off the excess were carefully placed on seeded plates (1:100 dilution). The plates were incubated for 24 hrs at 37^{0} for the bacteria and 2 - 7 days for fungi. Nystatin solution (100,000 IU/ml) was used as a standard antifungal agent and cephalexin was used for the antibiotics. The average diameter of zone of inhibition of triplicate tests was recorded. It is to be noted that although the various actual quantities of the essential oils were not determined, this method serves as a method for comparison of antimicrobial activity of different essential oils.

3.8.2. AGAR STREAK METHOD

The modified method of Mischer et al [181] was applied. For testing, a stock solution of each essential oil was prepared by dissolving 100 mg in 2 ml of acetone. Either Trypticase-soy agar, Sabouroud's agar or potato dextrose agar was prepared and sterilized in the usual fashion by autoclaving at 121° for 15 min in a portable autoclave. For preparation of 1000 µg/ml for example, 10 ml of agar was put into each petri dish before congealing and 0.2 ml of the dissolved essential oil from stock solution added. The petri dishes were carefully swirled until the agar began to set. Other concentrations were prepared in the same manner. Concentrations of 25 µg/ml to 3000 µg/ml were used for the tests.

The bacteria test organisms were maintained on trypticase-soy agar slants and recovered for testing by growing them in peptone water for 24 hours. The organisms were diluted 1:100 with sterile peptone water before streaking. The organisms were streaked in a radial pattern on agar for various test essential oils. A maximum of 6 organisms were streaked on a single plate. The plates were incubated at 37° and

examined after 24 hours. Complete suppression of growth was required for the oil to be declared active at a given concentration.

The fungi were streaked in the same manner. However the animal fungi (Candida albicans, Microsporum canis and M. audpuinii) were maintained in Sabouroud's agar plates. The plant fungi (Colletotrichum coffeanum, Fusarium solani, Cercopora species and Aspergillus species) were subcultured and streaked on Potato dextrose agar. A maximum of 3 differrent fungi were streaked on each plate to avoid cross-contamination among them. The plates were incubated for 2 - 7 days at room temperature (about 25^{0}).

Acetone had previously been demonstrated to have no effect on the micro-organisms. Control experiments containing no essential oil but inoculated with micro-organisms were included to demonstrate their viability and the ability of the media to support growth.

3.9. LARVICIDAL ACTIVITY OF ESSENTIAL OILS OF LIPPIA SPECIES

Mosquito larvae (*Aedes aegypti*) were used. Third instar larvae were used instead of fourth instar in order to avoid pupation during the experiments. A stock solution of each oil was prepared by dissolving 50 mg oil accurately weighed into 5ml acetone. To prepare for example 50 ppm dilution, 0.1 ml of stock solution was made up to 20 ml with water. Other concentrations were prepared by using the appropriate volumes of the stock solution by use of micro-pipettes. Concentrations of 25 to 200 ppm of the essential oils of *Lippia* species were used. Some of the chemical constituents of the oils were also tested at concentrations ranging from 10 to 200 ppm. For the control experiments, acetone concentrations in water without the essential oils were used. The essential oils or their constituents were bioassayed by placing 20 larvae in small uniform beakers (50ml) containing various concentrations and larvae mortality noted 24 hrs after being kept in a humidified room. The larvae were fed on powdered desiccated liver (Oxoid) during the assay. The tests were replicated at least six times.

3.10 ESSENTIAL OILS OF LIPPIA SPECIES AS MAIZE WEEVIL (SITOPHILUS ZEAMAIS MOTSCH.) REPELLANTS

The method recently developed and applied at the International Centre of Insect Physiology and Ecology (ICIPE) Nairobi, Kenya was used in this experiment [182]. A Y-tube olfactometer (made of glass) was used to bioassay the repellant activity of essential oils of Lippia species on maize weevils (Fig 3).

Different quantities of the essential oils (0.625 µl, 1.25 µl, 2.5 µl and 5.0 µl) in acetone were applied on the "test" filter paper discs (1.8 cm diameter). DEET (N,N-diethyltoluamide), a potent and well studiedsynthetic insect repellant was used for comparison at the same doses [182]. Discs with only acetone were used as controls. The acetone was allowed to evaporate from the filter paper discs. The treated and control discs were then placed either in compartment B or C of the olfactometer. Vacuum at low pressure was applied at outlet D (Fig 3).

For the bioassay, 80 randomly selected maize weevils of mixed sex and varying age were introduced into compartment A. The olfactometer, with the

exception of compartment A, was enclosed in a paper carton and compartment A was illuminated with light from a 60 Watt, bulb. Since the weevils are negatively phototropic, they moved from compartment A towards compartment B and C.

The experiment was left to run for 2 hours after which time the number of weevils in the control and treated arms of the olfactometer were counted. After each test, the olfactometer was thoroughly cleansed. The experiments were carried out in triplicates for the essential oils and 6 times for DEET.

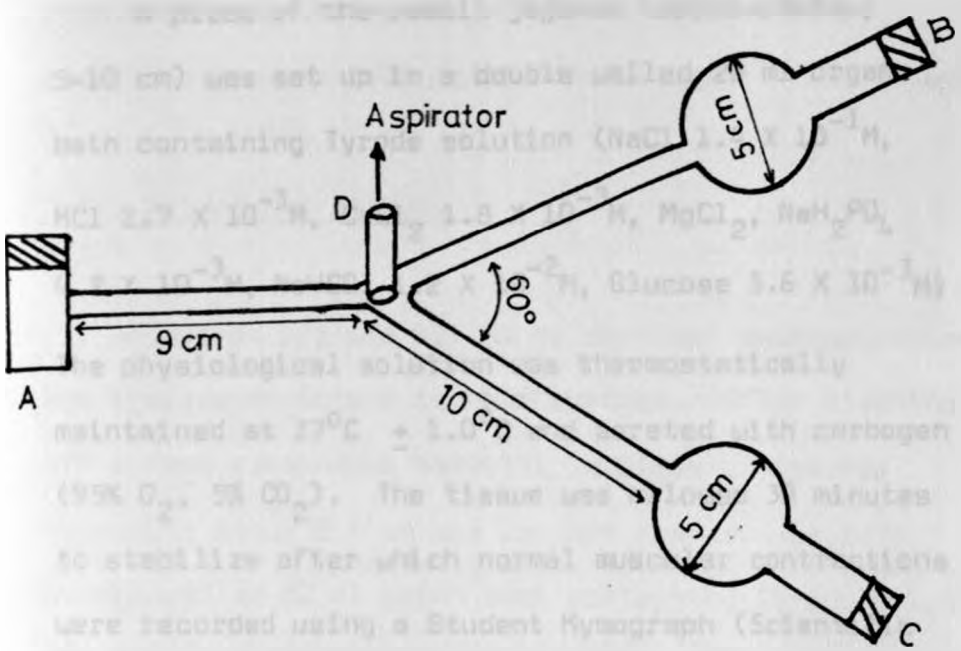
The percentage repellency (R) was calculated using the equation.

$$R = \frac{N_c - N_t}{N_c + N_t} \times 100$$

N_c = weevils in the control arm

N_t = weevils in the treated arm

Fig 3 Olfactometer for maize weevils (Sitophilus zeamais) repellency



3.11. EFFECT OF THE ESSENTIAL OILS OF LIPPIA SPECIES ON SMOOTH MUSCLES

Gastrointestinal tract

A piece of the rabbit jejunum (approximately 5-10 cm) was set up in a double walled 20 ml organ bath containing Tyrode solution (NaCl 1.4 X 10^{-1}M, KCl 2.7 X 10^{-3}M, $CaCl_2$ 1.8 X 10^{-3}M, $MgCl_2$, NaH_2PO_4 4.2 X 10^{-3}M, $NaHCO_3$ 1.2 X 10^{-2}M, Glucose 5.6 X 10^{-3}M). The physiological solution was thermostatically maintained at 37^{o}C $\pm$ 1.0^{o}C and aerated with carbogen (95% O_2, 5% CO_2). The tissue was allowed 30 minutes to stabilize after which normal muscular contractions were recorded using a Student Kymograph (Scientific and Research Instrument Ltd, England).

The essential oil suspended in 1% Tween 80 was added to the bath to give 125 µg/ml final bath concentration. The tissue response was recorded for about 3 minutes. The tissue was washed three times and left to recover. The cycle was repeated with other essential oils. Preliminary experiments had indicated that concentrations lower than 125 µg/ml caused minimal effect, whereas higher concentrations induced instant

and complete muscular paralysis. 1% Tween 80 alone also had no effect on the tissue.

Guinea pig ileum was also set up and the effect of the oils on acetylcholine and histamine induced contractions noted. Final bath concentrations of the essential oils used were 5-10 µg/ml while that of acetylcholine was 0.05 µg/ml and 0.3 µg/ml for histamine.

3.12 Guinea Pig Trachea

A guinea pig was killed by cervical exsanguination and a piece of intact trachea removed. After cleaning off excess extraneous material, a piece of trachea measuring about 2.5 cm was cut off and set up in a double-walled 20 ml organ bath containing Tyrode solution. The solution was maintained at $37^{o}C \pm 1^{o}C$ and aerated with a gas mixture containing 95% O_2 and 5% CO_2. The tissue was allowed one hour to equilibriate. Contractions and relaxations of smooth muscles of trachea after addition of the essential oil (in Tween 80) into organ bath caused changes of Tyrode solution in the fine bore tube attached to the trachea. Final bath concentrations of 125 µg/ml of each essential oil was used while 1 µg/ml final concentration of histamine was used. Tween 80 had been shown to have no effect on trachea at concentrations used.

3.13. ISOLATED PERFUSED RABBIT HEART

A rabbit was killed and the heart, with at least 1 cm of aorta attached, was removed as quickly as possible and placed in a dish of Ringer-Locke solution (NaCl 0.15 M, KCl 5.6 X 10^{-3}M, $NaHCO_3$ 2 X 10^{-3}M, $CaCl_2$ 2.2M, Glucose 5.6 X 10^{-3}M) at room temperature. The heart was then squeezed gently to wash out blood, freed from extraneous tissue and immediately attached through aorta onto the cannula at the base of the heart apparatus. The heart was then attached by a thread through a pulley system to a heart lever and the heart beat recorded by a Washington oscillograph (BioScience, England). Administration of the essential oils was through the cup just above the cannula and the heart was protected from drying by applying liquid paraffin or glycerine to the surface. Two dose ranges of 100ug and 200 ug of each essential oil in Tween 80 were used. Tween 80 at the highest concentration used in the essential oil had been shown to have no activity on the heart.

The Pharmacological experiments were carried out after Perry [183].

CHAPTER 4

RESULTS AND DISCUSSION

4.1. CULTIVATION

Introduction of *Lippia* species into cultivation was not very difficult. Preliminary experiments showed that the best method of introduction into cultivation of all the *Lippia* species was by clones (suckers or splits). Clones are progenies of a single plant obtained vegetatively. All members of a clone are identical, there is no variation within a clone either phenotypically or genetically. Any differences are therefore due to the environment. Cuttings did well for *Lippia ukambensis* chvar *camphor* and *L. carviodora*. Leaves were usually prominent after planting the stems of these species. Layering was very effective with *L. ukambensis* chvar *cineole* while rooting young stems in water followed by planting was only successful with *L. dauensis*. Roots of *L. dauensis* appeared in about 2 weeks. Germination of seeds took a long time (about 3 weeks for *L. javanica*, *L. dauensis*) and germination rate was very poor.

Application of a rooting hormone "Seradix" (4-indol-3-ylbutyric acid) did not seem to be effective in enhancing rooting. Application of a fertilizer (top-dressing with Calcium Ammonium Nitrate, 26% Nitrogen) to both varieties of Lippia ukambensis apart from increasing the amount of foliage did not have any significant effect on yield or composition of the essential oil (Table 4,5,6,7). Intercropping the two varieties with cabbages did not seem to affect them (Plate 1b). Due to their fibrous rooting system, Lippia species have the ability of holding soil together and therefore could be used in soil erosion controls.

4.2. MICROSCOPIC FEATURES

The microscopic features of Lippia grandifolia and L. javanica leaves through the midrib are shown in Fig. 4 and Fig. 5. respectively. These represent the typical features found in the leaves of Lippia species studied. Apart from leaves of L. javanica and L. dauensis which had palisade on both sides (isobilateral), all the others had palisade only on the upper side (dorsiventral). The palisade was not continuous at the midrib. The Lippia species examined had unicellular warty covering trichomes. The covering trichomes were

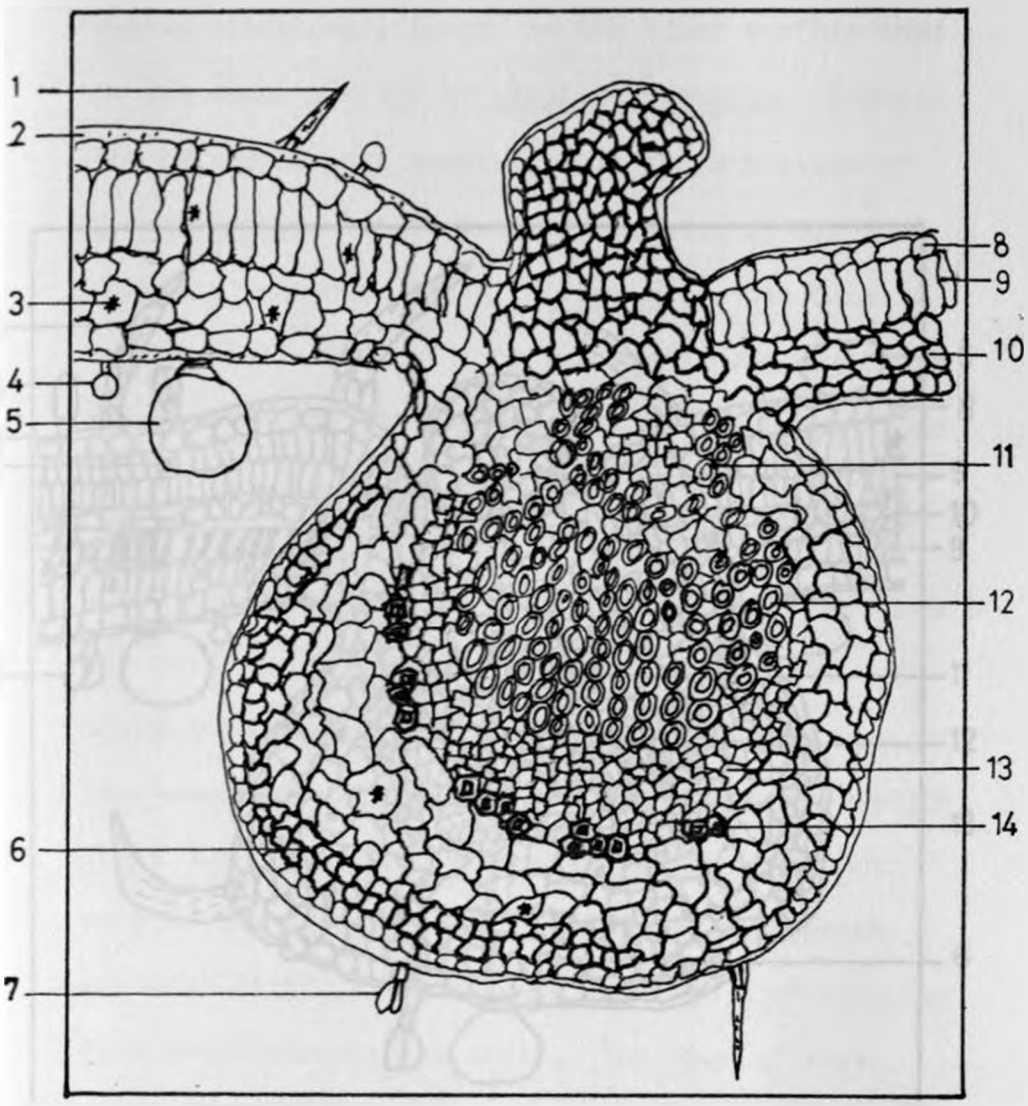


FIG. 4 Microscopic features of Lippia grandifolia

1. covering trichome
2. cuticle
3. calcium oxalate crystal
4. capitate gland
5. secreting gland
6. collenchyma
7. glandular trichome
8. epidermis
9. palisade layer
10. spongy mesophyll
11. accessory bundles
12. xylem vessels
13. phloem
14. pericyclic fibres

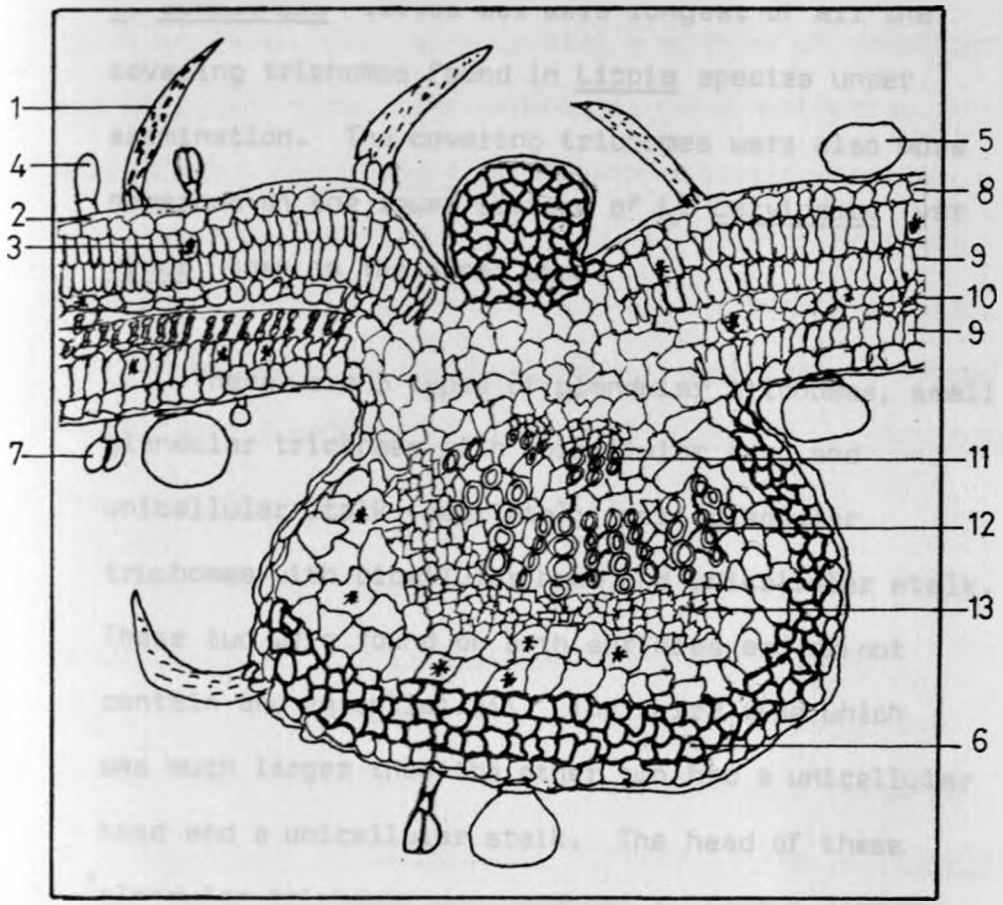


Fig 5 Microscopic features of Lippia javanica

*Numbering as in Fig 4

characteristically longer on the lower surface than on the upper surface of Lippia ukambensis leaves. The trichomes were equal in size on both sides of L. somalensis leaves but were longest of all the covering trichomes found in Lippia species under examination. The covering trichomes were also more numerous on the lower surface of L. carviodora var minor than on the upper side.

There were 3 types of glandular trichomes, small glandular trichomes with unicellular head and unicellular stalk (capitate); small glandular trichomes with bicellular head and unicellular stalk. These two were found on both surfaces and did not contain any essential oil. The third type which was much larger than the other two had a unicellular head and a unicellular stalk. The head of these glandular trichomes was expanded to form a bladder-like structure containing a light-yellow essential oil (i.e secreting glands). These glands occasionally contained a droplet of the essential oil. The secreting glands were more numerous on the lower surface of the leaves than on the upper side. The secreting glands of L.javanica also became more intensely yellow after

the lignification test with phloroglucinol and conc. HCl.

The stomata were present on the upper and the lower surfaces. They were usually a mixture of anomocytic and diacytic type. The anomocytic were dominating apart from L. somalensis where the diacytic stomata were numerous. Small calcium oxalate cluster crystals were found both in the lamina and at the midrib of the leaf in L. dauensis, L. javanica, L. ukambensis chemical varieties, L. somalensis, L. grandifolia and L. carviodora. The crystals were most numerous in L. dauensis.

Pericyclic fibres were found at the midrib in only L. grandifolia and L. wilmsii. They were lightly lignified in the former case and non-lignified in the latter case. Accessory vascular bundles were found in L. grandifolia, L. ukambensis chemovarieties, L. somalensis and L. javanica. Apart from L. grandifolia where there was only one circular accessory bundle, the others had 2-5 small bundles sometimes without any phloem. The epidermal walls were all wavy and striated cuticle could be located while collenchyma was found both on the lower and the upper side of the midrib.

From the above observations, it was concluded that L. carviodora and L. carviodora var minor apart from the morphological differences in the size of their bracts

(those of the latter being very large) had important microscopic differences. L. carviodora leaves have small calcium oxalate cluster crystals while none were detected in L. carviodora var minor. L. carviodora var minor had also more unicellular warty covering trichomes on the lower surface than on the upper surface while they were uniformly distributed on both sides in L. carviodora leaves. L. carviodora leaves also had more oil secreting glands on the lower surface than L. carviodora var minor.

Although morphological and microscopic features of L. ukambensis varieties were similar, it is possible to confuse the morphological features of L. somalensis with those of L. ukambensis varieties. However, microscopic observation revealed that L. ukambensis leaves had short unicellular warty covering trichomes on the upper surface and relatively long ones on the lower surface while L. somalensis leaves had unisize, very long trichomes on both surfaces. The surface preparation of the leaves also showed that although both diacytic and anomocytic stomata were present in L. ukambensis varieties and L. somalensis leaves, the latter

had more diacytic stomata than the anomocytic ones while L. ukambensis had more anomocytic.

Metcalfe and Chalk [184] described the general microscopic features found in Verbenaceae family. They rarely mentioned the features pertaining to Lippia species and certainly none on Lippia species found in Kenya. The detailed microscopic features of leaves from Lippia species described above were therefore reported for the first time in the present work.

4.3. TLC AND INFRARED (IR) SPECTROSCOPY

TLC indicated that the essential oils of Lippia species had many components. Some of these components could be easily identified by the use of the standards. The presence or absence of camphor (2) in L. ukambensis was easily recognised even by the use of TLC. The same case applied to 1,8-cineole (4) in the same plant. Preparative work on thicker plates indicated that some components could be isolated. Camphor for example was isolated from Lippia ukambensis and its IR spectrum confirmed by the use of a standard.

IR spectra of the essential oils (whole fraction) indicated the major functional groups in these oils. The spectra are given in Appendix 2 - 10. The IR analysis of the oils served as a quick check of the tentative contents of the essential oil. It was then easier to

guess which standards one would run in GLC in an attempt to identify the oil constituents. For example presence of strong C=O or C-O peaks as in the essential oil of L. wilmsii and L. somalensis would prompt one to look for functionalized essential oil constituents such as piperitone and 1,8-cineole respectively (appendices 4,9). Comparing the IR spectrum of the essential oil with that of the standard almost confirmed the identity of some components. The IR spectra of the total essential oil is usually recommended where such facilities as GC/MS are not readily available or simply to confirm the results of other analytical methods. For example essential oil of Lippia ukambensis chvar cineole which had been shown to contain almost no camphor (2) by TLC when run in IR spectrometer showed only a weak C=O peak whereas that which had shown the presence of camphor on TLC had a strong C=O peak in its IR spectrum (appendices 2,3).

4.4. ESSENTIAL OIL CONTENT AND PHYTOCHEMISTRY

Key for Tables on essential oil constituents of Lippia species

In the following tables the GLC peaks are listed in order of elution from the 12% Carbowax 20M column apart from those of *L. grandifolia* and *L. carviodora* var *minor* oils where 6% Carbowax 20M + 1% SE 30 column was applied.

Identification methods:

(a) GLC - 12% Carbowax 20M or 6% Carbowax 20M + 1% SE 30 on Chromosorb W-HP: direct comparison with authentic standards by retention times and peak enhancement (i.e co-injection with authentic standards).

(b) GC/MS - Methylsilicone capillary column (15 m)

(c) GC/MS - Carbowax capillary column (25 m)

(d) GC/MS - SP 2100 fused silica column (50 m)

(e) Infra-red spectrum

As stated in the experimental section, it was necessary to carry out the GLC analysis using different columns and conditions in order to identify as many components as possible. For example, separation of limonene (2) and 1,8-cineole (4) was only possible with carefully packed very polar columns such as 12% Carbowax 20M on Chromosrob W-HP or a Carbowax capillary column. Other less polar columns eluted these two compounds as a single peak.

Appendix I shows how complex and sometimes confusing the picture from a GC/MS can be. It should also be noted that even the most equipped GC/MS library data base will not provide the identity of each and every compound in such a complex mixture without additional data. However GC/MS provides a very useful guide towards the identity of the essential oil components and frequently serves as the only identification method.

Mass spectra (M.S)

The detailed M.S fragmentation patterns of some essential oil constituents were given in some cases. These constituents were considered to be important in the description of either chemical varieties (eg. L. ukambensis chvar camphor and L. ukambensis

chvar cineole) or closely related components in different essential oils (eg L. dauensis and L. javanica oils). The detailed fragmentation patterns of most of the other compounds was not considered necessary since these are readily available from the literature.

LIPPIA UKAMBENSIS

The essential oil content of leaves and flowers from various parts of the country estimated on moisture-free basis from 23 samples ranged from 1.1-3.0% (mean 1.8%) (Table 4).

The yield was at variance with the only recorded report for fresh leaves of L. ukambensis collected from Tanzania which yielded 0.3% oil [143].

TLC and GC/MS analysis revealed the presence of about 27 compounds (Table 5 and Fig. 6). The essential oil was rich in camphor (2) (average 37.3%). This agreed with the quantities of camphor (36.5%) reported in the essential oil from Tanzania Lippia ukambensis. A substantial amount of trans-sabinene hydrate (thujanol-trans-4) (76) was also found in the essential oil from L. ukambensis from Kenya (18.93%)

This again was in agreement with the reported value of compound (76) in the Tanzanian essential oil which was 18.5%. It was noted that whereas the Tanzanian plants yielded an oil containing a reasonable amount of 1,8-cineole (4) (11.3%) the Kenyan samples showed only traces of this compound (average 1.1%) (table 6). Borneol (15) (average 4.2%) was present in the Kenyan samples of oil instead of α-terpineol (14) (2.3%) reported in the Tanzanian sample. There was no major difference in the β-cubenene (77) content in both the essential oils from Tanzania and Kenya (average 6.6% and 6.5% respectively).

Chemical variation in L. ukambensis

Another sample of L. ukambensis, which appeared to be a chemical variety of the common L. ukambensis already described above was collected. This plant had the same morphological (confirmed by the East African Herbarium, Nairobi) and the histological features as L. ukambensis already described above. The essential oil yield from this plant was lower than that of the common L. ukambensis (mean 1.55%) against 1.81% (Tables 4,6).

The chemical composition also showed that the essential oil from 18 samples examined from this variety also had about 27 notable compounds (Table 7 and Fig 7). Comparison of the components in the essential oil of this new variety with those of the common L. ukambensis oil showed several differences (Table 5, 7 and Figs 6, 7). 1,8-Cineole (4) was very prominent in the new variety (average 23.7%) as compared with the previously described and common L. ukambensis oil in Kenya which had only traces of this compound. α-Terpineol (14) (mean 9.7%) was also present in the new variety but completely absent in the oil of the common L. ukambensis variety. Borneol (15) (average 4.2%) on the other hand was present in the common L. ukambensis oil but absent in the essential oil of the new variety.

Of the 27 compounds separated in each of the essential oil of L. ukambensis variety, 12 components which seemed to be present in both varieties were further statistically analyzed (Table 8). Four compounds were not significantly different between both varieties ($P > 0.05$). The rest were significantly different to some degree, five compounds being prominently significantly different ($p < 0.0001$).

Table 4 Collection sites and essential oil content of Lippia ukambensis chvar camphor

	Date of collection	Collection sites	% yield	Comments
I	17/8/83	Nakuru National Park	1.6	Leaves
II	17/8/83	Nakuru Town	3.0	Leaves
III	8/9/83	Kitui Town	1.5	Leaves
IV	8/9/83	Kitui Town	2.2	flowers
V	14/2/83	Nairobi Dam	2.0	Leaves
VI	3/2/84	Nairobi Dam	2.1	Leaves
VII	28/2/85	Kinungi (Limuru)	1.6	Leaves
VIII	21/9/85	Narok Town	1.6	Leaves
IX	22/9/85	Menengai Crater	1.9	Leaves
X	23/6/85	Mweiga (Nyeri)	2.3	Leaves
XI	23/6/85	Mweiga	1.8	flowers
XII	1/12/86	Gacharu (near Sagana)	1.2	Leaves
XIII	23/6/86	1 km from LUC	1.7	Leaves
XIV	23/8/86	3 km from LUC	1.8	Leaves
XV	5/6/86	Marsabit forest	2.6	Leaves
XVI	3/6/85	Kabete cultivation	1.4	Leaves
XVII	3/6/85	Kabete cultivation	1.8	Leaves from another shrub
XVIII	30/4/86	Kabete cultivation	1.1	Leaves CAN treated

LUC - Lippia ukambensis chvar cineole collection site

CAN - Calcium Ammonium Nitrate fertilizer

Table 4 continued

XIX	30/4/86	Kabete cultivation	1.3	Leaves not CAN treated.
XX	30/4/86	Kabete cultivation	1.6	flowers not CAN treated
XXI	7/8/86	Kabete cultivation	1.4	Leaves CAN, treated
XXII	24/9/86	Kabete cultivation	1.6	Leaves not CAN treated
XXIII	7/8/86	Kabete cultivation	2.6	Leaves not CAN treated

Mean 1.81%

Standard Error of the Mean

(SEM) $\pm$ 0.10

Table 5 Essential oil constituents of Lippia ukambensis chvar camphor

Peak no.	Constituent	Identification method	% of constituents in the samples																								
			I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI	XVII	XVIII	XIX	XX	XXI	XXII	XXIII	mean
1	α-pinene	a,b,c,d	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	–
2	α-thujene	a, b, d	2.7	3.5	2.0	1.5	1.5	2.2	2.5	1.2	3.5	3.1	1.5	0.4	1.7	1.3	T	0.3	T	3.2	1.5	1.5	3.8	3.4	3.7	2.0
3	camphene	a, b, c, d	11.1	12.6	7.9	7.0	5.8	6.2	10.2	4.4	11.2	10.0	7.3	1.3	14.3	3.8	8.5	3.8	T	3.4	5.8	5.1	16.7	10.4	12.9	7.8
4	β-pinene	a, b, c	1.2	1.3	0.9	T	0.6	1.2	0.8	0.5	1.0	0.7	0.7	0.4	1.2	0.8	0.8	2.8	T	1.0	0.8	1.1	0.9	1.0	1.0	0.9
5	3 carene	c	1.4	2.0	1.1	T	1.4	1.5	1.0	0.6	1.2	1.0	0.8	T	1.0	0.8	0.9	2.0	T	1.1	0.9	1.4	1.0	1.0	1.5	1.0
6	myrcene	a, b, c, d	3.0	3.3	1.0	T	1.0	0.8	3.0	2.1	1.5	3.8	3.1	0.4	1.0	2.2	4.5	1.0	T	2.0	1.2	3.0	1.4	T	1.0	1.8
7	β-phellandrene	a, b, c	1.6	2.0	0.5	T	0.5	0.2	0.9	2.2	1.5	1.7	0.4	T	T	1.0	1.2	0.7	T	0.7	1.0	0.8	1.0	T	1.0	0.8
8	α-terpinene	a, b, c	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	–
9	limonene	a, b, c, d	6.35	7.5	2.2	T	4.5	5.2	6.2	6.6	8.5	7.3	5.4	2.6	7.6	6.2	4.4	5.0	2.7	4.7	5.1	7.1	4.8	4.9	5.7	5.2
10	1,8-cineole	a, b, c, d	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	–
11	β-ocimene	a, c	2.3	3.0	0.5	T	0.9	T	T	T	1.9	2.0	1.3	T	T	2.6	3.6	2.7	3.0	1.3	3.3	2.7	1.8	2.5	2.4	1.6
12	γ-terpinene	a, c	2.1	2.4	0.7	T	1.2	T	T	T	0.8	2.9	1.7	1.7	2.4	1.9	3.2	2.0	1.6	1.1	1.8	2.5	1.7	2.5	2.2	1.6
13	p-cymene	a, b, c	1.7	1.3	1.6	T	0.6	T	T	T	2.5	0.4	T	1.7	T	1.6	2.2	1.6	T	2.1	T	2.5	T	1.7	0.7	1.0
14	terpinolene	a, c	1.7	1.8	0.8	T	1.0	T	T	T	T	1.5	1.5	1.7	T	1.3	T	1.0	1.2	T	T	3.0	T	T	1.5	–
15	unknown		T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	–
16	unknown		T	T	0.9	T	1.7	1.2	T	T	T	T	T	T	T	T	T	T	1.7	1.6	T	T	T	T	T	–
17	trans-sabinene hydrate	a, b, c, d	14.7	19.9	23.9	27.5	18.7	25.8	21.3	23.4	12.7	13.7	25.2	22.7	15.1	17.6	12.8	20.1	20.0	11.8	23.8	18.1	14.4	18.4	13.6	18.9
18	unknown		T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	–
19	camphor	a, b, c, d, e	40.1	30.9	43.5	43.6	43.7	35.3	40.8	50.4	39.3	33.4	33.4	37.1	40.6	24.1	37.0	36.5	32.0	40.2	35.5	27.9	33.2	39.6	40.8	37.3
20	linalool	a, b, c	T	T	0.5	T	T	5.2	T	T	T	T	T	7.2	T	6.0	T	T	9.3	T	T	5.1	4.8	T	T	–
21	cis-sabinene hydrate	c	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	–
22	terpinen-4-ol	a, b, c, d	3.2	4.4	2.8	3.4	4.3	3.7	2.5	1.1	4.5	5.7	3.6	7.2	3.0	7.1	4.9	2.5	2.5	5.8	2.6	8.0	T	4.2	4.4	4.0
23	thujyl alcohol	c	T	T	T	T	T	T	T	T	T	T	T	T	T	2.1	T	T	T	T	T	T	T	T	T	–
24	α-cadinene	c	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	–
25	sesquiterpene hydrocarbon	c	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	–
26	borneol	a, b, c, d	3.8	5.7	4.9	4.0	6.2	6.4	2.7	1.9	4.3	4.2	3.9	7.8	2.2	4.6	4.8	3.1	4.6	2.4	4.1	5.9	3.1	3.4	2.0	4.2
27	β-cubenene	b, c, d	3.2	5.0	3.7	T	6.3	1.5	1.8	3.1	5.6	9.0	10.2	8.5	9.0	12.3	8.0	14.6	12.7	8.9	6.7	3.4	7.1	6.7	4.2	6.6

T = Trace <0.2 % and regarded as zero in calculation of mean values

Table 6 Collection sites and essential content of Lippia ukambensis chvar cineole

Sample	Date collected	Collection sites	Yield %	Comments
I	3/6/84	Cultivation (100m from wild sample)	1.3	Leaves
II	3/6/84	"	1.4	Flowers
III	2/9/84	"	1.5	Leaves
IV	2/9/84	"	1.4	Flowers
V	28/10/84	"	1.9	Leaves
VI	28/10/84	"	1.1	Flowers
VII	1/11/84	"	1.6	Leaves
VIII	25/5/85	"	1.8	Leaves
IX	25/5/85	"	1.5	Flowers
X	6/10/85	Wild sample	1.8	Leaves
XI	16/6/85	cultivation (100 m from sample)	1.7	Leaves
XII	16/6/85	"	1.7	Flowers
XIII	3/2/85	"	1.5	Leaves
XIV	28/12/85	"	1.5	Leaves
XV	24/9/85	Kabete cultivation	1.6	Leaves not CAN* treated
XVI	15/4/86	"	1.7	Leaves not CAN* teated
XVII	15/4/86	"	1.5	Leaves CAN teated
XVIII	17/2/86	"	1.4	leaves CAN treated

NB. Wild sample found at Kianjege between Sagana and Karatina

Mean = 1.55% (SEM ± 0.05)

*CAN - Calcium Ammonium Nitrate.

Table 7 Essential oil constituents of Lippia ukambensis chvar cineole

Peak no.	Constituent	identification method	% of constituents in the samples I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI	XVII	XVIII	mean
1	α-pinene	a, b, c	0	0	T	0	T	0	T	0	T	T	T	0	0	0	T	0	T	T	—
2	α-thujene	b, c, d	1·8	1·7	2·3	1·6	2·3	3·0	1·7	3·0	2·7	1·8	1·7	T	2·4	2·3	2·5	0·6	2·9	4·0	2·1
3	camphene	b, c, d	T	0·4	0·2	0·4	0·2	0·5	0·4	T	0·5	T	T	T	T	0·2	T	0·2	0·5	0·5	—
4	β-pinene	a, b, c	T	T	0	0	T	0	T	0	0	T	0	T	T	T	T	T	T	T	—
5	3-carene	c	5·6	4·8	4·7	4·1	6·1	6·5	5·0	7·0	6·4	6·0	6·2	4·0	7·5	6·0	9·2	6·1	11·0	2·5	6·2
6	myrcene	a, b, c, d	1·7	1·8	2·0	1·0	1·9	1·7	1·6	2·0	1·9	T	1·8	1·4	2·1	4·0	2·1	2·6	3·4	3·1	2·0
7	phellandrene	b, c	T	T	T	T	T	T	T	0·8			0·4	0·3	T	T	T	T	T	T	—
8	α-terpinene	a, b, c	T	T	T	T	T	T	T	0·8	T	T	0·7	0·8	T	T	T	T	T	T	—
9	limonene	a, b, c	0·9	0·4	T	0·7	0·9	0·8	0·4	0·8	0·7	T	0·6	0·6	1·3	0·9	T	T	0·7	T	—
10	1,8-cineole	a, b, c, d	22·0	20·1	25·6	20·2	25·4	26·2	19·4	20·5	24·8	30·8	28·9	25·9	28·4	17·1	34·0	16·2	18·1	20·7	23·6
11	γ-ocimene	a, b, c	0·9	1·3	2·4	T	1·9	T	T	2·0	1·1	T	2·4	0·7	3·5	2·4	2·8	2·5	2·2	2·1	1·9
12	γ-terpinene	a, c	2·3	0·7	1·4	2·0	2·7	1·4	1·4	2·0	1·4	T	2·3	2·4	0·4	1·5	T	0·2	0·6	1·2	1·4
13	p-cymene	a, b, c	0·4	0·7	T	T	0·3	T	1·8	T	T	T	T	0	0·8	1·2	T	0·3	1·0	0·3	0·4
14	terpinolene	c, c	0·4	0·6	T	T	0·7	0·5	1·3	T	0·5	T	0·6	0·6	T	T	T	0	T	T	—
15	unknown		0·4	T	T	T	0·4	T	T	T	T	T	T	T	T	T	T	T	T	T	—
16	unknown		0·4	T	T	T	0·5	T	T	T	T	T	T	1·2	T	T	T	T	T	T	—
17	trans-sabinene hydrate	a, b, c, d	19·7	26·4	30·6	24·0	19·8	28·4	23·5	24·1	28·4	33·0	19·4	23·4	22·9	21·3	24·3	24·5	13·5	31·2	24·7
18	unknown		T	T	T	T	T	0·3	T	T	T	T	T	T	T	T	T	T	T	T	—
19	camphor	a, b, c	0·7	1·5	0·6	1·7	0·9	1·8	2·5	0·8	1·0	T	0·7	T	2·3	3·0	T	1·2	1·6	0·5	1·4
20	linalool	a, b, c	2·7	2·9	3·6	2·8	2·8	2·9	3·6	2·6	1·9	5·1	2·0	T	3·3	2·6	2·9	5·1	5·0	2·3	3·2
21	cis-sabinene hydrate	c	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	—
22	terpinen-4-ol	a, b, c	6·4	6·4	3·1	8·1	5·7	8·2	6·1	6·0	2·4	4·1	5·9	5·2	6·9	9·1	4·5	8·4	8·4	7·7	6·3
23	unknown		0·9	1·7	0·9	T	1·2	1·4	1·0	T	T	T	T	0	1·5	2·4	0	1·1	1·7	2·0	—
24	unknown		T	T	T	T	T	T	T	T	0·4	T	T	T	T	T	T	T	T	T	—
25	p-cymen-8-ol	b, c	T	T	T	T	T	T	T	T	0·4	T	T	T	T	T	T	1·1	T	T	—
26	α-terpineol	a, b, c, d	8·1	10·0	10·2	12·1	7·6	10·6	9·8	8·4	8·0	12·0	9·0	7·3	7·5	10·4	8·2	10·3	12·5	8·9	9·7
27	β-cubebene	b, c, d	12·4	5·1	13·5	20·6	16·0	14·3	17·9	16·0	16·0	7·2	16·0	24·5	7·5	11·8	8·0	13·4	10·8	8·5	13·5

T = Trace < 0·2% and regarded as zero in calculation of mean values

Table 8 Comparison of essential oil constituents of Lippia ukambensis chvar camphor and L. ukambensis chvar cineole

GLC Peak	Compound	% X̄ (SEM) L. ukambensis chvar camphor[a]	% X̄ (SEM) L. ukambensis chvar cineol[b]	T_{39} Test	Significance
	%yield of oil	1.81(0.10)	1.55(0.05)	2.15	*
2	α-thujene	2.0(0.25)	2.13(0.21)	0.38	NS
3	camphene	7.8(0.89)	0.22(0.05)	7.54	***
5	3-carene	1.03(0.11)	6.15(0.47)	11.85	***
6	myrcene	1.75(0.27)	2.02(0.21)	0.76	NS
9	limonene	5.24(0.41)	0.55(0.09)	7.08	***
10	1,8-cineole	Trace	23.7(0.21)	-	
11	β-ocimene	1.64(0.26)	1.57(0.26)	0.20	NS
12	γ-terpinene	1.58(0.20)	1.34(0.20)	0.84	NS
13	p-cymene	0.97 (1.0)	0.44 (0.13)	2.09	*
17	trans-sabinene hydrate	18.93(0.98)	24.67(1.0)	4.06	**
19	camphor	37.34(1.21)	1.11(0.18)	26.27	***
22	terpine-4-ol	3.97(0.40)	6.26(0.45)	3.81	**
26	borneol[c]	4.17(0.67)	absent	-	
25	α-terpineol[c]	absent	9.65(0.65)	-	
27	β-cubenene	6.59(0.79)	13.53(1.22)	4.96	***

NS, not significant $P > 0.05$; *, $P < 0.05$; ** $P < 0.001$; *** $P < 0.0001$

$\bar{X}$ (SEM) = mean (Standard Error of the mean)

[a] n = 23; [b] n = 18; c= compounds absent in one or other chemovariety

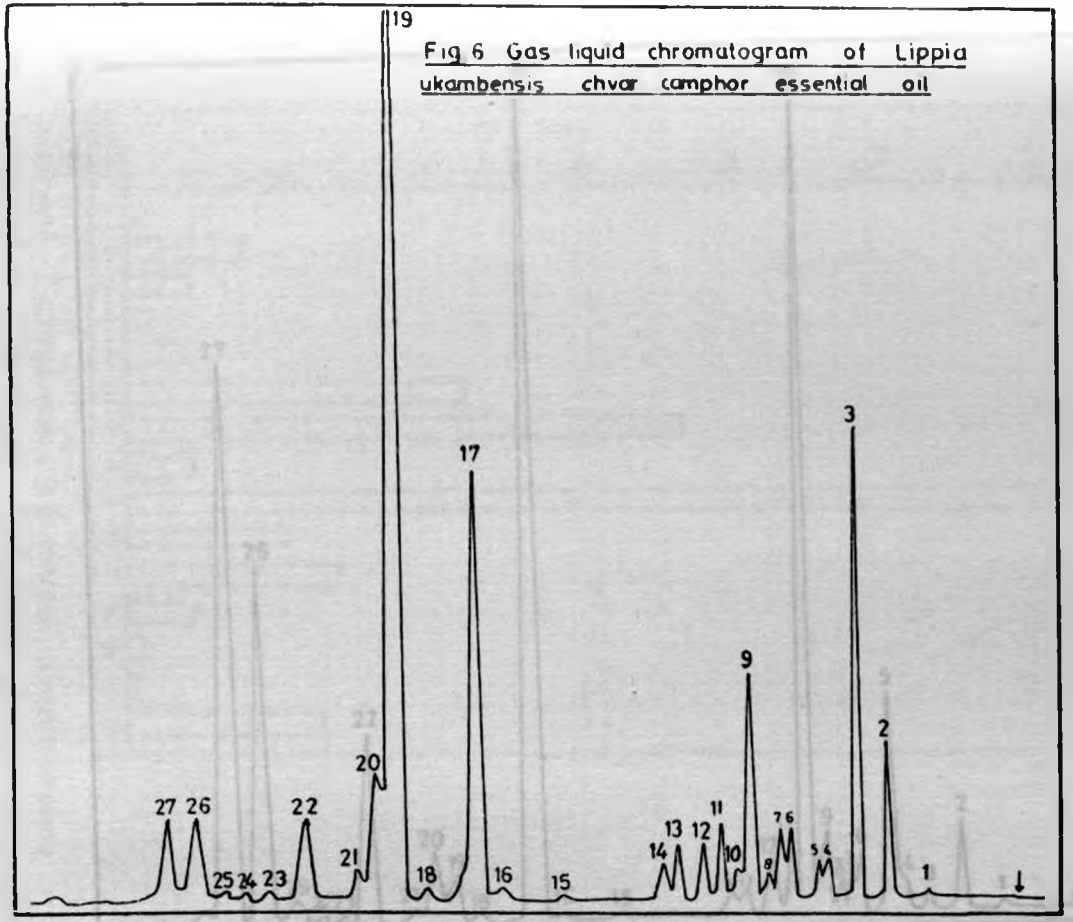


Fig 6 Gas liquid chromatogram of Lippia ukambensis chvar camphor essential oil

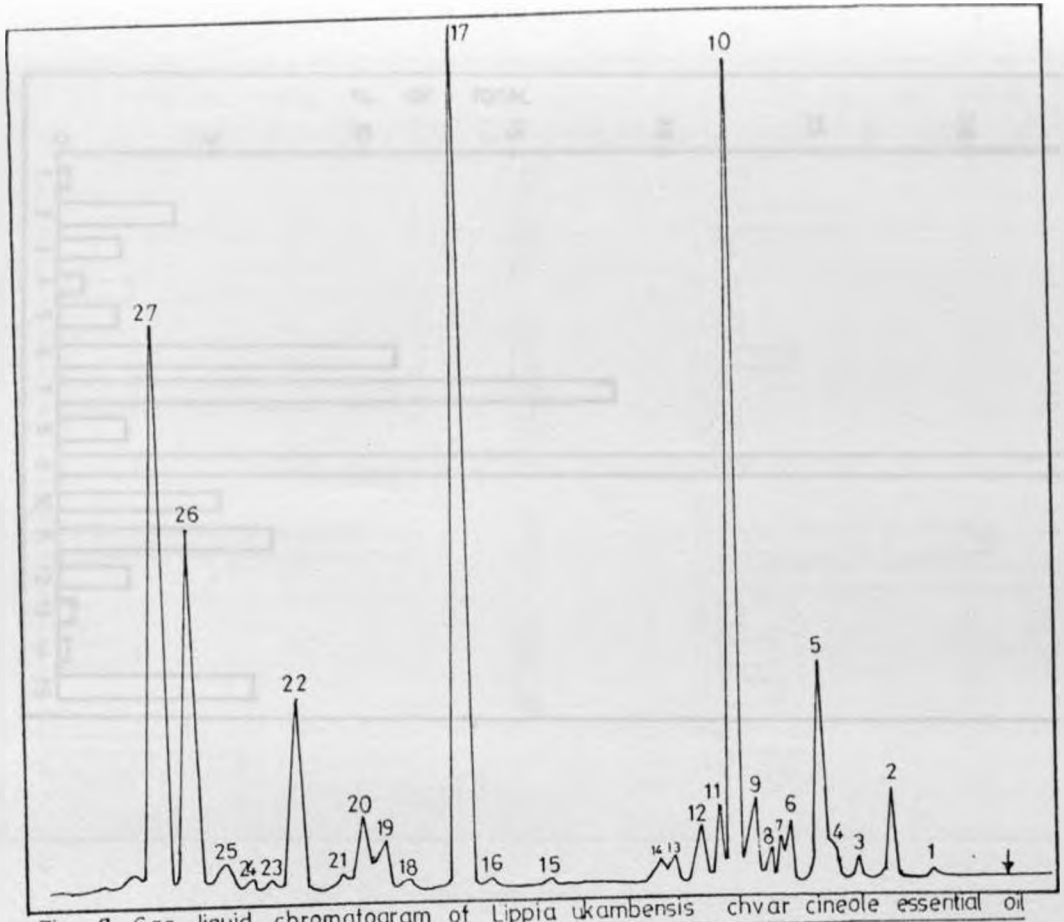
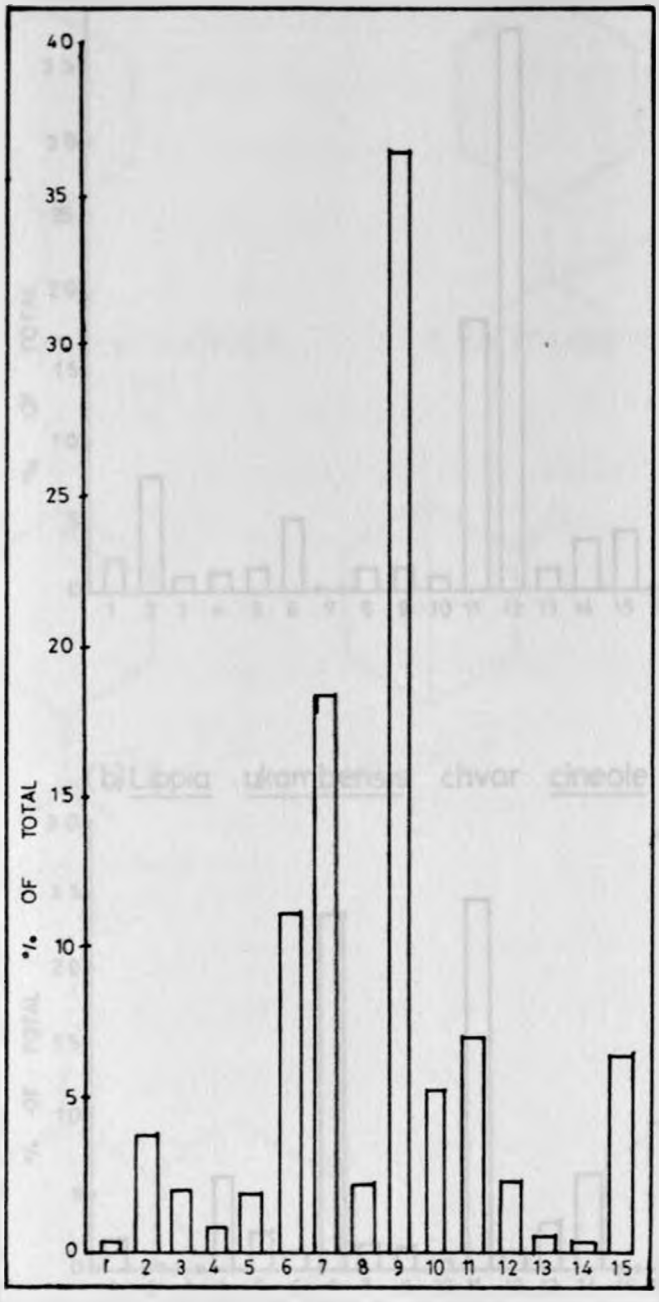


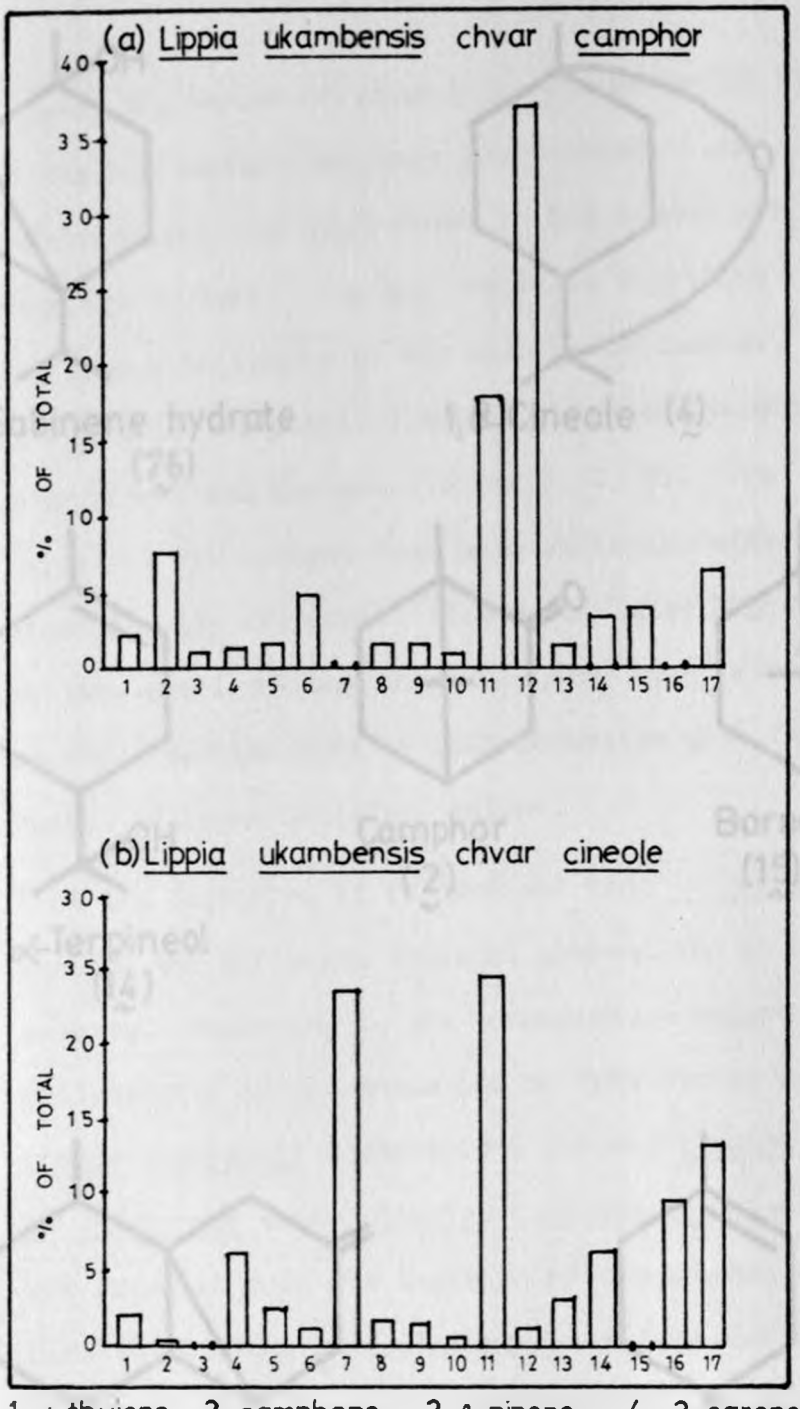
Fig 7 Gas liquid chromatogram of Lippia ukambensis chvar cineole essential oil

Fig. 8 Essential oil of Lippia ukambensis from Tanzania [143]



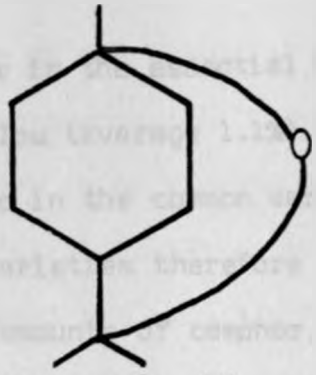
1. α-pinene 2. camphene 3. β-pinene 4. monoterpene hydrocarbon 5. limonene 6. 1,8-cineole 7. sabinene hydrate 8. monoterpene alcohol 9. camphor 10. monoterpene alcohol 11. monoterpene alcohol 12. α-terpineol 13. sesquiterpene hydrocarbon 14. sesquiterpene hydrocarbon 15. β-cubenene

Fig. 9 Essential oil of Lippia ukambensis chemical varieties from Kenya



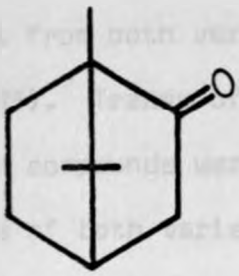
1. ∝-thujene 2. camphene 3. β-pinene 4. 3-carene
5. myrcene 6. limonene 7. 1,8-cineole 8. ocimene
9. γ-terpinene 10. p-cymene 11. sabinene hydrate
12. camphor 13. linalool 14. terpinen-4-ol
15. borneol 16. ∝-terpineol 17. β-cubenene

Sabinene hydrate
(76)

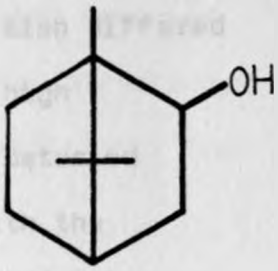


1,8-Cineole (4)

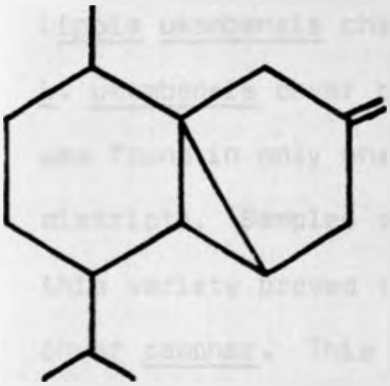
α-Terpineol
(14)



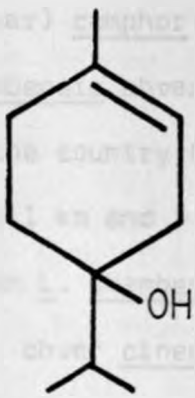
Camphor
(2)



Borneol
(15)



β-Cubenene (77)



Terpinen-4-ol (19)

Indeed, the amount of camphor in the essential oil of the new variety was very low (average 1.1%) in comparison with that found in the common variety (average 37.34%). The two varieties therefore differed principally in the amounts of camphor, 1,8-cineole, camphene, 3-carene, limonene, β-cubenene, α-terpineol and borneol (Tables 5, 7, 8). The essential oil content from both varieties also differed significantly ($P < 0.05$). Traces of other high boiling-point unknown compounds were also detected in the essential oils of both varieties with the methyl silicone capillary column.

From the foregoing it is apparent that L. ukambensis showing the different chemical composition is a chemical variety. According to the nomenclature suggested by Tetenyi [185], these can be referred to as Lippia ukambensis chemovariety (chvar) camphor and L. ukambensis chvar cineole. L.ukambensis chvar cineole was found in only one location of the country (Kirinyaga district). Samples collected about 1 km and 3 km from this variety proved to be the common L. ukambensis chvar camphor. This L. ukambensis chvar cineole was cultivated in nearby garden (100 m away from the wild plant) to preserve it. This continued to exhibit

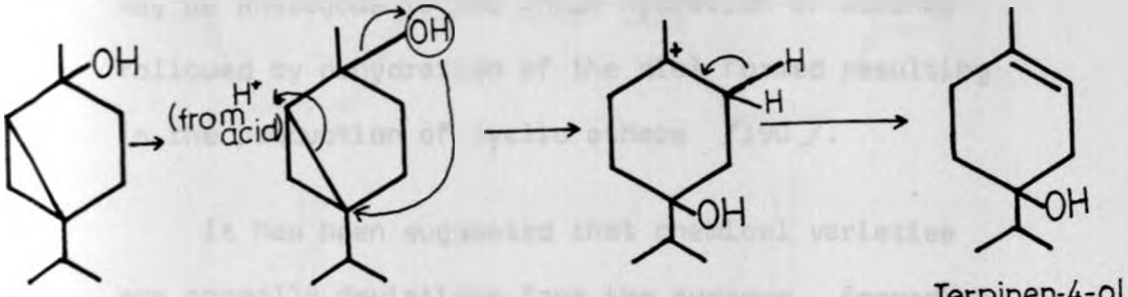
the same chemical composition of the essential oil as the wild sample. Subsequently, introduction of L. ukambensis chvar camphor and L. ukambensis chvar cineole in a completely different geographical location (at Kabete, Nairobi, in the University farm) and only 3 m from each other revealed that the essential oil composition of these varieties did not depend on geographical factors. Application of a fertilizer (Calcium Ammonium Nitrate) only increased the amount of foliage but did not affect the chemical composition of the essential oil from these varieties (Table 5 and 7).

The main differences between the essential oil from L. ukambensis from Tanzania and that from Kenya L. ukambensis oil is an intermediate in respect with the Kenyan two varieties. It has a high camphor (2) content and a reasonable amount of 1,8-cineole (4). It is therefore suggested that it should be referred to as L. ukambensis chvar camphor - cineole.

1,8-Cineole (4) is supposed to be biosynthesised from α-terpineol (14) which in turn is envisaged to be from a common cation (11) with borneol (15). Borneol would easily be converted into camphor by oxidation [5, 19]. This is plausible since L. ukambensis chvar

camphor oil had some borneol (15) while α-terpineol (14) was not detected in this oil. Essential oil of L. ukambensis chvar cineole on the other hand had some α-terpineol while borneol was absent. All the chemical varieties including that from Tanzania had essential oil with significant amounts of sabinene hydrate (76) although L. ukambensis chvar cineole had slightly more (Fig 7 and 8). Under condition of sterilization, sabinene hydrate yields terpine-4-ol (19) [186]. In vitro, trans-sabinene hydrate is also transformed to terpinene-4-ol by an acid catalysed reaction [187]. The cyclopropane ring of α-thujene (20) is unstable and readily undergoes fission [45]. This is not unusual as it is known that in alicyclic systems, the relief of strain can provide a powerful driving force for rearrangement [188]. The biosynthesis of compound (19) in L. ukambensis possibly follows the same pathway. Although the proposed biosynthetic pathway for the major oxygenated compoundsin L. ukambensis varieties is shown below, it may be that with more biosynthetic studies, closer relationship among these compounds will be found. Camphor (2) for example has been shown to be derived from α- terpineol (14) during biosynthesis [189].

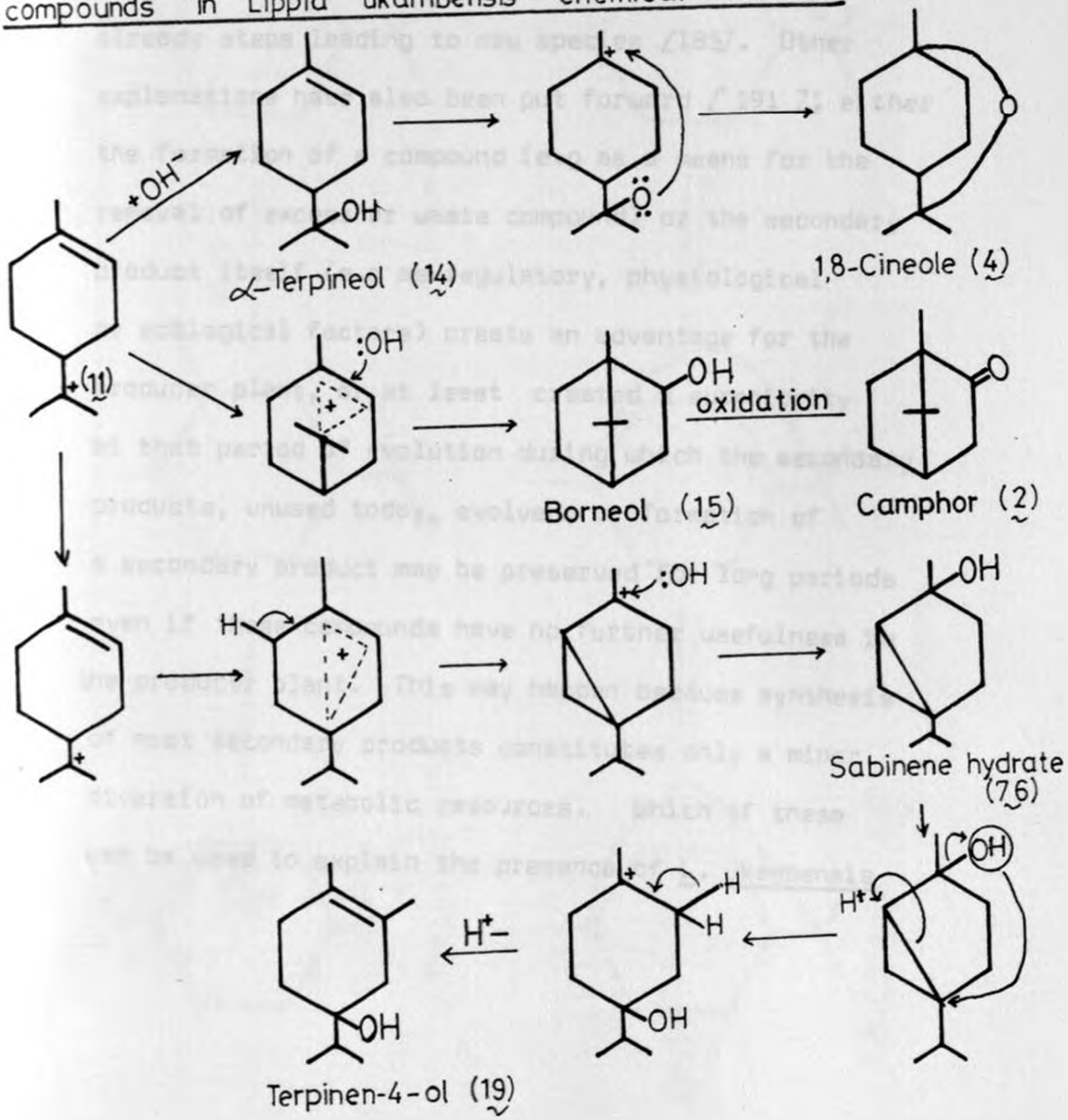
Conversion of sabinene hydrate to terpinen-4-ol by acid



Sabinene hydrate (76)

Terpinen-4-ol (19)

Proposed biosynthetic pathway for the major oxygenated compounds in Lippia ukambensis chemical varieties



α-Terpineol (14)

1,8-Cineole (4)

Borneol (15)

Camphor (2)

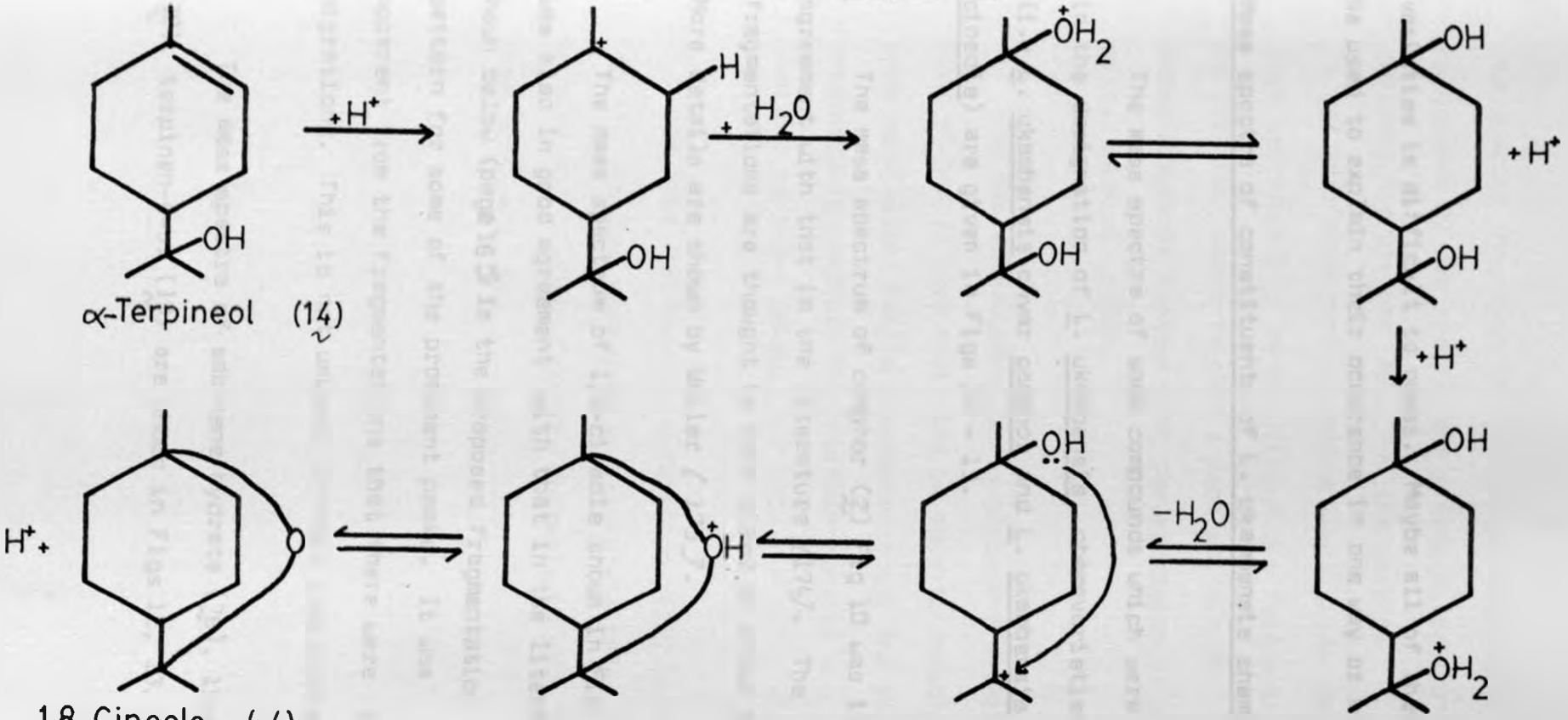
Sabinene hydrate (76)

Terpinen-4-ol (19)

The conversion of compound α-terpineol into 1,8-cineole may be analogous to the known hydration of alkenes followed by dehydration of the diol formed resulting in the production of cyclic ethers [190].

It has been suggested that chemical varieties are normally deviations from the average. According to this opinion, the chemical varieties are actually the representatives of a gradual transition. They are already steps leading to new species [185]. Other explanations have also been put forward [191]; either the formation of a compound (e.g as a means for the removal of excess or waste compound) or the secondary product itself (e.g as regulatory, physiological or ecological factors) create an advantage for the producer plant, or at least created a superiority at that period of evolution during which the secondary products, unused today, evolved; or formation of a secondary product may be preserved for long periods even if these compounds have no further usefulness in the producer plant. This may happen because synthesis of most secondary products constitutes only a minor diversion of metabolic resources. Which of these can be used to explain the presence of L. ukambensis

Conversion of α-terpineol into 1,8-Cineole



α-Terpineol (14)

1,8-Cineole (4)

varieties is difficult to guess. Maybe all of them can be used to explain their occurence in one way or another.

Mass spectra of constituents of L. ukambensis chemovarieties

The mass spectra of some compounds which were used in the designation of L. ukambensis chemovarieties (i.e L. ukambensis chvar camphor and L. ukambensis chvar cineole) are given in Figs 10 - 13.

The mass spectrum of camphor (2) Fig 10 was in agreement with that in the literature [174]. The fragmentations are thought to take place as shown below. More details are shown by Waller [175].

The mass spectrum of 1,8-cineole shown in Fig 11 was also in good agreement with that in the literature [176] shown below (page 165) is the proposed fragmentation pattern for some of the prominent peaks. It was apparent from the fragmentations that there were hydrogen migrations. This is not unusual in mass spectrocospy [173].

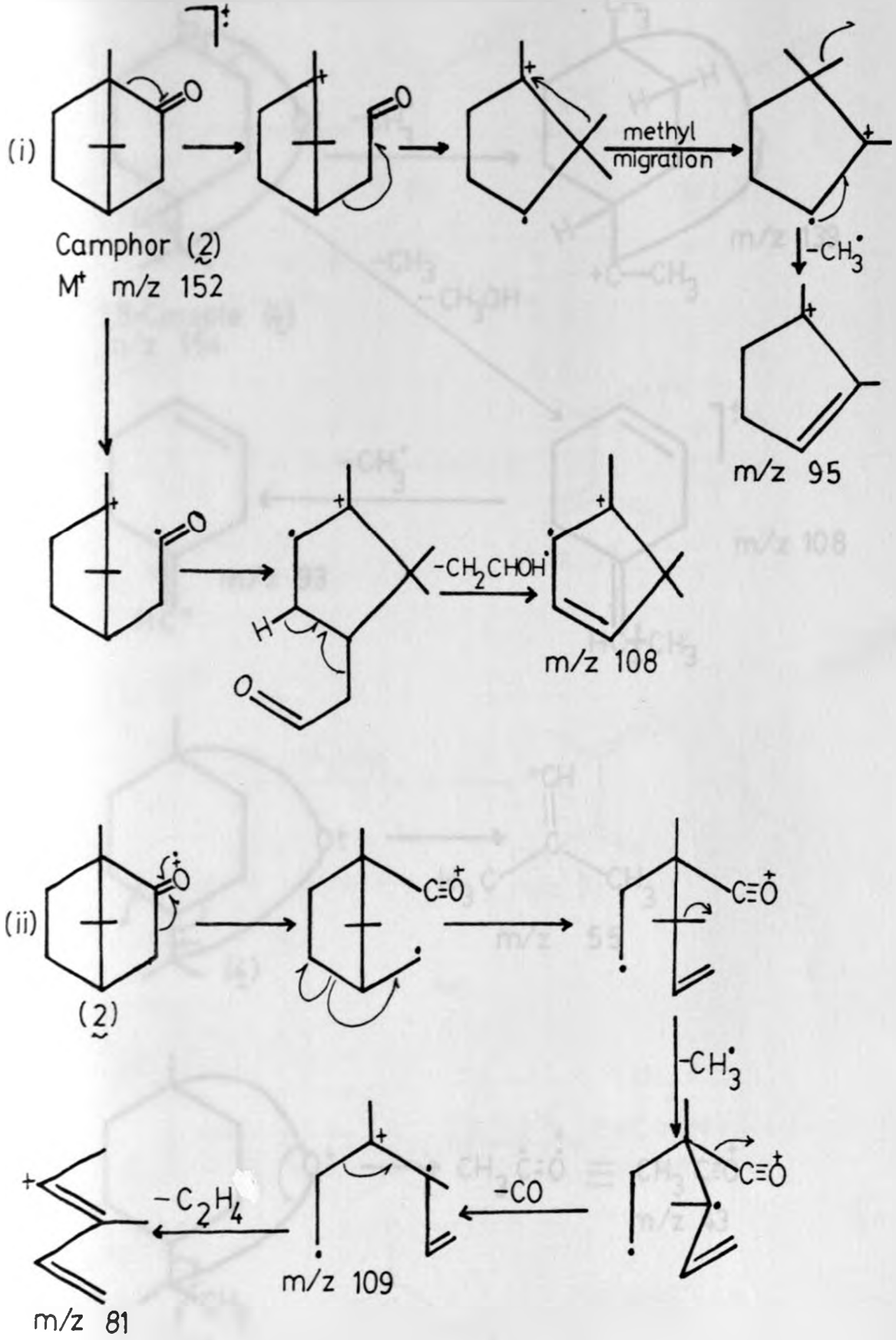
The mass spectra of sabinene hydrate (76), linalool (30), terpinen-4-ol (19) are shown in Figs 12, 13, 14.

The spectra show that the base peak of linalool and terpinen-4-ol was m/z 71 and although that of sabinene hydrate was m/z 43 the peak at 71 was very prominent. Terpinen-4-ol gives the base peak through a retro-Diels-Alder fragmentation on electron impact to give a peak at m/z 86, which fragments further with the expulsion of a methyl radical /192/.

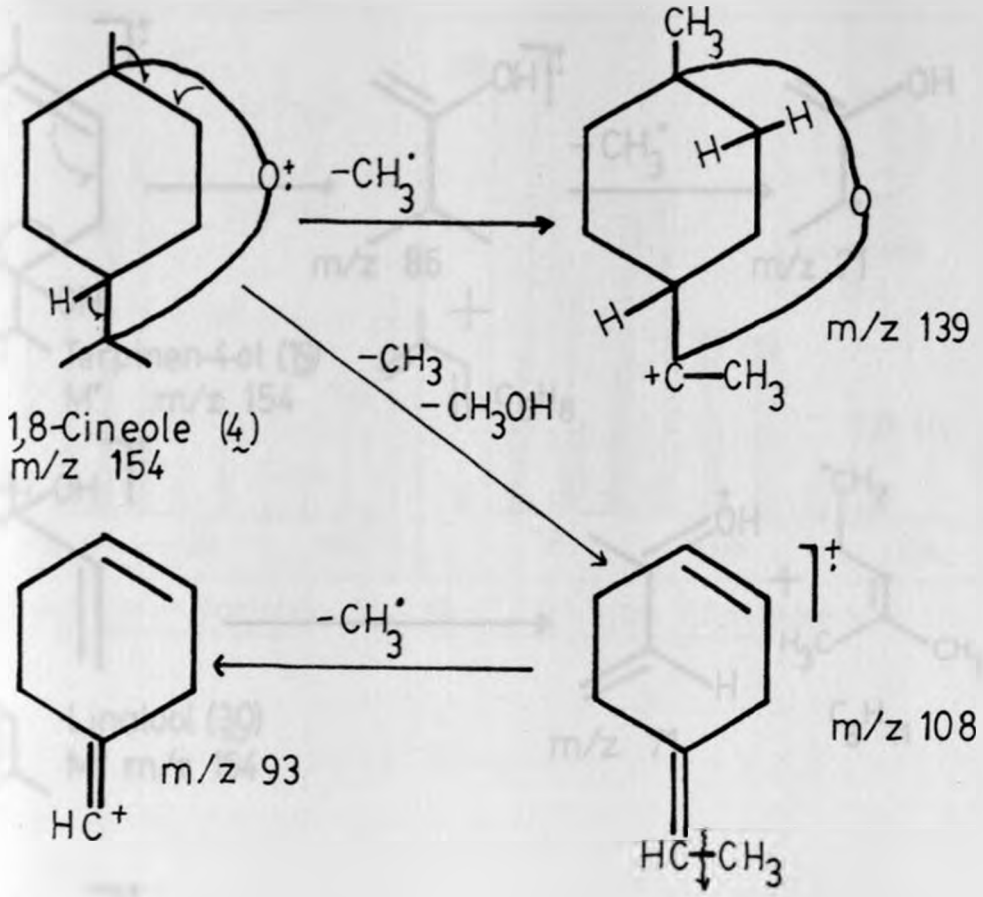
For the other compounds to produce a prominent peak at m/z 71 they must also be able to readily undergo similar facile fragmentations. The proposed fragmentation pattern for sabinene hydrate and linalool is shown below.

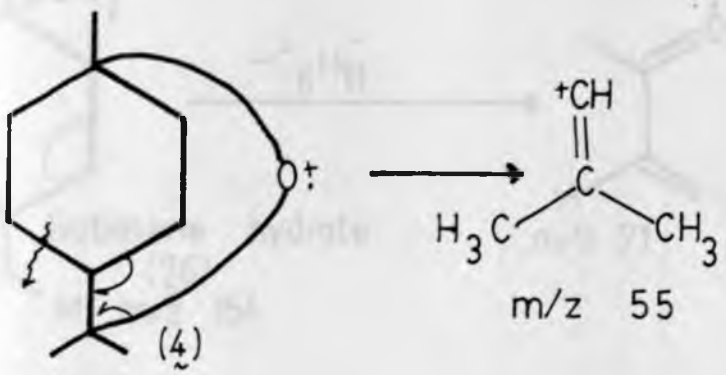
The mass spectrum of ∝-terpineol (14) is shown in Fig 15. It also undergoes a retro-Diels-Alder cleavage to give an ion which further fragments to give the base peak m/z 59.

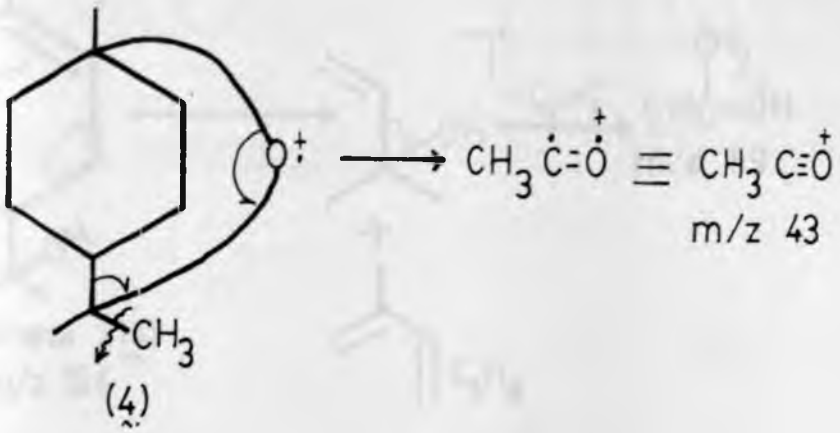
MS fragmentation of camphor



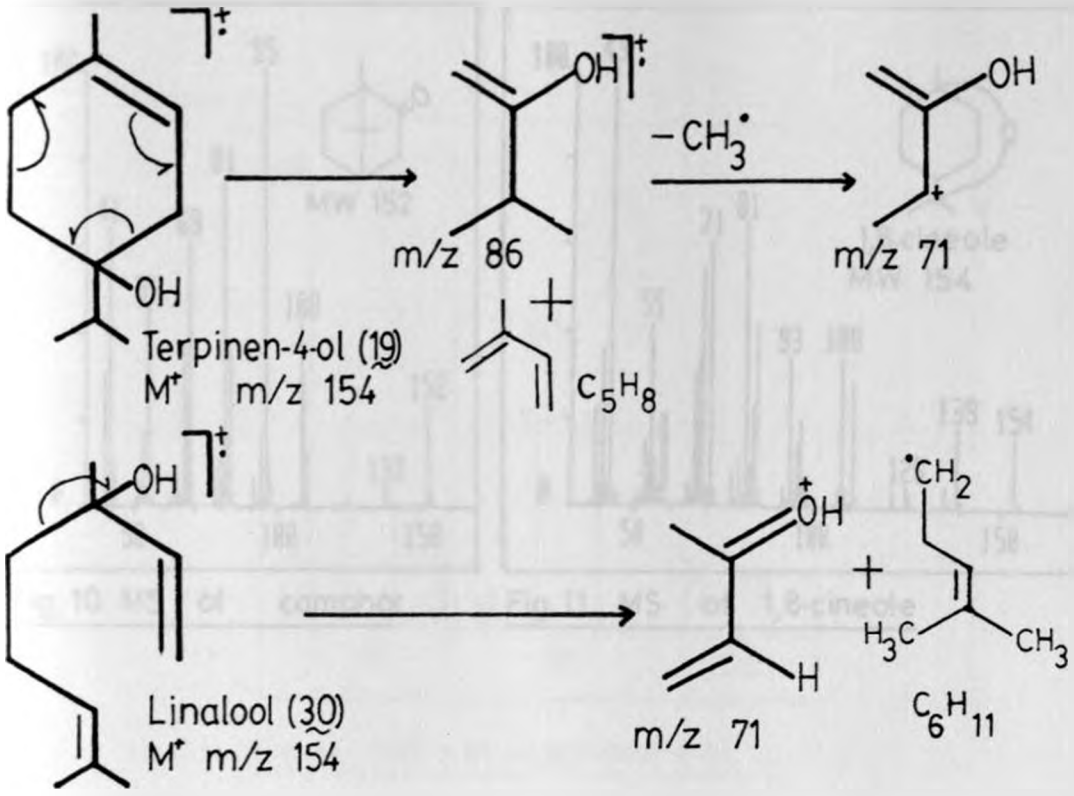
Suggested MS fragmentation of 1,8-cineole

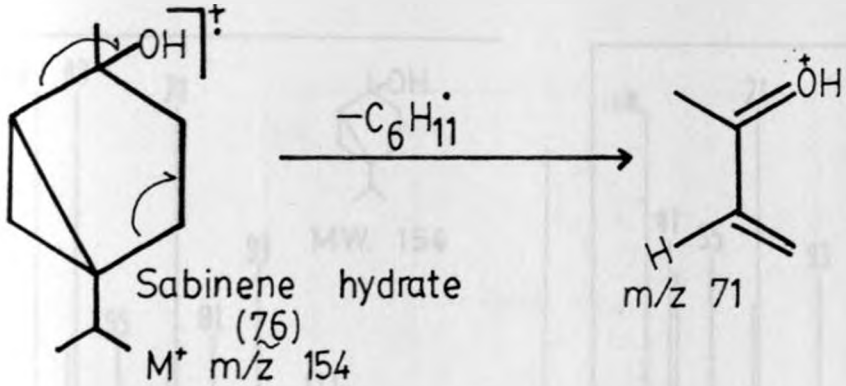


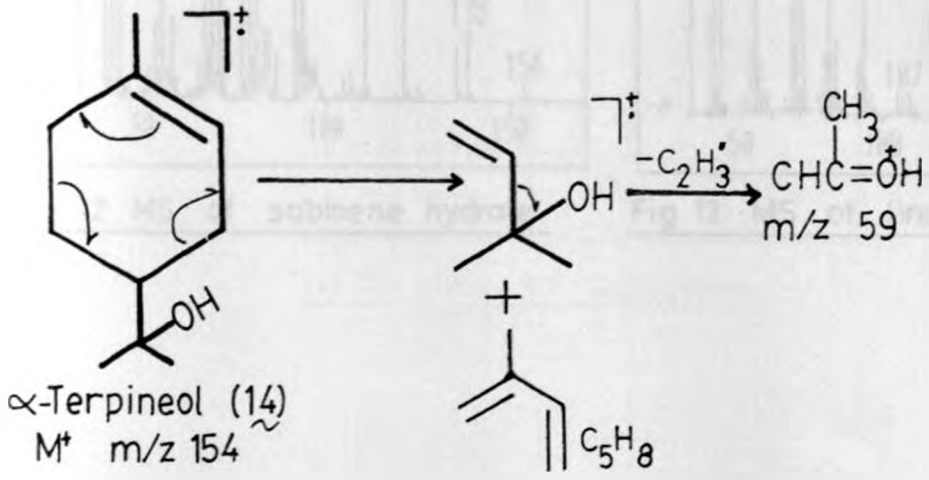




Possible source of m/z 71 in MS of terpinen-4-ol, linalool, sabinene hydrate and m/z 59 in ∝-terpineol







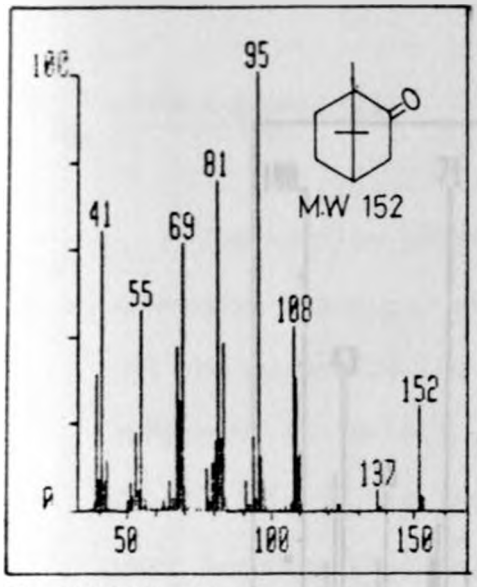


Fig. 10 MS of camphor

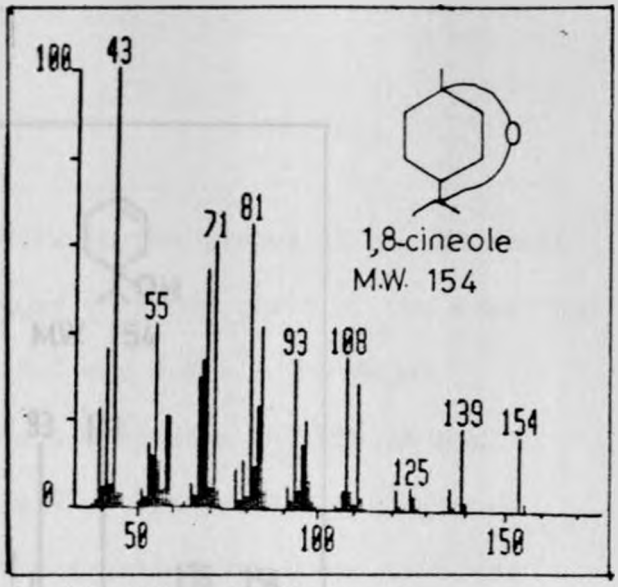


Fig. 11 MS of 1,8-cineole

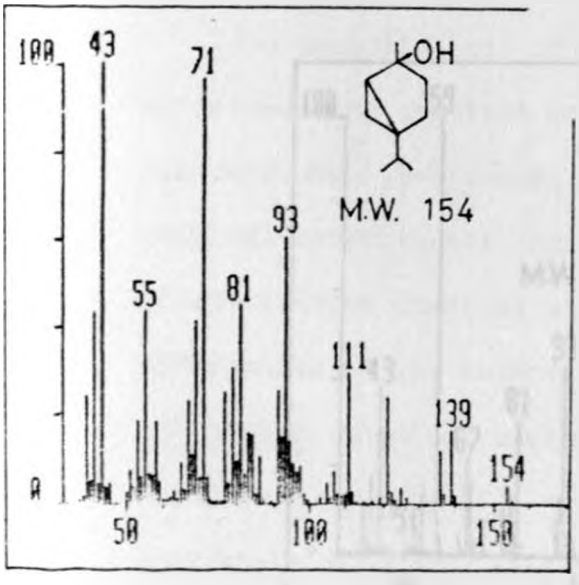


Fig. 12 MS of sabinene hydrate

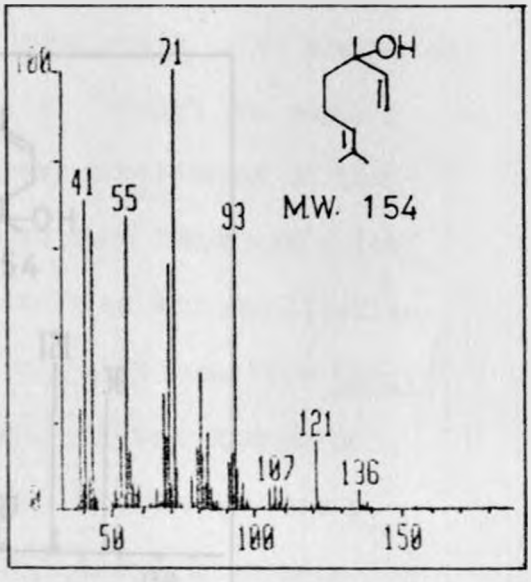


Fig 13 MS of linalool

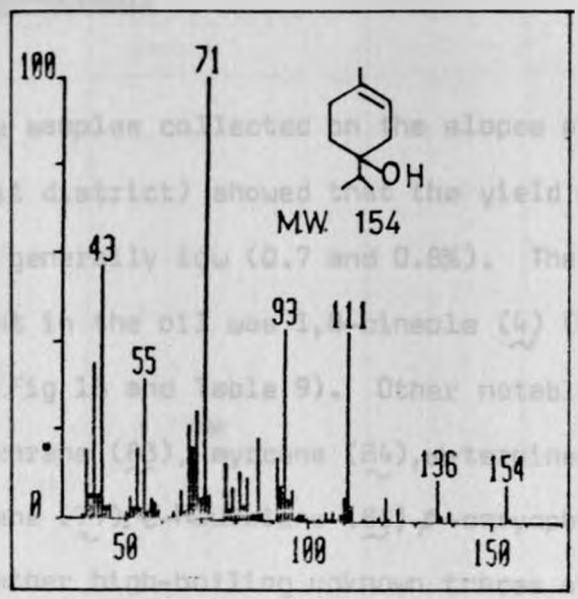


Fig. 14 MS of terpinen-4-ol

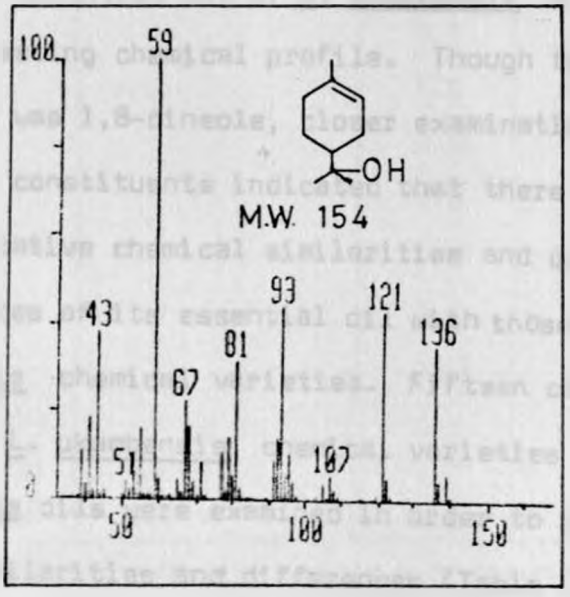


Fig 15 MS of α-terpineol

LIPPIA SOMALENSIS

The samples collected on the slopes of Mt. Marsabit (Marsabit district) showed that the yield of the essential oil was generally low (0.7 and 0.8%). The major component in the oil was 1,8-cineole (4) (26.4% and 37.4%) (Fig 16 and Table 9). Other notable compounds were 3-carene (83), myrcene (84), α-terpineol (14) and β-cubenene (77). ϵ-Muurolene (85) β-caryophyllene (39) and 18 other high-boiling unknown traces of compounds were also detected by the methyl silicone capillary column.

The essential oil of L. somalensis also presented an interesting chemical profile. Though the major compound was 1,8-cineole, closer examination of the chemical constituents indicated that there was a lot of qualitative chemical similarities and quantitative differences of its essential oil with those from Lippia ukambensis chemical varieties. Fifteen compounds found in L. ukambensis chemical varieties and L. somalensis oils were examined in order to compare their similarities and differences (Table 10).

Table 9 Essential oil constituents of Lippia somalensis

Peak No.	Constituent	Identification method	% of constituents in samples I	III	Mean
1	α-pinene	a,b	T	0	-
2	α-thujene	a,b	1.9	2.0	1.95
3	Camphene	a,b,c	T	T	-
4	β-pinene	a,b	T	T	-
5	3-carene	a,b	11.9	11.0	11.45
6	myrcene	a,b	12.3	9.8	11.05
7	β-phellandrene	a	T	T	-
8	limonene	a,b	10.7	8.9	9.8
9	1,8-cineole	a,b	26.4	37.4	31.9
10	β-ocimene	a,b	2.7	1.5	2.1
11	γ-terpinene	a,b	3.0	4.3	3.65
12	p-cymene	a,b	3.6	6.5	5.05
13	unknown		0.3	1.9	1.1
14	unknown		2.2	2.2	2.2
15	unknown		0.56	1.3	0.93
16	unknown		1.2	0.9	1.05
17	terpinen-4-ol	a,b	1.3	1.2	1.25
18	verbenal	b	1.2	1.9	1.55
19	unknown		0.9	T	-
20	p-cymen-8-ol	c	0.3	T	-
21	α-terpineol	a,b	2.7	4.6	3.65
22	β-cubenene	b,c	15	4.6	9.8

T = trace

Sample I = yield 0.7% collected on 27/11/85
II = yield 0.8% collected on 7/2/86
Both samples collected at Mt. Marsabit

Table 10 Comparison of essential oils of L. ukambensis chemical varieties and L. somalensis

Compound	% Mean		
	LU	LUH	LS
% yield of oil	1.81	1.55	0.75
α-thujene	2.0	2.13	1.95
camphene	7.8	0.22	Trace
3-carene	1.03	6.15	11.45
myrcene	1.75	2.02	11.05
limonene	5.2	0.55	9.8
1,8-cineole	Trace	23.7	31.9
β-ocimene	1.64	1.57	2.1
γ-terpinene	1.58	1.34	3.65
p-cymene	1.0	0.44	5.05
camphor	37.3	1.1	Absent
Trans-sabinene hydrate	18.93	24.67	Absent
terpinen - 4-ol	3.97	6.26	1.25
α-terpineol	Absent	9.7	3.65
borneol	4.2	Absent	Absent
β-cubenene	6.59	13.53	9.8

LU - L. ukambensis chvar camphor
LUH - L. ukambensis chvar cineole
LS - L. somalensis

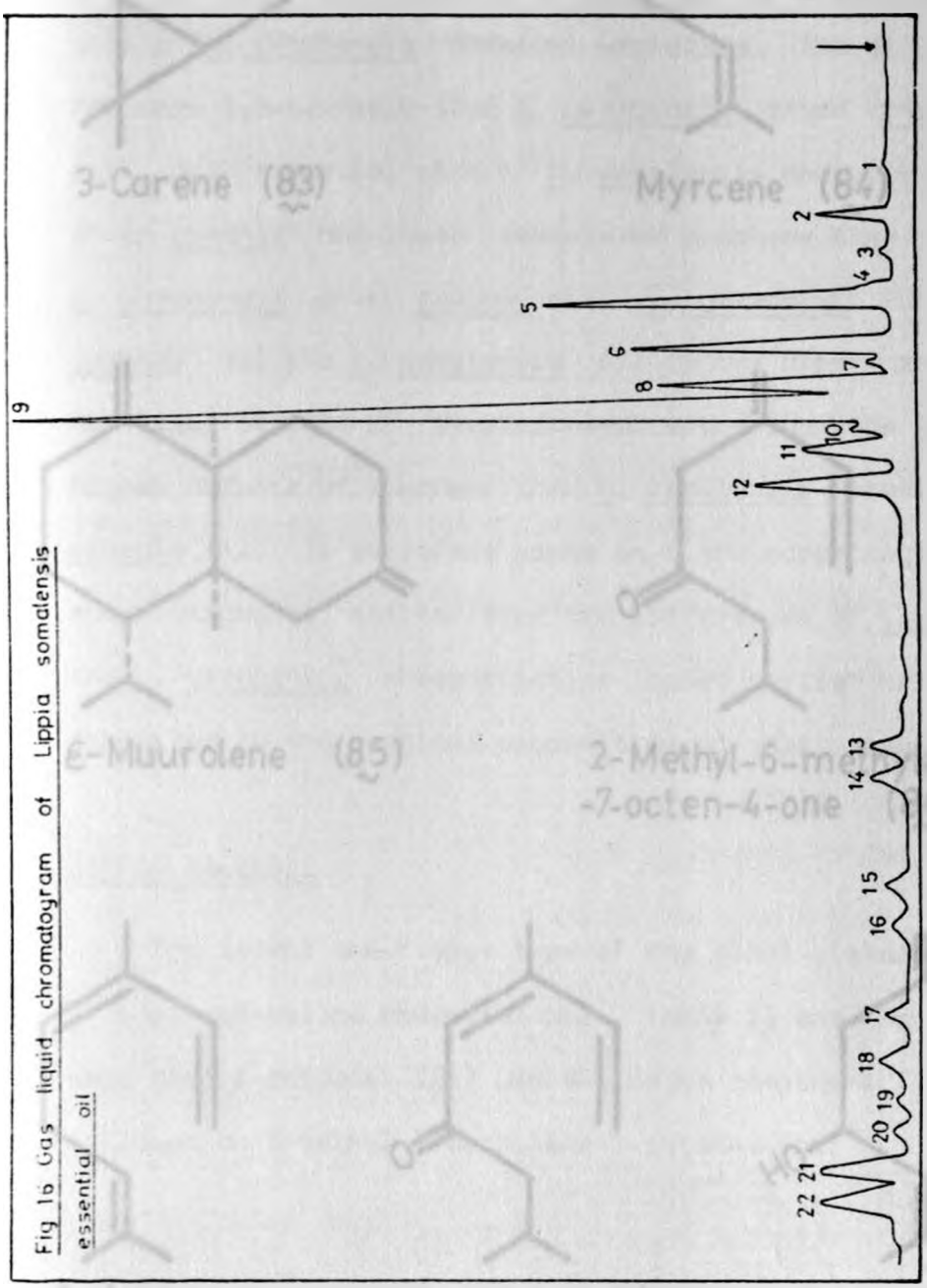
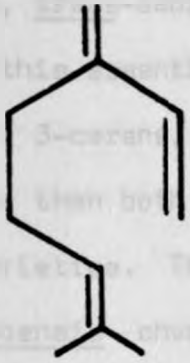
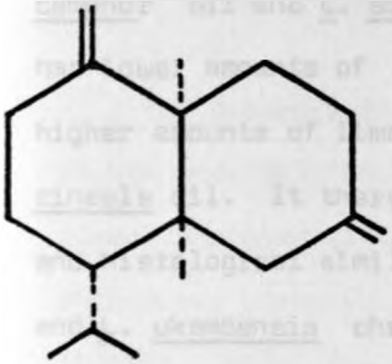


Fig 16 Gas liquid chromatogram of Lippia somalensis essential oil

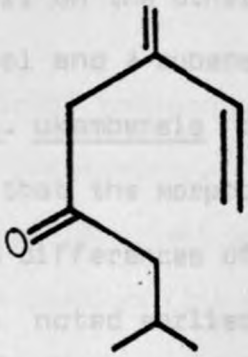
3-Carene (83)



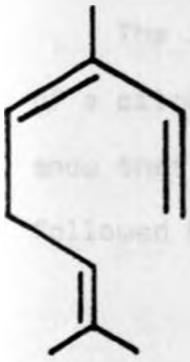
Myrcene (84)



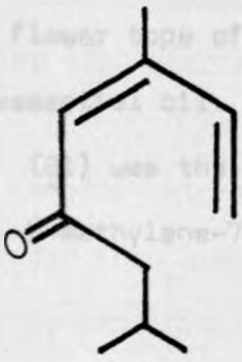
ℰ-Muurolene (85)



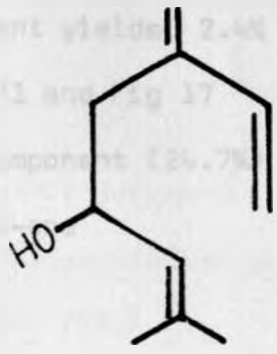
2-Methyl-6-methylene -7-octen-4-one (86)



β-Ocimene (81)



Cis-tagetone (87)



2-Methyl-6-methylene-2,7-octadien-4-ol (88)

The table shows that apart from low yield of essential oil from L. somalensis, no camphor, trans-sabinene hydrate and borneol were found in this essential oil. L. somalensis oil had more myrcene, 3-carene, p-cymene, limonene, β-ocimene and γ-terpinene than both essential oils of L. ukambensis chemical varieties. The oil also had more 1,8-ocineole than L. ukambensis chvar cineole oil. Both essential oils of L. somalensis and L.ukambensis chvar cineole had lower amounts of camphene than in L. ukambensis chvar camphor oil. L. ukambensis chvar camphor oil and L. somalensis oil on the other hand had lower amounts of terpinen-4-ol and β-cubenene but higher amounts of limonene than L. ukambensis chvar cineole oil. It therefore seems that the morphological and histological similarities and differences of L.somalensis and L. ukambensis chemovarieties noted earlier are further exhibited in the chemical composition of their essential oils.

LIPPIA DAUENSIS

The leaves and flower tops of the plant yielded 2.4% of a citrus-yellow essential oil. Table 11 and Fig 17 show that β-ocimene (81) was the major component (24.7%) followed by 2-methyl-6-methylene-7-octen-4-one

(86) (15.7%), myrcene (84) (12.9%), cis-tagetone (87) (11.0%), 2-methyl-6-methylene-2, 7-octadien-4-ol (ipsdienol) (88) (9.4%) and dihydrotagetone (89). Trans-tagetone (90) accounted for only 3.3%. Others identified using the Carbowax 20m capillary column but in small quantities included limonene (42), chrysanthenone (91), p-cymen-8-ol (92), linalool (30), β-caryophyllene (39), terpinen-4-ol (19), thymol (23) carvacrol (24) and 13 other traces of unknown compounds

The presence of high amounts of compounds (88) (9.4%) and (86) (15.7%) is very interesting. The former compound is one of the most important sex pheromones produced by male bark beetles (Ips confusus) [54, 168, 193]. The sex pheromone attracts both sexes but more to the female [153]. This is the first reported work on presence of 2-methyl-6-methylene-2,7-octadien-4-ol (88) from a plant. This compound has always been isolated from the bark beetles (Ips species).

Compound (86) is also interesting. This compound can easily be converted into 2-methyl-6-methylene-7-octen-4-ol (ipsenol) (93) by reduction with $NaBH_4$ [194]. Compound (93) is another important sex attractant pheromone produced by the same male bark beetle [168, 194]. Although

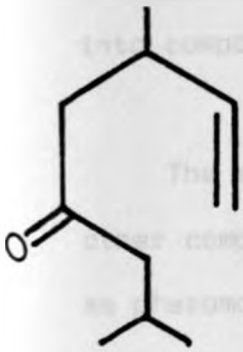
Table 11 Essential oil constituents of Lippia dauensis

Peak No.	Constituent	Identification method	% of constituents in the sample
1	α-thujene	b	2.2
2	3-carene	b	0.8
3	myrcene	a,b	12.9
4	α-terpinene	a,b	3.4
5	β-ocimene	a,b,c	24.7
6	p-cymene	a,b,c	5.8
7	dihydrotagetone	a,b,c	6.8
8	2-methyl-6-methylene-7-octen-4-one	a,b	15.7
9	trans-tagetone	a*, b, c	3.3
10	cis-tagetone	a*, b, c	11.0
11	unknown		1.1
12	2-methyl-6-methylene-2-7-octadien-4-ol	c	9.4
13	α-terpineol	c	1.4
14	β-cubenene	c	1.4

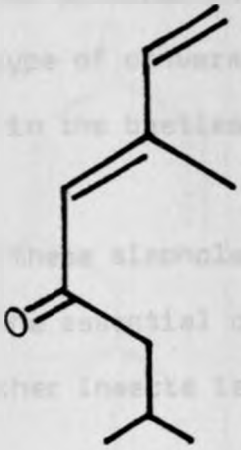
* Essential oil of Tagetes minuta used for peak enhacement of these components /170/.

Sample collected on 9/6/86 at Turbi on Marsabit-Moyale road. Yield 2.4% oil

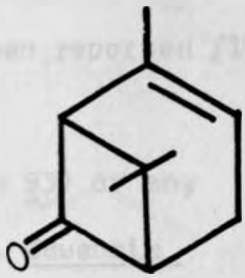
Fig 17 Gas liquid chromatogram of Lippia dauensis essential oil



Dihydrotagetone
(89)

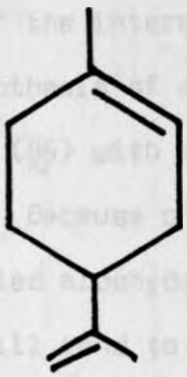


Trans-tagetone
(90)

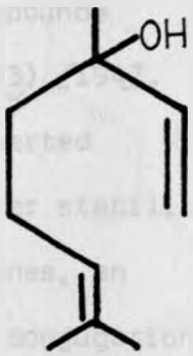


Chrysanthenone
(91)

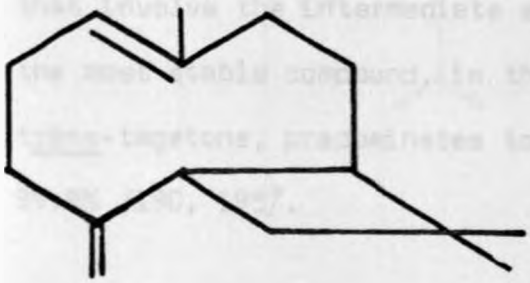
p-Cymen-8-ol
(92)



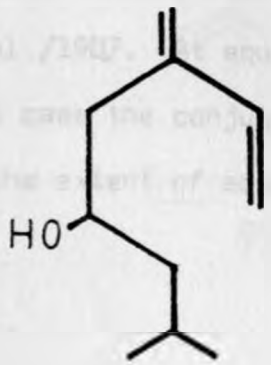
Limonene
(42)



Linalool
(30)



β-Caryophyllene
(39)



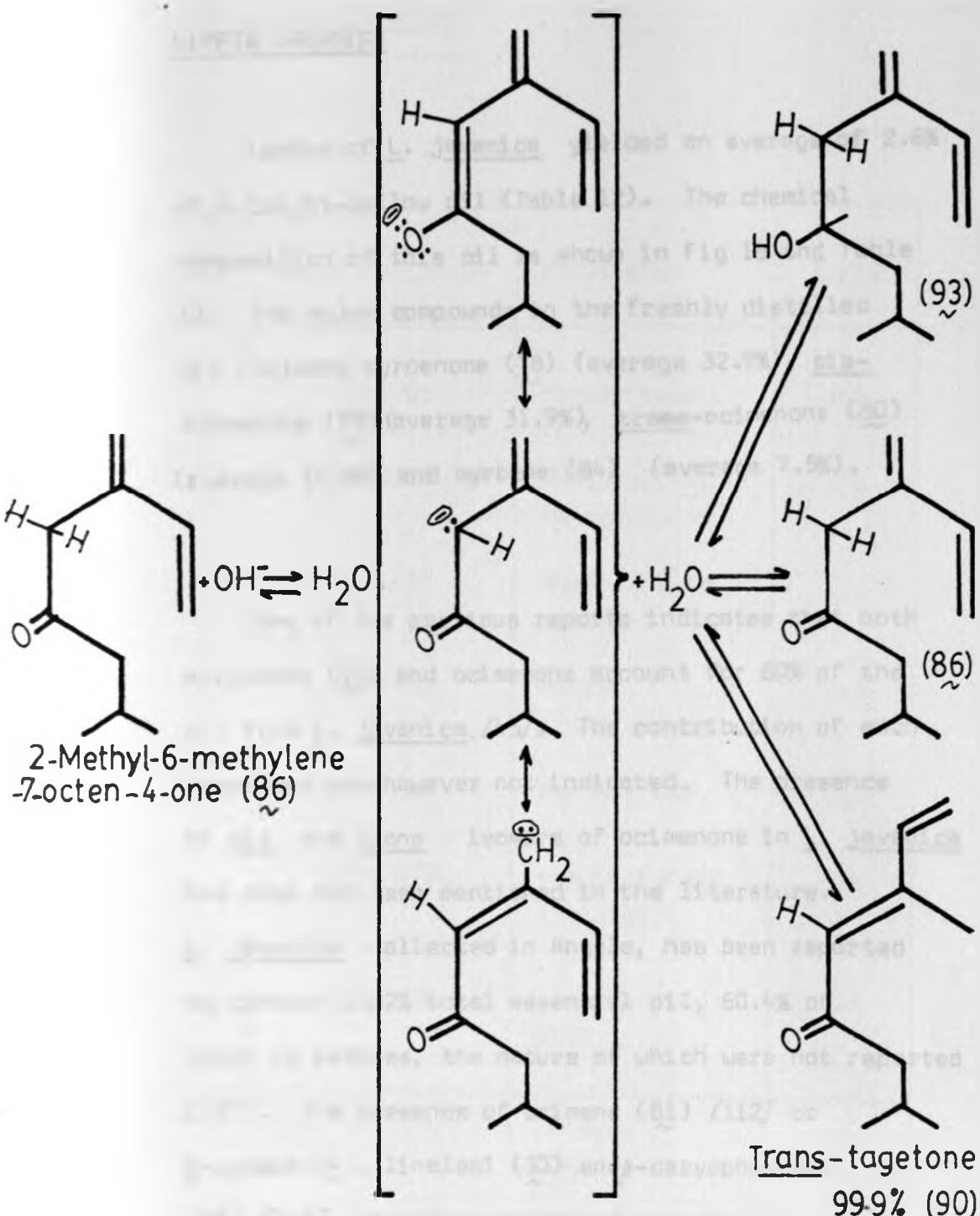
2-Methyl-6-methylene
7-octen-4-ol (93)

this compound was not detected in the essential oil of L. dauensis, this type of conversion of compound (86) into compound (93) in the beetles has been reported [194].

The effect of these alcohols (88 and 93) or any other compound in the essential oil of L. dauensis as pheromones to other insects is still unknown.

The presence of compound (86) has also not been previously reported in plants. This compound is only reported as one of the intermediate compounds during the chemical synthesis of compound (93) [194]. Treatment of compound (86) with alkali converted it into compound (90). Because of the greater stability of conjugated unsaturated aldehydes and ketones, an isolated double bond will tend to move into conjugation if a suitable pathway is available. This double-bond migration is especially facile for double bonds that are $\beta\gamma$ to the carbonyl group by base catalysed reactions that involve the intermediate enol [190]. At equilibrium the most stable compound, in this case the conjugated trans-tagetone, predominates to the extent of about 99.9% [190, 195].

Treatment of 2-Methyl-6-methylene-7-octen-4-one with an alkali



LIPPIA JAVANICA

Leaves of L. javanica yielded an average of 2.6% of a bright-yellow oil (Table 12). The chemical composition of this oil is shown in Fig 18 and Table 13. The major compounds in the freshly distilled oil included myrcenone (78) (average 32.9%), cis-ocimenone (79) (average 31.9%), trans-ocimenone (80) (average 15.8%) and myrcene (84) (average 7.5%).

One of the previous reports indicates that both myrcenone (78) and ocimenone account for 80% of the oil from L. javanica [45]. The contribution of each component was however not indicated. The presence of cis and trans isomers of ocimenone in L. javanica has also not been mentioned in the literature. L. javanica collected in Angola, has been reported to contain 1.02% total essential oil, 60.4% of which is ketones, the nature of which were not reported [157]. The presence of ocimene (81) [112] or p-cymene (34), linalool (30) and β-caryophyllene (39) [142] were not established as major components of the essential oil of L. javanica in any of the samples examined.

Table 12. Collection sites and essential oil content of Lippia javanica

Sample	Date collected	Collection	Yield %
I	18/8/83	Nakuru Town	3.0
II	29/8/83	Mutituni (Machakos)	2.8
III	27/1/85	Ngoina hills (Kericho)	2.5
IV	24/9/85	Kabete (cultivated)	2.3
V	15/9/86	Nairobi Dam	2.6
VI	14/12/86	Umoja Estate (Nairobi)	3.2
VII	30/12/86	Langata Road (Nairobi)	1.8
		Mean	2.6

Table 13 Essential oil constituents of Lippia javanica

Peak No	Constituent	Identification method	% of constituents in samples I	II	III	IV	V	VI	VII	Mean
1	unknown		T	0.1	0	T	T	T	T	
2	β-pinene	a,b	T	1.5	0	T	T	0	T	-
3	myrcene	a,b	8.3	8.2	5.8	5.7	7.0	12.7	4.7	7.5
4	unknown		T	0.4	0	T	T	T	T	-
5	unknown		T	0.2	0	T	0.6	0.6	T	-
6	unknown		T	0.4	T	T	0.3	0.6	T	-
7	2-methyl-6-methylene -7 octen-4-ol	b	0.8	1.9	0	0.8	2.2	1.3	T	-
8	dihydrotagetone	b	0.6	3.6	0	T	0.4	2.6	T	-
9	unknown		1.1	0.6	0	T	0	2.0	1.0	-
10	unknown		2.4	T	0	T	T	0.3	T	-
11	unknown		0	T	0	T	0	0.6	0	-
12	unknown		T	1.1	T	0.4	0.7	1.2	0	-
13	linalool		T	0.5	T	T	0	0.4	0	-
14	trans-tagetone	a*,b	T	4.6	0	T	0.9	1.3	1.5	-
15	cis-tagetone	a*,b,d	1.4	2.9	1.5	1.5	2.5	3.0	1.5	2.0
16	myrcenone	b,d	36	20.9	49.7	39.3	27.3	25.2	32.2	32.9
17	unknown		T	1.2	0	T	T	1.2	1.8	-
18	unknown		T	1.0	0	TO	T	1.3	T	-
19	trans-ocimenone	a*,b,d	11.4	19.3	15.5	13.3	20.6	16.5	14.1	15.8
20	cis-ocimenone	a*,b,d	35.3	26.2	27.8	34.3	35.6	24.9	39.9	31.9
21	unknown		T	2.4	0	T	T	3.3	3.7	-
22	β-caryophellene	a,b,d	T	1.2	0	T	T	T	1.0	-
23	β-cubenene	b	T	1.3	0	T	0	1.0	0	-

T=Traces Essential oil of Tagetes minuta used for peak enhancement of these components [70].

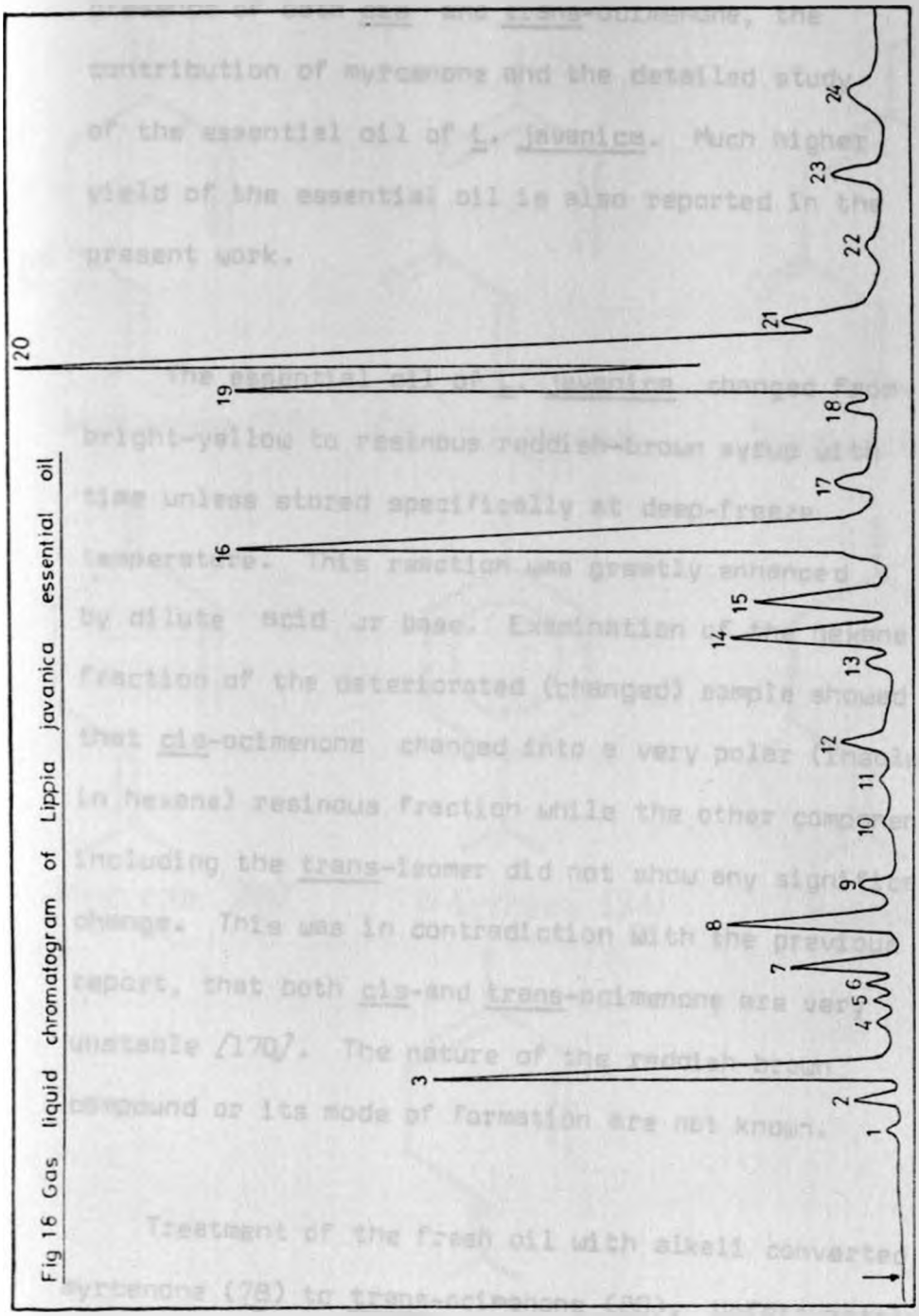
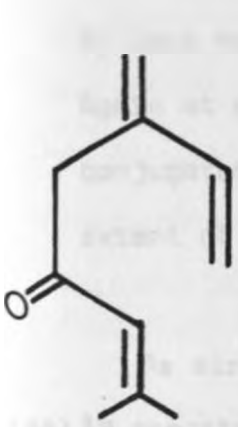


Fig 16 Gas liquid chromatogram of Lippia javanica essential oil

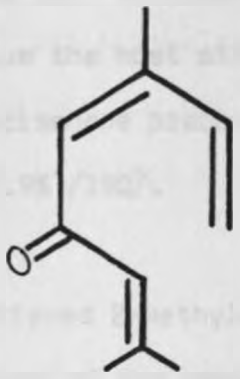
This is therefore the first report to show the presence of both cis and trans-ocimenone, the contribution of myrcenone and the detailed study of the essential oil of L. javanica. Much higher yield of the essential oil is also reported in the present work.

The essential oil of L. javanica changed from bright-yellow to resinous reddish-brown syrup with time unless stored specifically at deep-freeze temperature. This reaction was greatly enhanced by dilute acid or base. Examination of the hexane fraction of the deteriorated (changed) sample showed that cis-ocimenone changed into a very polar (insoluble in hexane) resinous fraction while the other components including the trans-isomer did not show any significant change. This was in contradiction with the previous report, that both cis-and trans-ocimenone are very unstable [170]. The nature of the reddish-brown compound or its mode of formation are not known.

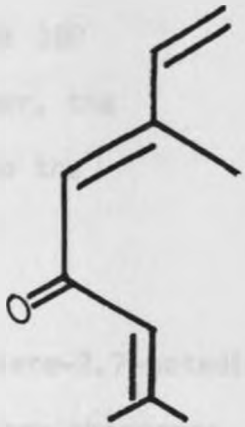
Treatment of the fresh oil with alkali converted myrcenone (78) to trans-ocimenone (80). Unfortunately this reaction also resinifies the cis-ocimenone (79) as has already been mentioned. The reaction mechanism for the



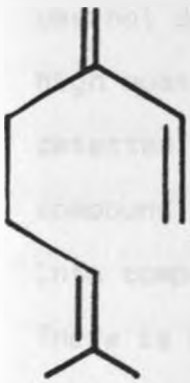
Myrcenone (78)



Cis – ocimenone (79)



Trans–ocimenone (80)



Myrcene (84)

p-Cymene (34)

Linalool (30)

β-Ocimene (81)

conversion of myrcenone into trans-ocimenone by base has already been discussed on page 180 Again at equilibrium the most stable isomer, the conjugated trans-ocimenone predominates to the extent of about 99.9% /190/.

As already mentioned 2-methyl-6-methylene-2,7-octedien-4-ol (88) is reported to be one of the most potent sex pheromone produced by certain bark beetles. Although this compound was not detected in the essential oil of L. javanica, high quantities of myrcenone (average 32.9%) were detected. This compound could be the precursor of compound (88) since myrcenone can easily be converted into compound (88) by reduction even in the laboratory. There is also a possibility that consumption of myrcenone (78) by certain insects leads to its conversion into the sex pheromone in the insect. Similar conversion of myrcene into compound (88) and (93) by various Ips species is known /194/.

Considering the structures of the major compounds found in both L. dauensis and L. javanica, one finds that they are probably very related to each other biogenetically As already mentioned, treatment of myrcenone with alkali produced the more stable trans-ocimenone. Likewise,

treatment of 2-methyl-6-methylene-7-octen-4one (86) produced trans-tagetone. Until the function of these compounds in the plants and their relationship to the overall physiology of the plants are known, it will not be possible to explain why the cis-isomers of these compounds are present in higher quantities in the concerned plants.

The presence of dihydrotagetone (89), cis and trans tagetone (87, 90) and cis and trans-ocimenone (79, 80) in either L. dauensis or L. javanica is probably of chemotaxonomic significance because the only other plants reported to have these components in their essential oils are Tagetes species which belong to the Compositae family [196, 197, 198]. Indeed the main substance in Tagetes glandulifera is cis-tagetone, although small amounts of trans-isomer occur [198], a situation which was also noted for L. dauensis in the present work.

Mass spectra of essential oil constituents of L. dauensis and L. javanica

The mass spectra of some major compounds in the essential oils of L. dauensis and L. javanica are discussed in the following text. These plants, although belonging to different species were found to contain a number of very structurally related ketones. It was therefore necessary to look at their different

fragmentation patterns more closely. The mass spectra of myrcenone (78), trans-ocimenone (80) are shown in Figs 19, 20. The base peak for myrcenone (78) m/z 83 arises from the loss of an alkyl group attached to the carbonyl function leading to an oxonium ion followed by elimination of CO to give m/z 55 peak. This mode of fragmentation is explained in the literature [192]. The base peak at m/z 135 in trans -ocimenone (80) (Fig 20) arises from the loss of a methyl group. M^+ (m/z 150) is prominent due to the stability of this compound conferred by the extended conjugation. The cleavage of the bonds adjacent to the carbonyl group was responsible for peaks m/z 83, 55, 95 and 67. This fragmentation pattern agreed very well with the reported literature [170] which indicated that the fragmentation was m/z 150 (77%), 135 (100%), 107 (58%), 95 (43%), 91 (55%), 83 (51%), 67 (58%), 55 (60%), 41 (58%), 39 (80%)

The mass spectra of 2-methyl-6-methylene-7-octen-4-one (86), cis-tagetone (87) and dihydrotagetone are shown in Figs 21, 22, 23. The base peak m/z 85 from compound (86) arises due to the formation of the oxonium ion.

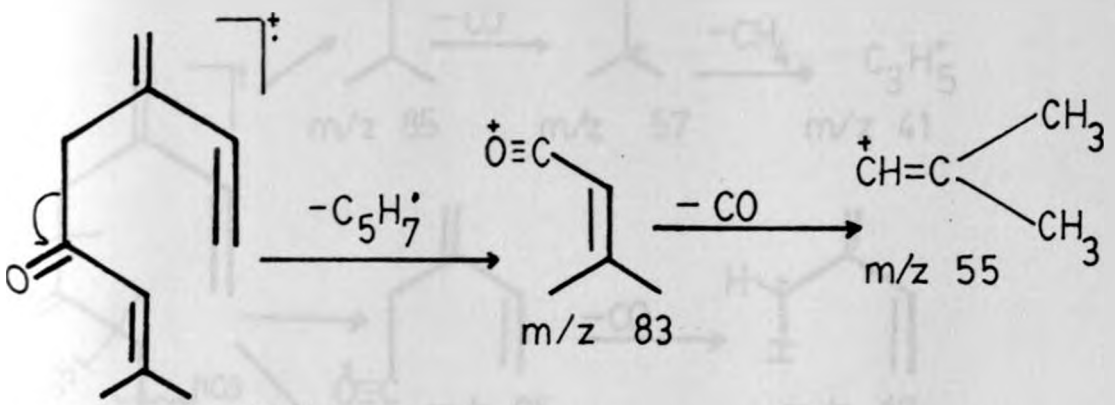
The base peak of cis-tagetone (87) m/z 95 (Fig 22) arises through the same fragmentation method as that of compound (86). Peak m/z 137 was due to loss of CH_3 group.

The spectrum of compound 87 (Fig 22) generally agreed with the reported literature [170] which had shown the fragmentation as m/z 152(10%),151 (4%), 137 (8%), 110 (6%), 109 (20%), 95 (100%), 67 (62%), 65 (14%) 41 (60%), 44 (41%).

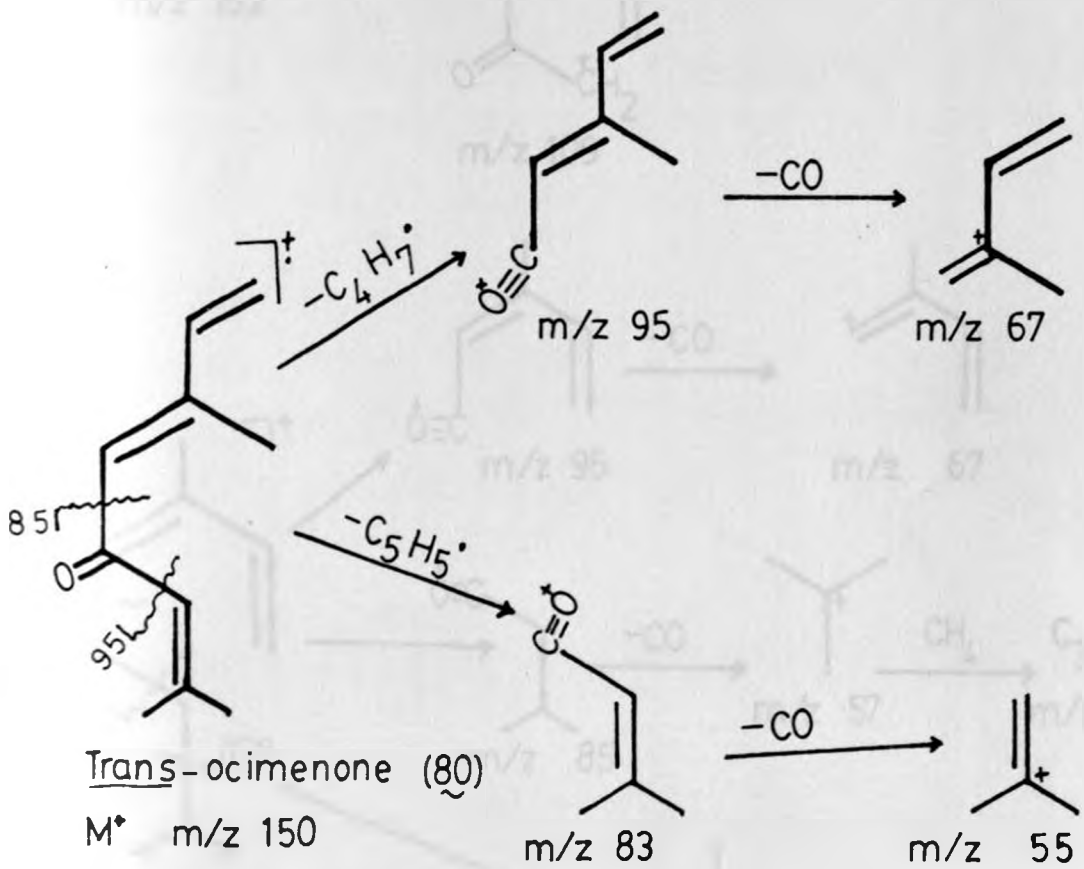
The mass spectrum of dihydrotagetone (89) is shown in Fig. 23. The base peak m/z 85 was due to the formation of the oxonium ion while the source of the others was as explained for compounds (80), (86) and (87). The fragmentation pattern of compound (89) generally agreed with published data [170] which showed the important peaks from this compound to be at m/z 154 (5%), 97 (23%), 85 (100%), 69 (35%), 57 (64%), 55 (27%), 43 (13%), 41 (27%).

The mass spectrum of 2-methyl-6-methylene-2, 7-octadien-4-ol (88) is shown in Fig 24. The base peak m/z 85 in this compound arises due to the formation of

MS fragmentation of myrcenone and trans-ocimenone

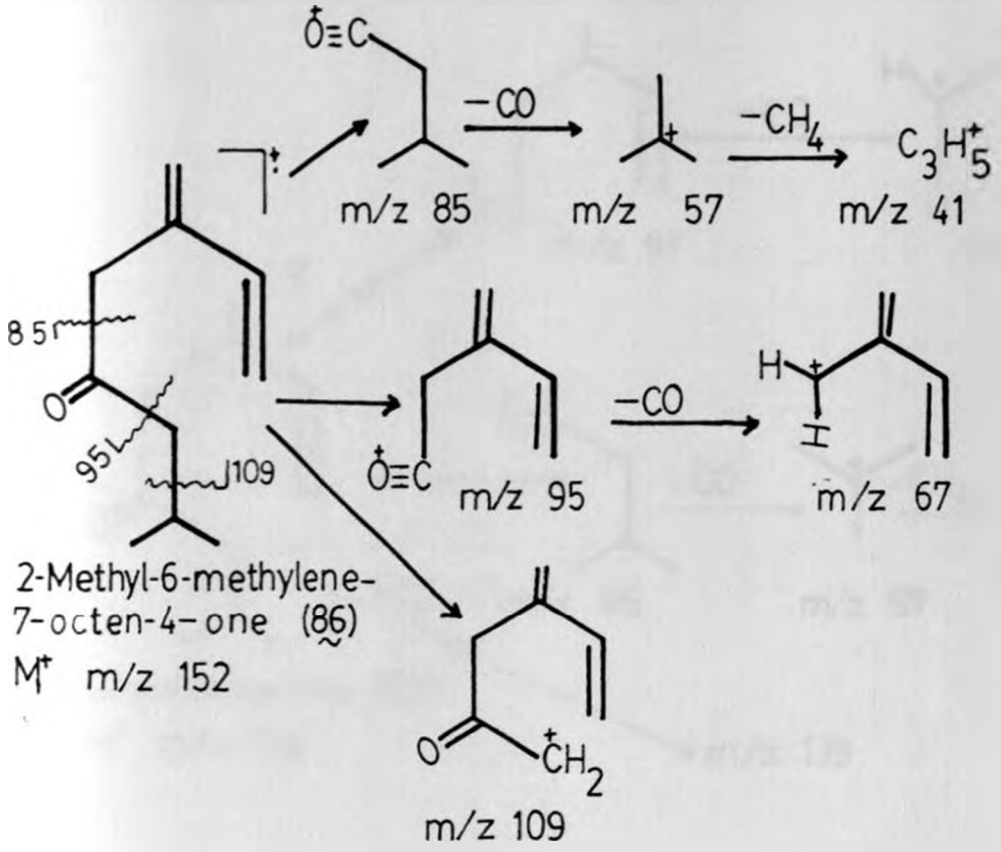


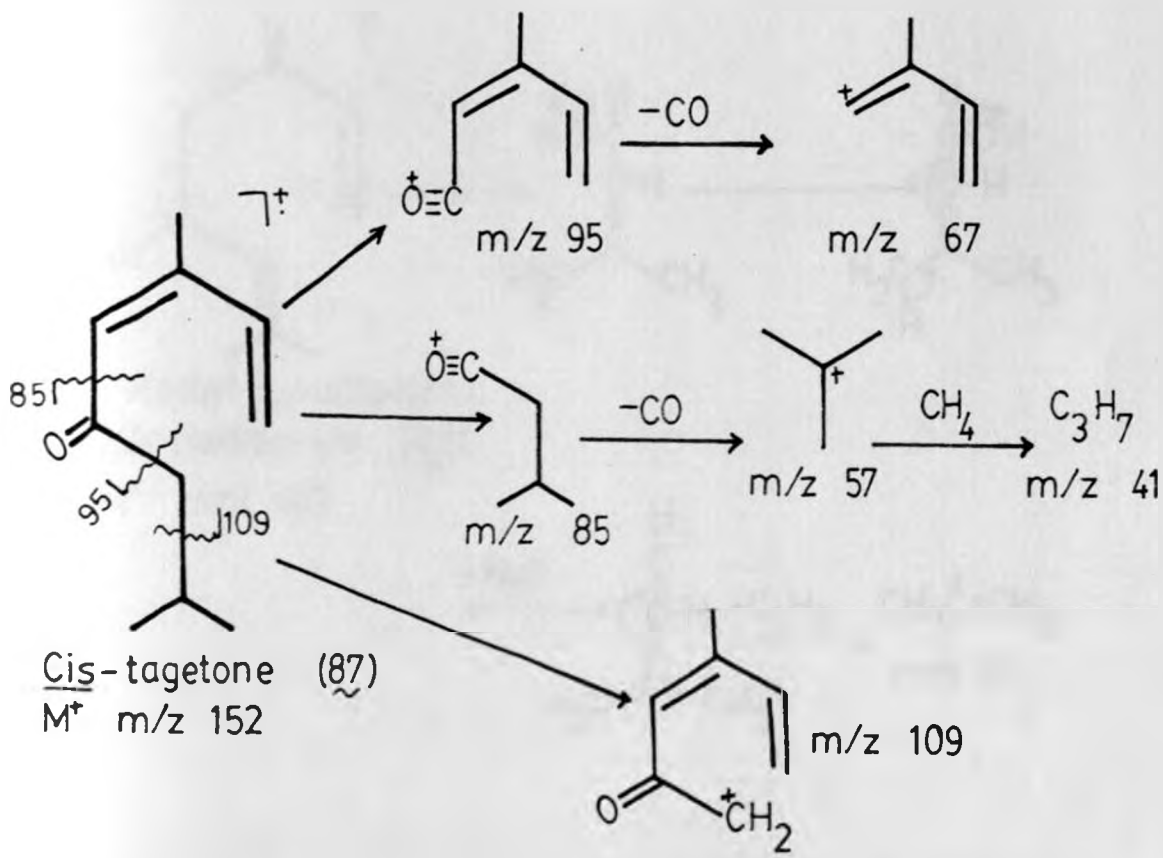
Myrcenone (78)
M^+ m/z 150



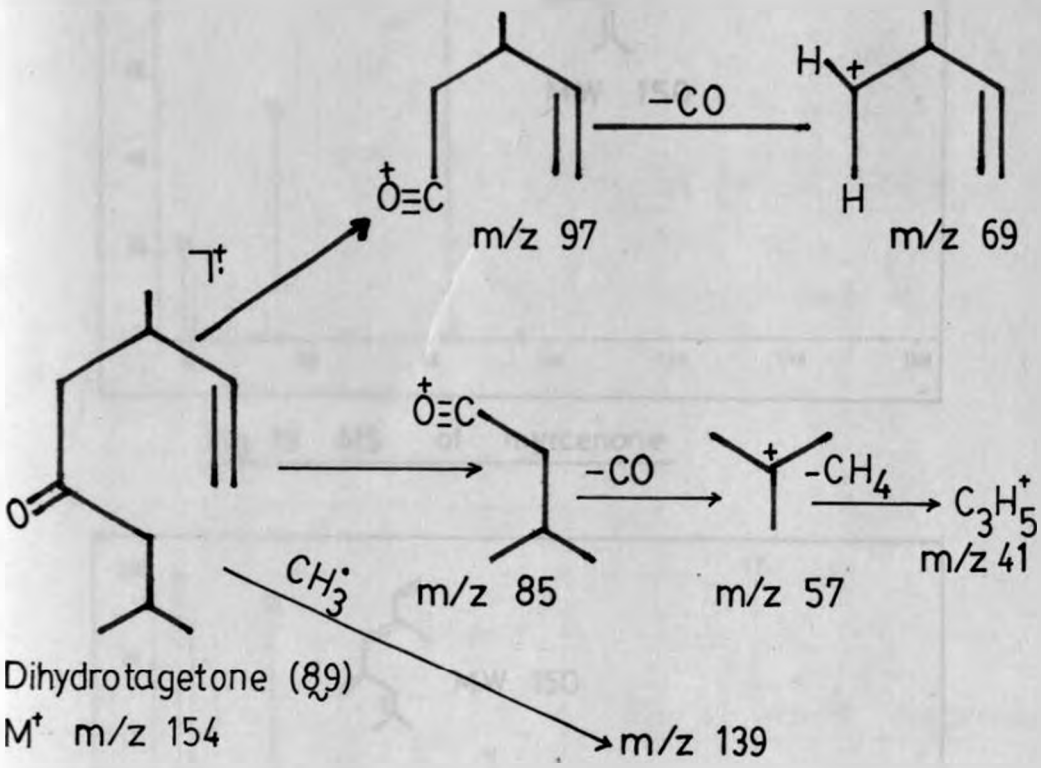
Trans-ocimenone (80)
M^+ m/z 150

MS fragmentation of 2-methyl-6-methylene-7-octen-4one and cis-tagetone

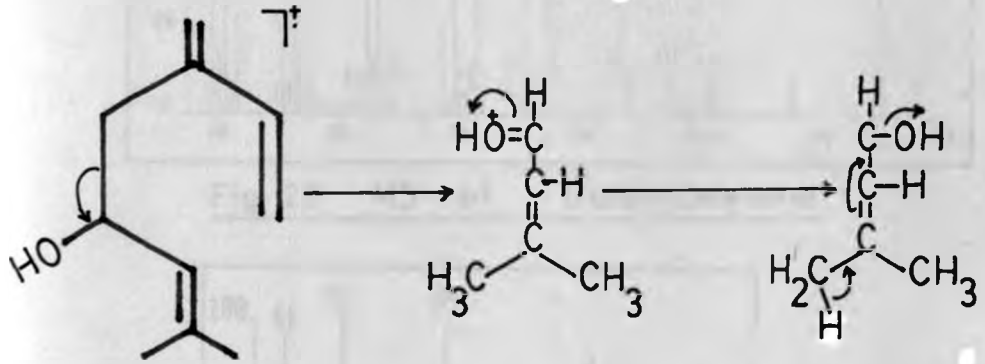




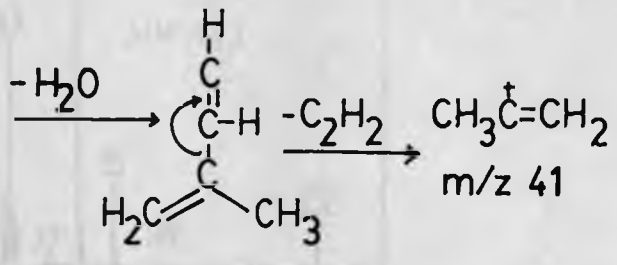
MS fragmentation of dihydrotagetone and 2-methyl-6-methylene-2,7-octadien-4-ol



Dihydrotagetone (89)
M+ m/z 154



2-Methyl-6-methylene
-2,7-octadien-4-ol (88)
M+ m/z 150



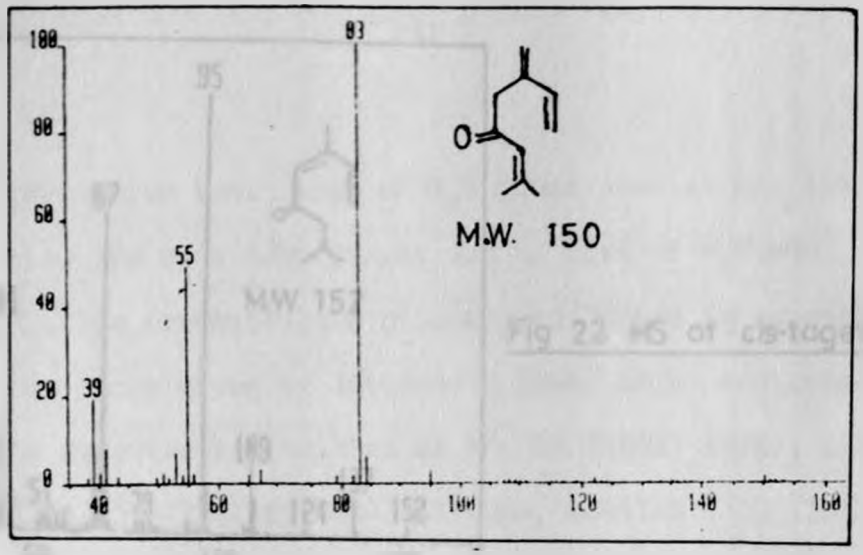


Fig. 19 MS of myrcenone

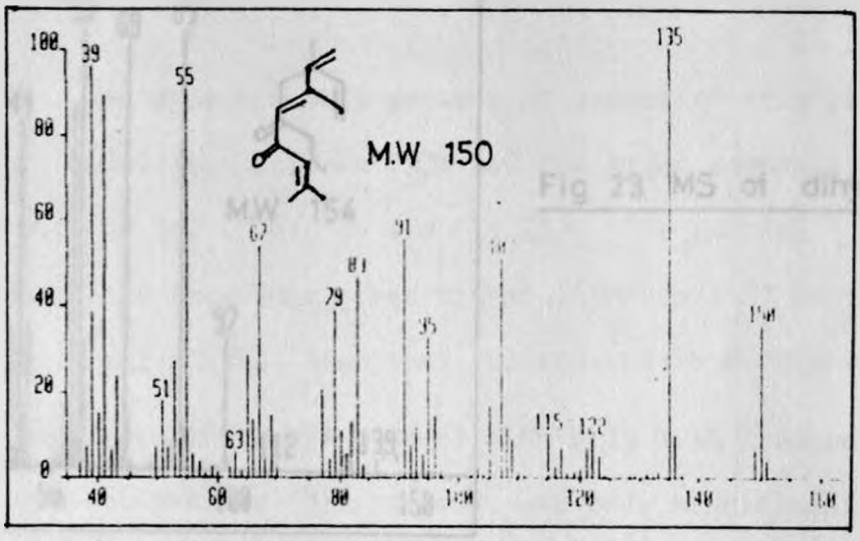


Fig. 20 MS of trans-ocimenone

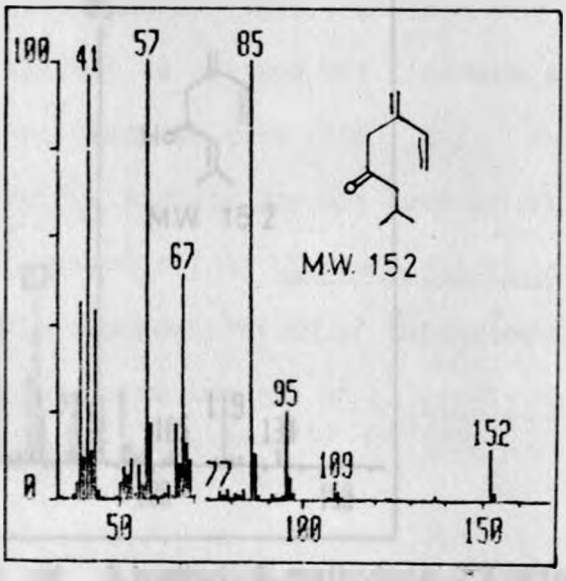


Fig. 21 MS of 2 methyl-6-methylene-7-octen-4-one

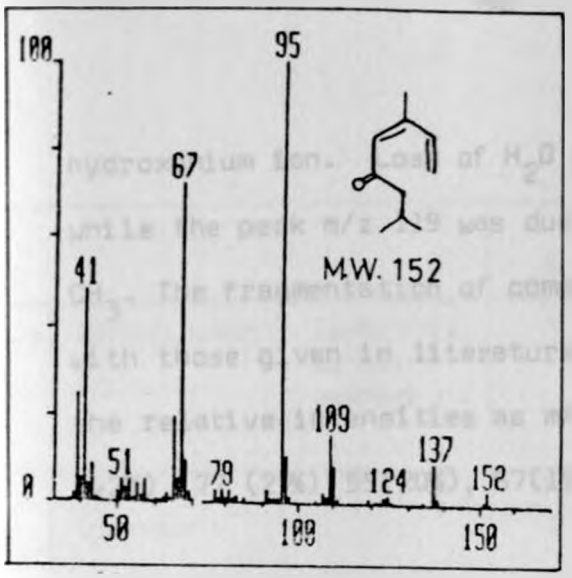


Fig 22 MS of cis-tagetone

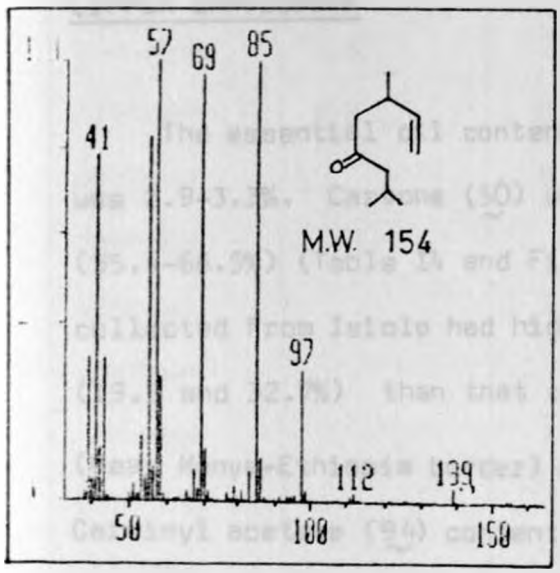


Fig 23 MS of dihydrotagetone

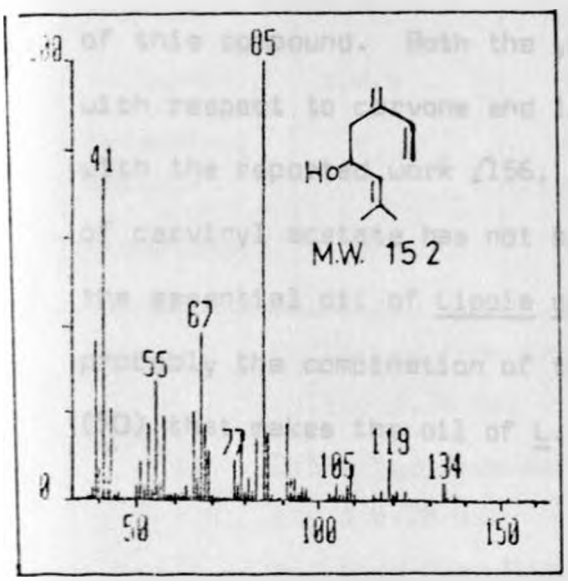


Fig 24 MS of 2-methyl-6-methylene-2,7-octadien-4-ol

hydroxonium ion. Loss of H_2O gives peak at m/z 134 while the peak m/z 119 was due to loss of H_2O and CH_3. The fragmentation of compound(88)was in agreement with those given in literature [168] which indicated the relative intensities as m/z 85 (100%) (71%), 119 (42%), 77 (27%) 55(20%), 67(19%), 134(18%), 105(16%).

LIPPIA CARVIODORA

The essential oil content of leaves of this plant was 2.9-3.3%. Carvone (50) was the major component (55.4-66.5%) (Table 14 and Fig 25). The samples collected from Isiolo had higher limonene (42) content (19.5 and 32.7%) than that collected from Furole-Turbi road (Near Kenya-Ethiopia border) with only 2.4% limonene. Carvinyl acetate (94) content was only significant in samples I and III with 10.6% and 19.3% respectively of this compound. Both the yield and chemical composition with respect to carvone and limonene seemed to agree with the reported work [156, 164]. However, the presence of carvinyl acetate has not been previously reported in the essential oil of Lippia carviodora. It is probably the combination of this compound and carvone (50) that makes the oil of L. carviodora have such a

Table 14 Essential oil constituents of Lippia carviodora

Peak Nos	Constituent	Identification method	% of constituents in samples I	II	III
1	unknown		0.5	0	0
2	unknown		0.5	0	0
3	p-cymene	a,d	1.1	5.7	T
4	limonene	a,d	19.5	32.7	2.4
5	unknown		5.4	1.4	0.7
6	unknown		0	0	0.7
7	unknown		0.5	0	4
8	unknown		0.5	0	2
9	unknown		0.5	0.9	2
10	piperitenone	d	1.6	1.4	0.7
11	unknown		0.5	T	1.2
12	unknown		0.2	T	1.2
13	d-carvone	a,d,e	58.1	55.4	66.5
14	I-carvinyl acetate	d	10.6	0	19.3
15	∝-copaene	d	0.5	0	0

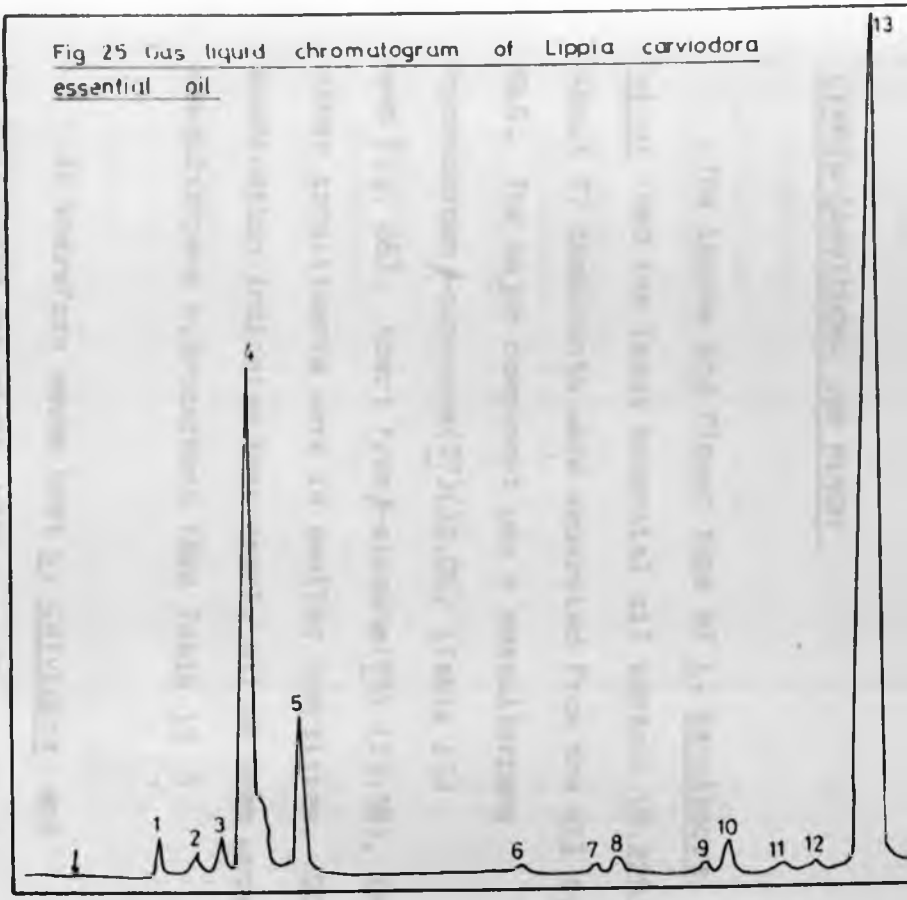
Sample I - Collected from Archers Post (Isiolo) 5/9/85 yield 3.3% oil

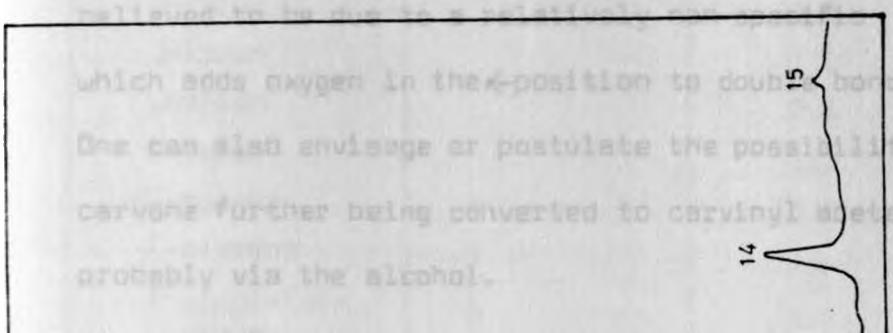
II - Collected from Buffalo Springs (Isiolo) 24/11/85, yield 2.8% oil

III - Collected from Furole (Kenya-Ethiopia border) yield 2.9% oil

T = trace

Fig 25 Gas liquid chromatogram of Lippia carviodora essential oil




15
14

pleasant odour.

Carvone is postulated to be biosynthesised from limonene [9]. The addition of an oxygen atom is believed to be due to a relatively non-specific enzyme which adds oxygen in the α-position to double bonds. One can also envisage or postulate the possibility of carvone further being converted to carvinyl acetate, probably via the alcohol.

LIPPIA CARVIODORA VAR MINOR

The leaves and flower tops of L. carviodora var minor had the least essential oil content (0.22%). About 27 components were separated from the oil by GLC. The major component was a sesquiterpene hydrocarbon β-cubenene(77)(32.0%) (Table 15) and Fig. 26). Apart from β-elemene(95) (13.5%), the other constituents were in smaller quantities. GC/MS examination indicated that nearly all of them were sesquiterpene hydrocarbons (See Table 15)

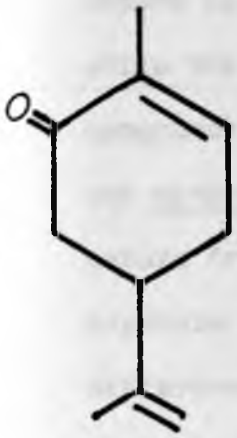
It therefore seems that L. carvidora and L. carviodora var minor have very fundamental differences in respect of their essential oil content and chemical composition. While L. carviodora

Table 15 Essential oil constituents of Lippia carviodora var minor

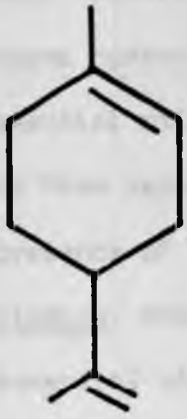
Peak No	Constituent	Identification method	% of constituents in the sample
1	limonene	a,b	1.3
2	unknown		1.5
3	unknown		1.0
4	unknown		0.4
5	unknown		1.3
6	α-copaene	b	3.9
7	β-elemene	b	13.7
8	β-trans-farnesene	b	2.2
9	unknown		0.4
10	α-cedrene	b	4.6
11	α-humulene	a,b	5.6
12	β-caryophyllene	a,b	2.5
13	unknown		2.1
14	d-γ-cadinene	b	2.8
15	β-cubenene	b	32.0
16	γ-muurolene	b	5.9
17	unknown	b	7.2
18	unknown		0.5
19	δ-cadinene	b	3.1
20	unknown		0.8
21.	γ-patchoulene	b	1.0
22	unknown		2.0
23	unknown		1.6
24	unknown		0.7
25	unknown		0.8
26	cadinol	b	0.3
27	unknown		0.5

Collected on 15/12/85 in Tsavo West National Park, yield 0.2% oil

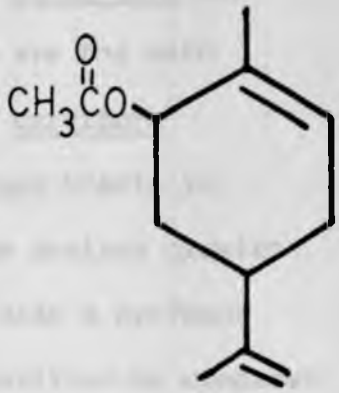
Fig 26 Gas liquid chromatogram of Lippia carviodora var minor essential oil



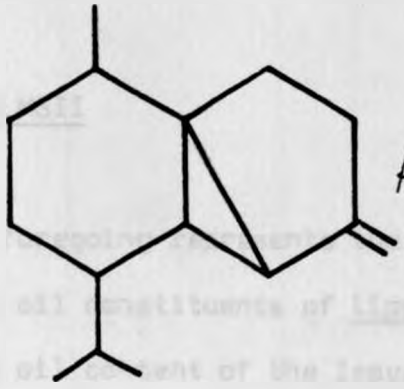
Carvone
(50)



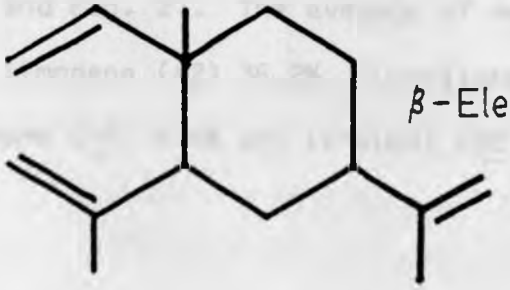
Limonene
(42)



Carvinyl acetate
(94)



β-Cubenene (77)



β-Elemene (95)

contains a substantial amount of oil, L. carviodora var minor has scanty oil. Carvone (50), a monoterpene ketone is the dominant feature in L. carviodora oil while the sesquiterpene hydrocarbons are the main compounds in the essential oil of L. carvidora var minor. So apart from very enlarged bracts in mature fruits and presence of calcium oxalate cluster crystals in L. carviodora, there is also a profound difference in its essential oil composition as compared with that of L. carviodora var minor. The presence of essential oil in L. carviodora var minor and its composition is reported for the first time in the present work.

LIPPIA WILMSII

The foregoing represents the first report on the essential oil constituents of Lippia wilmsii. The essential oil content of the leaves of this plant was 1.6% on average. The chemical composition is shown in Table 16 and Fig. 27. The average of major constituents included limonene (42) 36.2%, Piperitone (45) 27.2%, Piperitenone (96) 9.4% and linalool (30), 7.2%.

Table 16 Essential oil constituents of Lippia wilmsii

Peak No	Constituent	Identification method	% of constituents in the samples I	II	III	IV	MEAN	SEM
1	unknown		0.7	0.5	1.0	T	0.6	± 0.18
2	unkown		T	T	1.1	T	-	-
3	unknown		0.7	0.7	1.1	T	0.6	± 0.20
4	β-phellandrene	a, d	3.0	3.4	1.2	1.6	2.3	± 0.46
5	limonene	a, d	33.7	39.0	29.9	42.3	36.2	± 2.38
6	1,8-cineole	a,b,c	5.3	3.4	2.7	T	2.9	± 0.95
7	p-cymene	a,b	5.2	3.1	2.7	6.0	4.3	± 0.7
8	γ-terpinene	a,d	3.3	3.6	6.0	T	3.2	± 1.07
9	unknown		3.3	1.8	1.9	T	1.8	± 0.59
10	linalool	a,b,d	8.1	5.8	5.6	9.1	7.2	± 0.75
11	unknown		T	T	T	1.4	-	-
12	unknown		T	T	T	1.4	-	-
13	α-terpineol	a,d	T	T	T	T	-	-
14	unknown		T	T	T	T	-	-
15	piperitone	a,d,b	24.0	24.6	35.6	24.6	27.2	± 2.43
16.	unknown		T	T	0	T	-	-
17.	piperitenone	b,d	9.3	7.0	11.0	10.3	9.4	± 0.76
18.	β-elemene	d,b	T	T	T	T	-	-

All samples were collected from the slopes of Menengai Crater, (Nakuru)

Samples: I - collected on 24/11/84 yield 2.2%

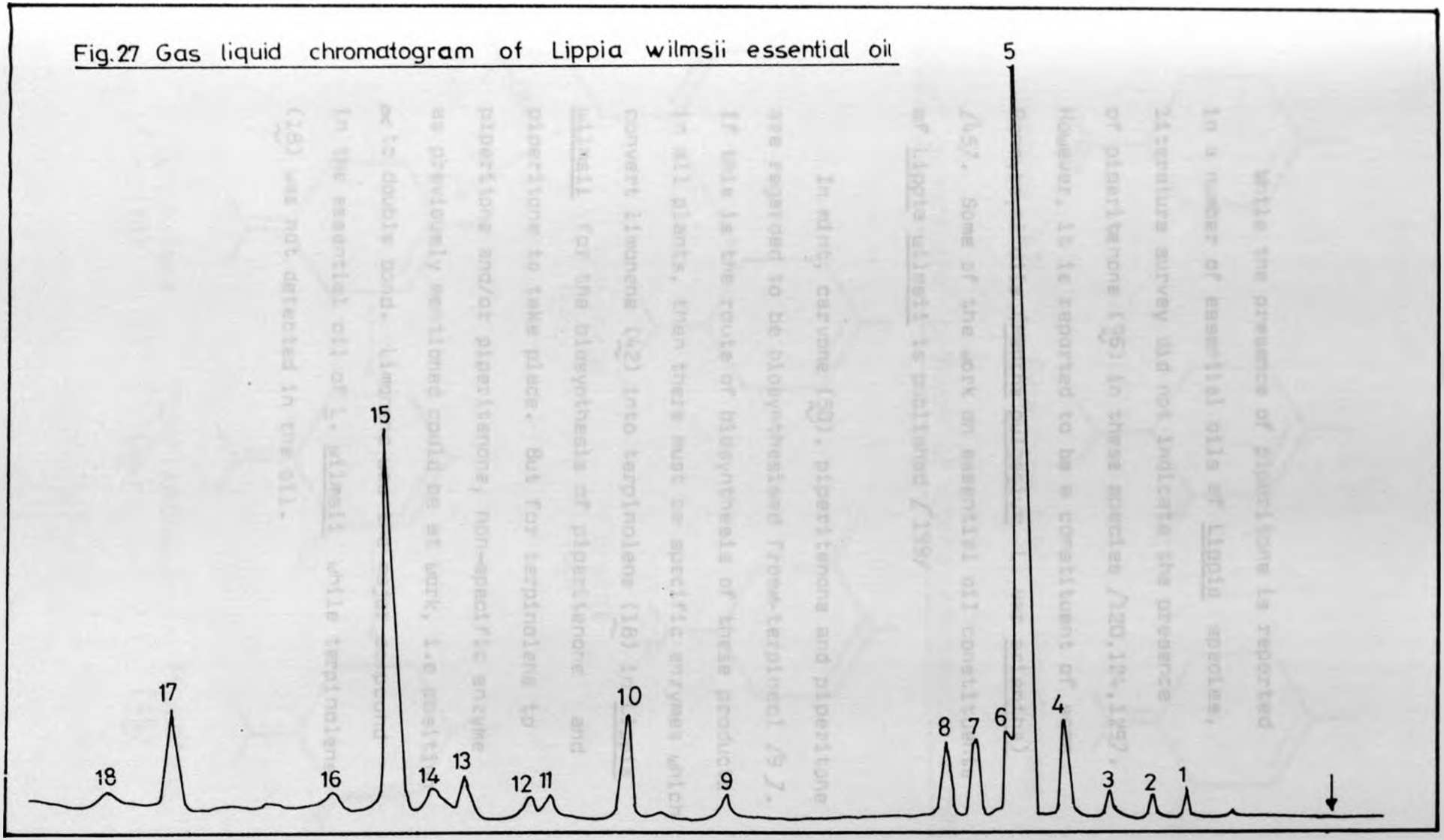
II - collected on 28/2/85 yield 1.4%

III - Collected on 29/9/85 yyield 1.1%

IV - Collected on 5/7/86 yield 1.6%

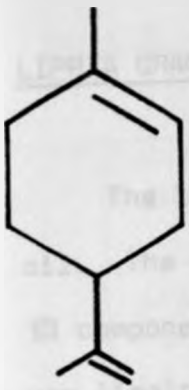
T - trace which is regarded to be zero in the calculations.

Fig.27 Gas liquid chromatogram of Lippia wilmsii essential oil

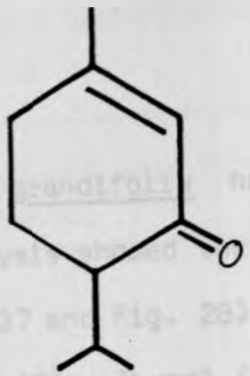


While the presence of piperitone is reported in a number of essential oils of Lippia species, literature survey did not indicate the presence of piperitenone (96) in these species [120,124,129]. However, it is reported to be a constituent of some Pennyroyal oils (Mentha pulgegium L. var erientha) [45]. Some of the work on essential oil constituents of Lippia wilmsii is published [199]

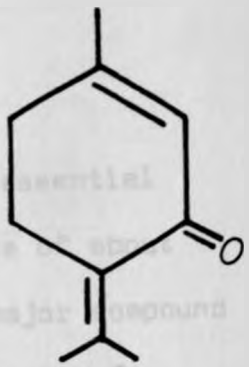
In mint, carvone (50), piperitenone and piperitone are regarded to be biosynthesised from α-terpineol [9]. If this is the route of biosynthesis of these products in all plants, then there must be specific enzymes which convert limonene (42) into terpinolene (18) in Lippia wilmsii for the biosynthesis of piperitenone and piperitone to take place. But for terpinolene to piperitone and/or piperitenone, non-specific enzyme as previously mentioned could be at work, i.e position α to double bond. Limonene was the major compound in the essential oil of L. wilmsii while terpinolene (18) was not detected in the oil.



Limonene (42)

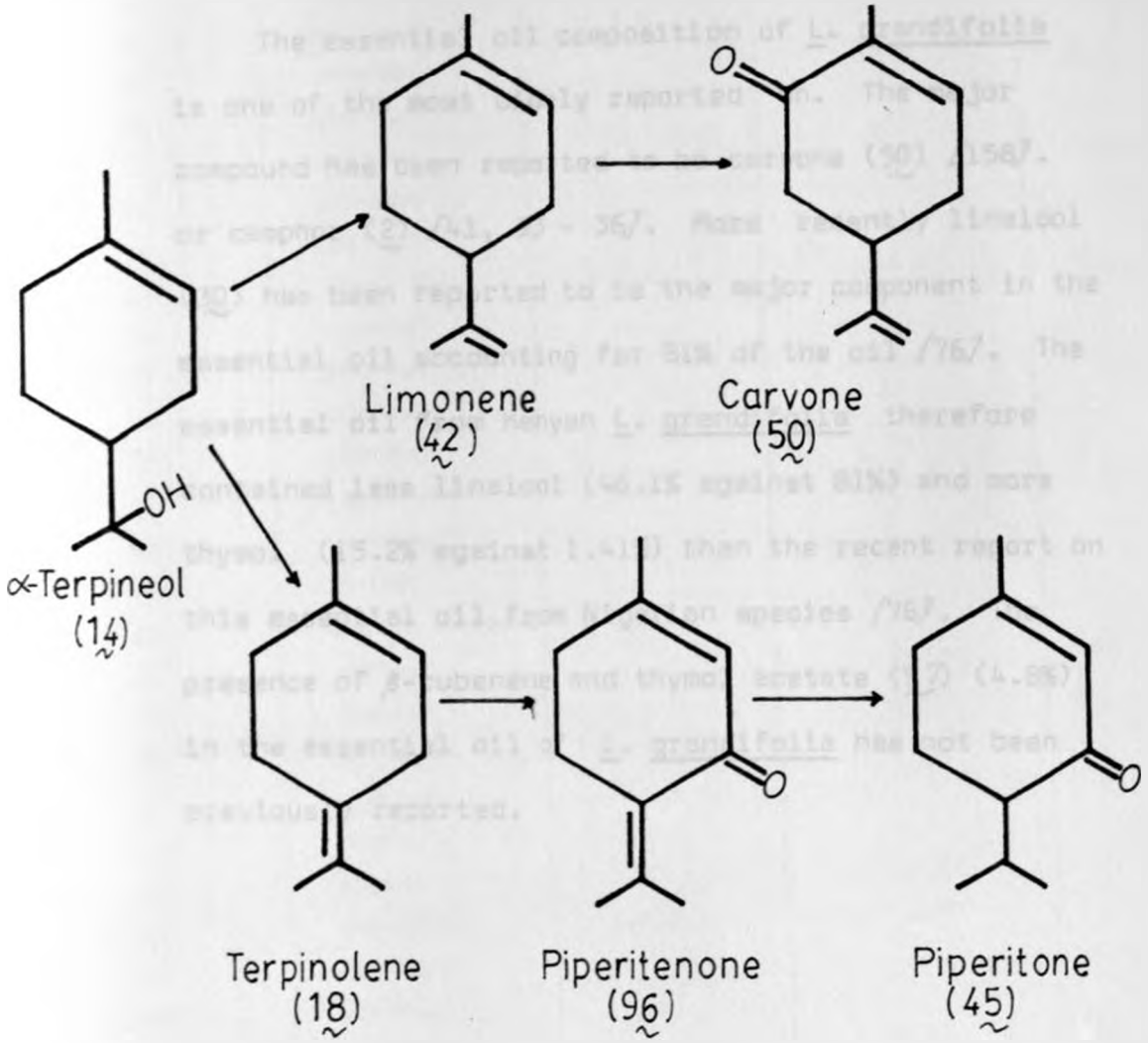


Piperitone (45)



Piperitenone (96)

Proposed biosynthetic pathway of some compounds in mint



LIPPIA GRANDIFOLIA

The leaves of L. grandifolia had 0.7% essential oil. The oil on analysis showed the presence of about 30 components (Table 17 and Fig. 28). The major compound was linalool (30) (46.1%). Thymol (23) accounted for 15.2% of the oil. The other significant components were p-cymene (34) (10.4%) and β-cubenene (77) (11.7%).

The essential oil composition of L. grandifolia is one of the most widely reported on. The major compound has been reported to be carvone (50) [158]. or camphor (2) [41, 33 - 36]. More recently linalool (30) has been reported to be the major component in the essential oil accounting for 81% of the oil [76]. The essential oil from Kenyan L. grandifolia therefore contained less linalool (46.1% against 81%) and more thymol (15.2% against 1.41%) than the recent report on this essential oil from Nigerian species [76]. The presence of β-cubenene and thymol acetate (97) (4.8%) in the essential oil of L. grandifolia has not been previously reported.

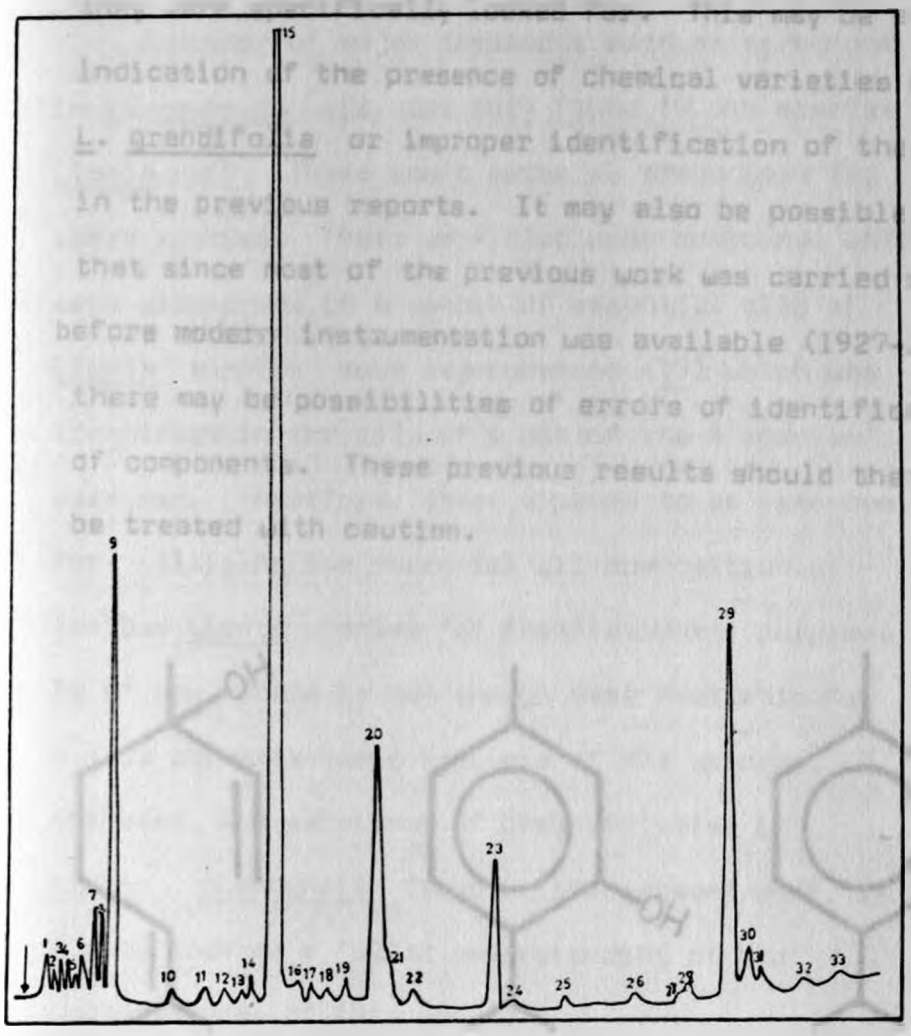
Table 17 Essential oil constituents of Lippia grandifolia

Peak No.	Constituent	Identification method	% constituents in the sample
1	α-thujene	c	0.7
2	unknown		0.3
3	3-carene	c	0.8
4	myrcene	a,c	0.5
5	α-terpinene	a,c	0.3
6	limonene	a,c	0.3
7	β-ocimene	a,c	2.4
8	γ-terpinene	c	3.5
9	p-cymene	a,b,c	10.4
10	unknown		0.4
11	unknown		1.4
12	unknown		T
13	unknown		T
14	unknown		T
15	linalool	a,b,c	46.1
16	unknown		T
17	unknown		T
18	unknown		T
19	unknown		T
20	β-cubenene	b,c	11.7
21	unknown		T
22	uknown		T
23	Thymol acetate	c	4.8
24	unknown		T
25	unknown		T
26	unknown		T
27	unknown		T
28	unknown		T
29	thymol	a,b,c	15.2
30	carvacrol	a,b,c	1.7
31	unknown		T
32	unknown		T
33	unknown		T

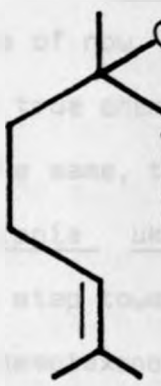
Yield 0.7% oil Collected at Cherangani Hills 22/11/86

T - Trace

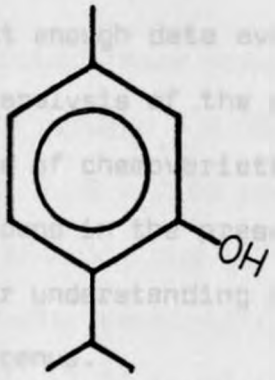
Fig 28 Gas liquid chromatogram of Lippia grandifolia essential oil



No carvone (50) or camphor (2) was detected in the sample of L. grandifolia leaf oil examined although they were specifically looked for. This may be an indication of the presence of chemical varieties of L. grandifolia or improper identification of the plant in the previous reports. It may also be possible that since most of the previous work was carried out before modern instrumentation was available (1927-1964) there may be possibilities of errors of identification of components. These previous results should therefore be treated with caution.

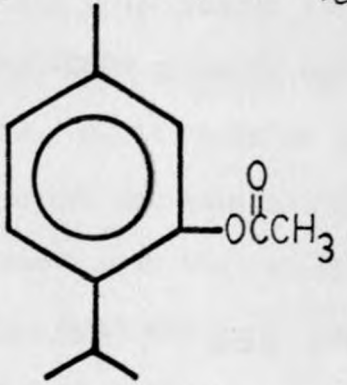


Linalool (30)



Thymol (23)

p-Cymene (34)



Thymol acetate (97)

CHEMOTAXONOMIC SIGNIFICANCE

A number of major compounds such as myrcenone in *L. javanica* oil were only found in one species of *Lippia* oil. These could serve as characters for identifying these species. There were also some compounds which were widespread in a number of essential oils of *Lippia* species such as β-cubenene (77) which was identified in the oils of 6 out of the 8 species examined. Therefore, there appears to be some basis for utilising the essential oil composition of various *Lippia* species for chemotaxonomic purposes. As of now, there is not enough data available for a true chemotaxonomic analysis of the species. All the same, the existence of chemovarieties of *Lippia ukambensis* found in the present work, is a step towards a fuller understanding of the chemotaxonomy of this genus.

NON-VOLATILE CONSTITUENTS OF LIPPIA CARVIODORA VAR MINOR

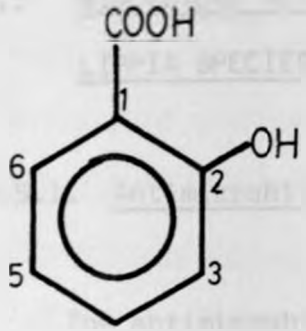
The white needle-like crystalline compound isolated from Lippia carviodora var minor was found to be salicylic acid (98). The melting point 155 - 157° agreed well with the literature value (about 160°) [200]. Both the isolated compound and pure salicylic acid had similar R_f values on TLC. The UV spectrum gave λ_{max} (MeOH) 301 nm (ε = 1629.58) (Appendix 11). The infra-red spectrum (KBr Appendix 12) was consistent with the structure of the compound and agreed with that reported in the literature [209].

Compound (98) showed three groups of multiplets in its ^{1}H-NMR spectrum between 6.78 - 7.92 (Appendix 13) The multiplet between 7.80 - 7.92 accounting for one aromatic hydrogen is probably due to the C-6 ring proton which undergoes greater deshielding by -COOH group. The multiplet at 7.36 - 7.56 (1-H) is probably due to the C-4 proton which, due to its para position relative to the -COOH group is deshielded. The multiplet at about 6.783 - 6.962 could be assigned to C-3 and C-5 protons (2-H) which are shielded by the OH group. This was in close agreement with the calculations of the chemical shifts of ortho, meta and para protons in mono-substituted benzenes [192].

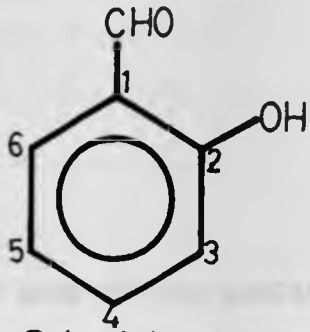
The ^{13}C-NMR spectrum of compound (98) Appendix 14 was compared with that of salicyladehyde (99) [201]. The ^{13}C-NMR spectra of both compounds are shown below.

C	Salicylaldehyde (99)	Salicylic acid (98)
1	121.00	113.96
2	161.40	163.12
3	117.40	118.13
4	136.60	136.52
5	119.60	120.05
6	133.60	131.52
7	196.70	173.41

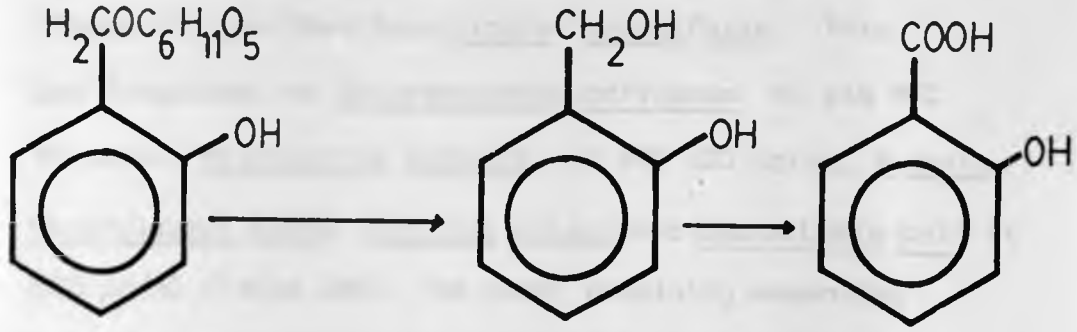
The presence of salicylic acid (98) as such in L. carviodora var minor is rather unusual. Salicin (100), a phenolic glycoside which is found in a number of willows (Salix species) [11, 202] is reported to act as a prodrug which is metabolised to a saligenin, salicyl alcohol (101), in the intestines and to salicylic acid after absorption [202].



Salicylic acid (98)



Salicylaldehyde (99)



Salicin (100) Salicyl alcohol (101) (98)

4.5. BIOLOGICAL ACTIVITIES OF ESSENTIAL OILS OF LIPPIA SPECIES

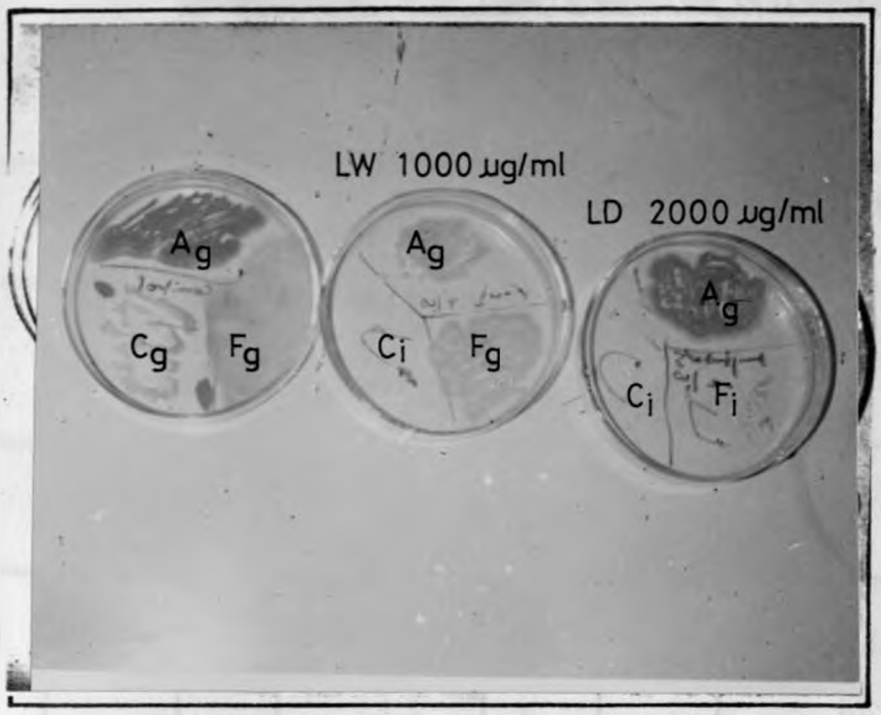
4.5.1. Antimicrobial Activity

The antimicrobial activity of some of the isolated oils was tested by both the filter paper disc and the streaking method (Plates 9a and 9b).

The most active oil, tested by streaking method (Table 18) was that from Lippia grandifolia. This was fungicidal to Colletotrichum coffeanum at its MIC 50 µg/ml, Microsporum audouinii at MIC 100 µg/ml, M.canis, Staphyloccus albus, Bacillus cereus and Escherichia coli at 500 µg/ml (Table 18). The other promising essential oil was fresh Lippia javanica which was fungicidal to Candida albicans and Colletotrichum coffeanum at its MIC 500 µg/ml while it was antimicrobial to S. aureus, S. albus, E. coli, Microsporum canis and M. audouinii at 1000 µg/ml. The Table shows that most of the other essential oils had antimicrobial activity at MIC 1000 µg/ml or higher concentrations, indicating that they cannot be regarded as suitable antibacterial agents since most of the commonly used antibacterial agents such as ampicillin, tetracycline

and chloramphenicol have MIC lower than 10 µg/ml to most bacteria [166]. However they may serve as antiseptic antibacterial agents in some skin preparations. No essential oil had any activity on Pseudomonas aeruginosa at the concentrations tested (up to 3000µg/ml). Apart from Colletotrichum coffeanum, the oils were not phytofungicidal to the tested plant fungi at concentrations lower than 2000 µg/ml. However, the animal pathogenic fungi tested were susceptible to a number of oils at 1000µg/ml or lower concentrations. The weakest oil as an antimicrobial agent was L. somalensis oil.

The filter paper disc method using neat oils is presented in Table 19. The essential oil of L. grandifolia had the largest zones of inhibition. In fact the zone of inhibition for the oil was the same as that produced by discs impregnated with 30 µg cephalexin for Staphylococcus albus. The results also showed that while nystatin discs soaked in a solution 100,000 of nystatin 1U/ml had a zone of inhibition of 20 mm for Candida albicans, plates containing discs soaked in L. grandifolia oil showed no growth for the organism. The same effect was noticed for Colletotrichum coffeanum



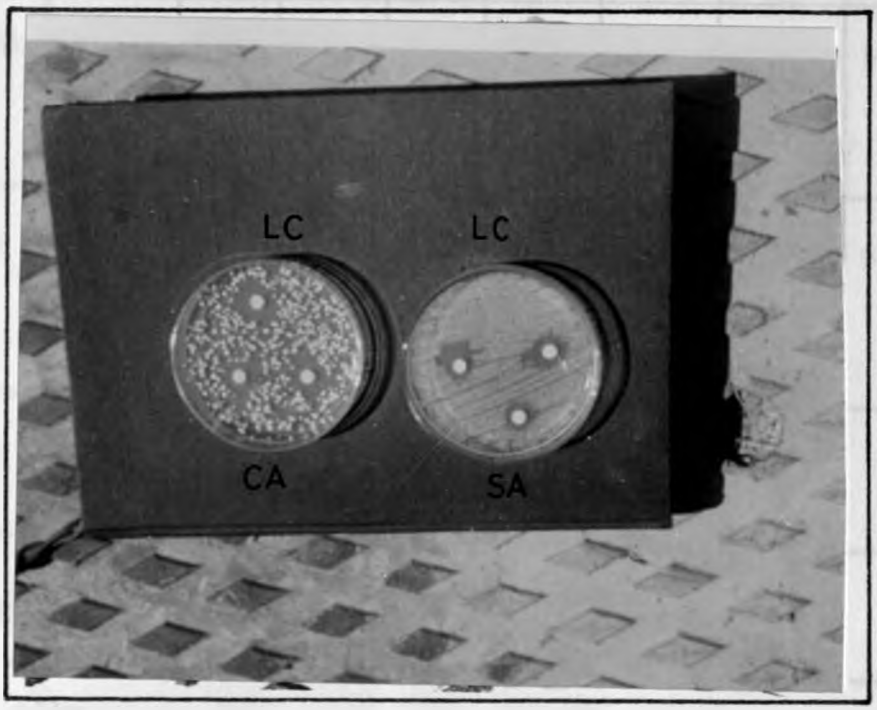


PLATE 9 Antimicrobial activity of essential oils of Lippia species (a) Streaking method (b) Filter paper disc method

A = Aspergillus species, C = Colletotrichum coffeanum
F = Fusarium solani CA = Candida albicans
SA = Staphylococcus aureus LW = Lippia wilmsii oil
LD = L. dauensis oil LC = L. carviodora oil
g = growth i = growth inhibited

Table 18 Minimum inhibition concentration (MIC) of Essential oils of Lippia species (streaking method)

Essential oils VS micro-organisms								
MIC µg/ml	LS	LC	LU	LUH	LD	LW	LJ	LG
50								8
100								13
500							7,8	2,3,4,6 7,12
1000	13	12, 13	7,8			2,4,5 6,13, 8	2,3,4 6,12, 13	5
2000	2,3,4	2,3,4 5,6,7 8,10	2,3,4 5,6, 12,13	2,3, 4,5 7,8 6,13	2,4,5 5,7,8 9	3,7	5,10	9,10,11
3000		9,11		12	13	10		
>3000	12		10	10	12	9		

Essential oils

LS - Lippia somalensis

LC - L. carviodora

LU - L. ukambensis chvar camphor

LUH - L. ukambensis chvar cineole

LD - L. dauensis

LW - L. wilmsii

LJ - L. javanica

LG - L. grandifolia

Table 18 continued

Micro - organisms

1. Pseudomonas aeruginosa
2. Staphylococus aureus
3. S. albus
4. Bacillus cereus
5. Klebsiella species
6. Escherichia coli
7. Candida albicans
8. Colletotrichum coffeanum
9. Fusarium solani
10. Cercospora species
11. Aspergillus species
12. Microsporum canis
13. M. audouinii

Table 19 Antimicrobial activity of neat essential oil Lippia species by filter paper Disc Method

Micro-organism	Diameter of inhibition in mm*										
	LS	LC	LU	LUH	LD	LW	LJ	LG	NA	CE	C
Pesudomonas aeruginosa	0	0	0	0	0	0	0	0		0	
Staphyllococcus aureus	4	6	2	4	12	2	8	14		24	
S. albus	4	4	2	2	4	2	4	24		24	
Bacillus cereus	6	4	4	6	12	4	14	(-)			
Escherischia coli	0	2	4	4	4	2	2	12		14	
Klebsiella species	4	4	2	4	4	2	2	12		10	
Candida albicans	8	12	4	4	4	9	15	(No)	20		(No)
Colletotrichum coffeanum	0	(No)	4	4	4	(No)	(No)	(No)			
Fusarium solani	0	(No)	4	0	4	6	(No)	6	14		

* Diameter of discs excluded (6.5mm)

LS - Lippia somalensis
LC - L. carviodora
LU - L. ukambensis chvar camohor
LUH - L. ukambensis chvar cineole
LD - L. dauensis
LW - L. wilmsii
LJ - L. javanica
LG - L. grandifolia
NA - Nystatin 100,000 IU/ml
CE - Disc containing 30µg of cephalexin
C - Carvone pure
(No) - Complete inhibition in the whole plate

for this oil and a few other microorganisms for other essential oils (Table 18). It was also noticed that even where zones of inhibition appeared (especially in oils which were active) the colonies of the organisms seemed very scarce and isolated (Plate 9b). This may indicate that there may have been diffusion of the oil through the agar or the vapours may have had an adverse effect on the microorganisms.

The high antimicrobial activity of the oil from L. grandifolia was most likely due to the presence of thymol (23) (15.2%) and thymol acetate (99) (4.8%) Thymol is reported to be twenty times more active than phenol as an antiseptic while linalool (30) has a very low phenol coefficient [23]. It is not known what contribution β-cubenene(77) (11.7%) had as an antimicrobial agent. It is to be noted that L. javanica oil was the second in potency as an antimicrobial agent and contained myrcenone (78) (average 32.9%), trans-ocimenone (80) (average15.8%) and cis-ocimenone (79) (average 31.9%)

Fungicidal activity of some essential oils and their constituents have been reported. For example oils of bay, cinnamon bark and leaves, cloves and thyme have been reported to completely inhibit mycelial growth of a number

fungi at concentrations of 1000 µg/ml and 500 µg/ml. It is also reported that essential oils of mint and spearmint inhibited the growth of Aspergillus flavus and A. niger at concentrations of 1.1% i.e 11,000 µg/ml [102]. Other reports indicate that both of these phytopathogenic animal fungi were inhibited by various essential oils being fungistatic at 500-1000 ug/ml and fungicidal at 0.9 - 1.6% [102]. The essential oils of Lippia therefore had very significant activity as antifungal agents in comparison with other reported essential oils (Table 18). The same could also be said about the antibacterial activity of essential oils from Lippia species.

4.5.2. Mosquito Larvicidal activity

Table 20 shows the larvicidal activity of essential oils of Lippia species. Figs 29, 31, 33 show the plots of % mortality v/s concentration while Figs 30,32,34 show their respective probit v/s log concentration graphs. Table 21 shows the larvicidal activity of the constituents. By the use of a computer, multiple regression analysis of probit v/s log concentration was also carried out. Using the gradient of slopes and y-intercepts obtained it was possible to do t-test for any pair of essential oils

or constituents. Representative comparisons of essential oils and the constituents are shown in Table 22.

Table 21 and Figs 35, 36 show some of the hydrocarbon monoterpenes found in the essential oils of Lippia species examined for their larvicidal activity. All of them had LD_{100} at 35 ppm or lower. β-Ocimene (81) was the most active hydrocarbon terpene. Examination of the oxygenated monoterpenes (Table 21 and Figs 37, 38) indicated that they had much lower larvicidal activity compared with the hydrocarbon momoterpenes. However, thymol (23) which is an oxygenated compound had also a high larvicidal activity with LD_{100} at 50 ppm. Table 23 shows that most of the essential oils of Lippia species had LD_{50} at lower than 100 ppm except L. ukambensis chemovariety oils whose LD_{50} concentrations were higher.

The above information was subjected to further statistical analysis. Comparison of the slopes of the graphs fo number of essential oils such as L. ukambensis chvar camphor and L. ukambensis chvar cineole oils, L. javanica and L. dauensis oils among others, showed that they were not significantly different in their larvicial activity ($P > 0.05$ for gradient and y-intercepts) (Table 22).

Table 20 Larvicidal activity of essential oil of Lippia species

Concentration ppm	Concentration ppm/ Mortality rate % (In brackets mean ± SEM)						
	25	50	75	100	125	150	200
Lippia somalensis oil	0 %	7.2 % (2.9)	48.3 % (4.01)	79.6 % (3.66)	92.0 % (3.15)	100 %	
L. grandifolia oil	0 %	4.0% (2.0)	18.7% (4.4)	70.0% (3.77)	93.2 % (2.0)	100 %	
L. ukambensis chvar camphor oil	—	0%	1.9% (0.91)	10.8% (1.43)	35.8% (4.34)	77.5% (4.78)	100%
L. ukambensis chvar cineole oil	—	—	0%	2.0% (0.76)	7.7% (1.7)	28.8% (4.4)	81.1% (3.72)
L. wilmsii oil	1.7% (1.05)	38.3% (3.8)	63.3 % (3.3)	78.2 % (4.52)	91.1% (3.09)	100%	
L. carviodora oil	0%	10.5% (3.33)	32.0% (3.3)	76.3% (3.08)	93 % (4.02)	97.5% (4.41)	100%
L. dauensis oil	0%	9.0% (3.49)	6 % (5.2)	93.8% (2.2)	98.8% (2.0)	100%	
L. javanica oil (fresh, yellow)	0 %	8.3% (3.1)	55.0% (5.49)	95.0% (1.66)	97.8% (0.87)	100%	
L. javanica oil (deteriorated, reddish)	0 %	0.8 % (0.83)	5.5 % (1.3)	11.1% (2.8)	22.7% (5.07)	31.1% (5.0)	60 % (3.4)

Table 21 Larvicidal activity of some essential oil contituents of Lippia species

	Concentration ppm / Mortality rate % (In brackets mean ± SEM)															
Conc. ppm	10	12	14	15	16	20	25	30	35	40	50	75	100	125	150	200
∝-Pinene	5 % (2.23)	17 % (3.0)	—	19.2 % (3.78)	—	53 % (8.74)	74 % (5.09)	83.1 % (4.71)	—	100 %						
p-Cymene	5 % (2.7)	18 % (4.6)	—	35 % (0)	57 % (8.6)	86.3 % (3.14)	100 %									
Ocimene	62.5 % (10.89)	81 % (1.87)	—	91 % (3.67)	—	94 % (2.44)	98 % (2)	100 %								
Limonene	39.2 % (4.9)	—	—	77.5 % (6)	—	80.5 % (4.9)	97.5 % (1.18)	99 % (0.88)	100 %							
Thymol	3.8 % (3.75)	—	34 % (7.64)	—	—	71 % (4.3)	77.5 % (1.44)	83.8 % (1.25)	—	—	100 %					
Camphor											0 %	16 % (3.67)	67.5 % (7.38)	91 % (3.3)	97 % (2.0)	100 %
Linalool											0 %	45 % (7.9)	82.5 % (9.24)	98.8 % (1.25)	100 %	
Piperitone											0 %	15 % (2.88)	30 % (5.77)	50 % (2.88)	78.3 % (6.6)	91.7 % (1.44)
1,8-Cineole											0 %	5 % (2.88)	10 % (0)	32 % (2.5)	73.3 % (0.3)	100 %

Table 22 Comparison of larvicidal activity between different Lippia species oils and constituents

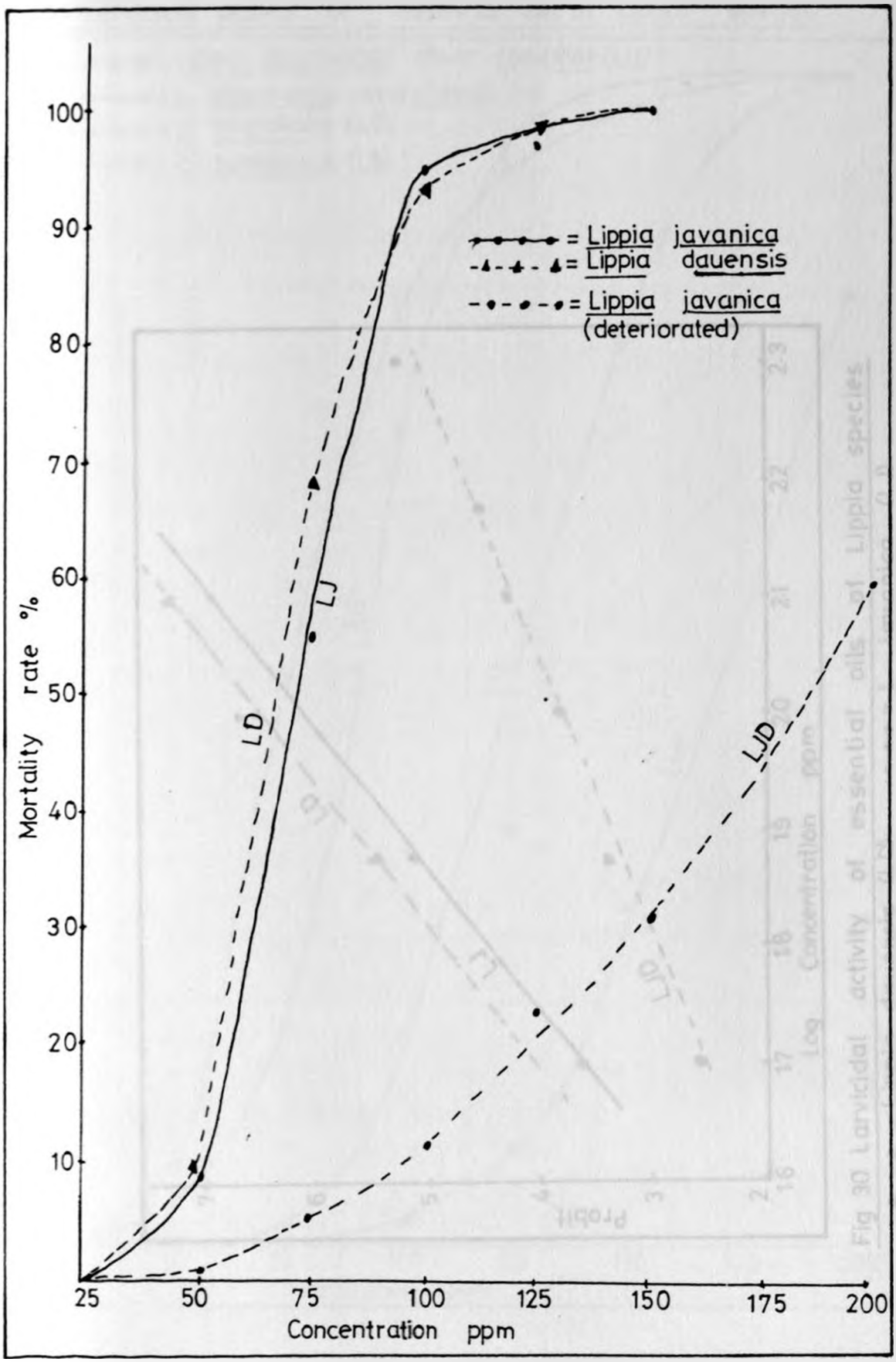
Comparison	t - test	
	Slope gradient	y-intercept
LU-LUH	0.93(NS)	1.3(NS)
LJ-LD	0.01(NS)	0.84(NS)
LG-LD	0.68(NS)	0.26(NS)
LS-LC	0.23(NS)	0.22(NS)
LJ -LW	1.5(NS)	1.07(NS)
LJD-LJ	8.64(P<0.001)	5.09 P<0.0025)
LU-LW	4.12 (P< 0.01)	4.79 (P< 0.005)
LUH-LD	0.9(NS)	2.87 (P< 0.05)
LW-LC	3.40 (P< 0025)	3.55(P<0.025)
LU-camphor	0.02(NS)	0.33(NS)
LG-linalool	1.38 (NS)	1.2(NS)
LUH-1,8-cineole	1.5(NS)	1.7(NS)
LS-1,8-cineole	1.5(NS)	0.36(NS)
LW-piperitone	1.2(NS)	2.2(NS)
LW-limonene	0.67(NS)	1.77(NS)
LG-thymol	0.38(NS)	2.8(P<0.05)
LC-limonene	1.2(NS)	4.0(P < 0.01)
LD-β-ocimene	8.9 (P<0.001)	15.39 (P< 0.0001)
α-pinene-piperitone	0.27 (NS)	3.66(P< 0.01)
camphor-limonene	1.73 (NS)	3.35 (P< 0.05)
Piperitone-limonene	0.05(NS)	4.04(P< 0.01)
β-ocimene-linalool	4.78 (P< 0.005)	6.5 (P< 0.0025)

LU - Lippia ukambensis chvar camphor, LUH - L. ukambensis chvar cineole, LJ = L. javanica, LD - L. dauensis, LG-L. grandifolia LS-L.somalensis, LJD - L. javanica deteriorated oil, LW - L.wilmsii, LC- L. carviodora, NS, Not significant P > 0.05

Table 23 Larvicidal activity of Lippia essential oils and their constituents at LD_{50} and LD_{90}

Essential oils or Components	Concentration in ppm	
	LD_{50}	LD_{90}
Lippia dauensis oil.....	66.1	95.5
L. javanica oil (fresh yellow oil)	74.1	107.2
L. somalensis oil	74.1	120.2
L. grandifolia oil	88.1	123.0
L. carviodora oil	79.4	123.0
L. wilmsii	61.0	126.0
L. ukambensis chvar camphor oil	128.8	166.0
L. ukambensis chvar cineole	167.9	216.3
L. javanica oil (deteriorated reddish oil)	195.0	398.1
β-ocimene	8.6	15.8
limonene	13.0	20.7
p-cymene	15.13	21.1
thymol	16.5	31.6
∝-pinene	19.5	34.3
linalool	81.2	105.9
camphor	93.3	125.8
piperitone	121.6	192.8
1,8-cineole	140.4	218.8

Fig. 29 Larvicidal activity of essential oils of Lippia species



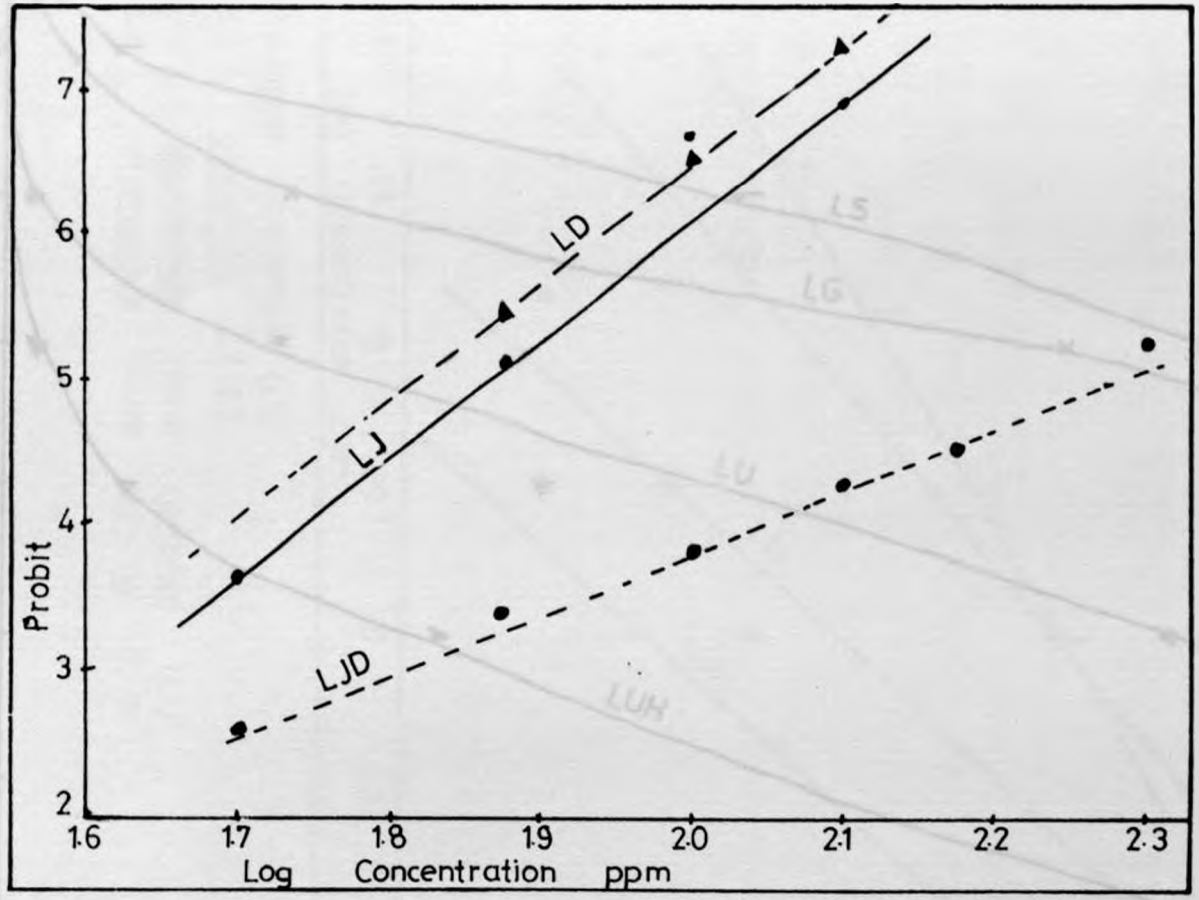


Fig 30 Larvicidal activity of essential oils of Lippia species
-▲ -▲- = Lippia dauensis (LD) —•—•— = L. javanica (LJ)
-•--•- = L. javanica (deteriorated)

Fig. 31 Larvicidal activity of essential oils of Lippia species

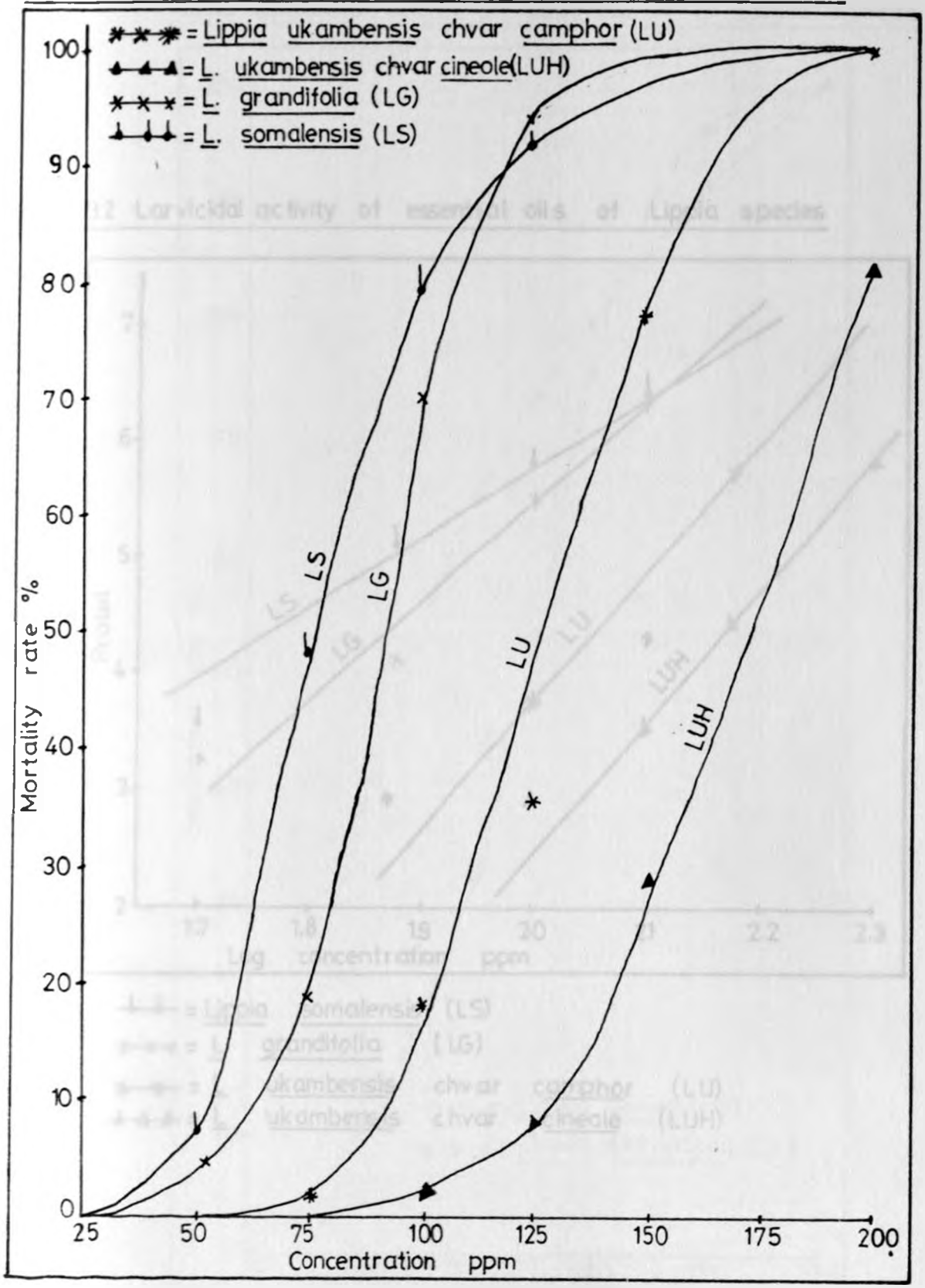
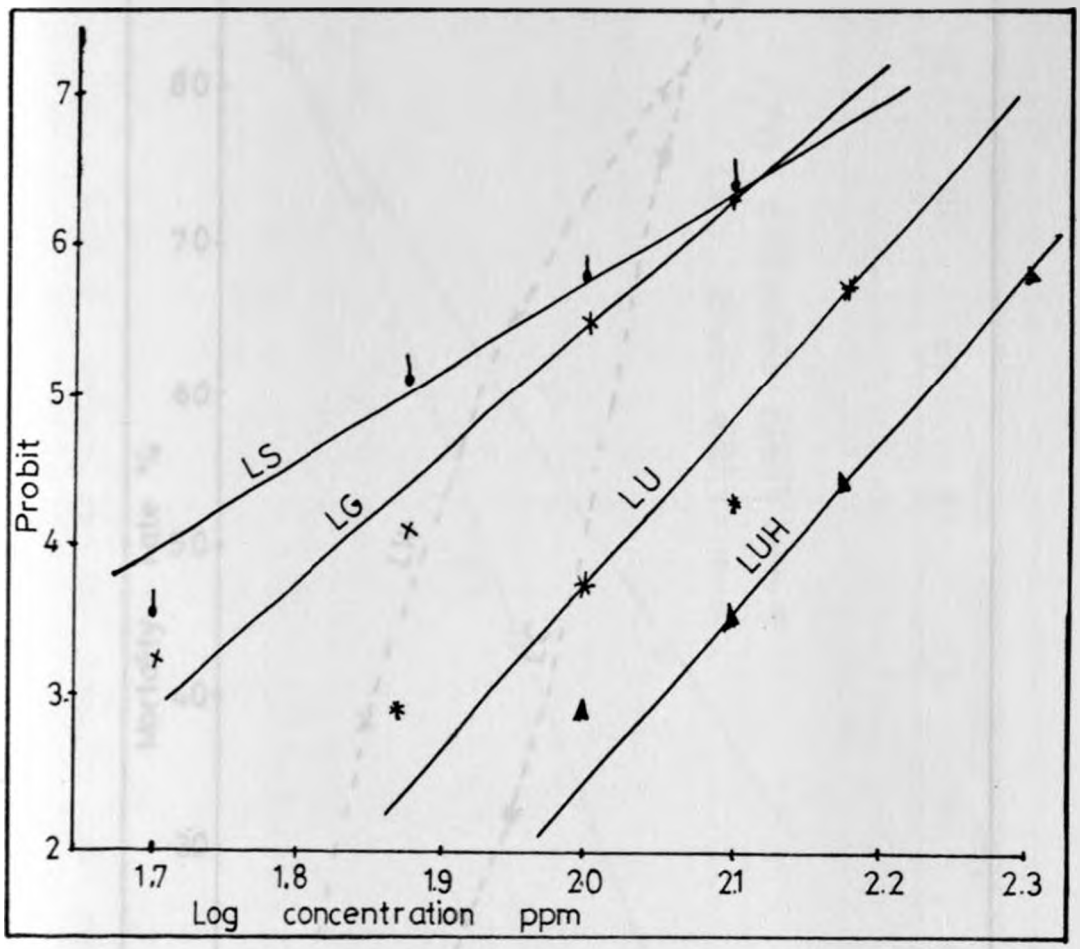


Fig 32 Larvicidal activity of essential oils of Lippia species



= Lippia somalensis (LS)
= L. grandifolia (LG)
= L. ukambensis chvar camphor (LU)
= L. ukambensis chvar cineole (LUH)

Fig 33 Larvicidal activity of essential oil of Lippia species

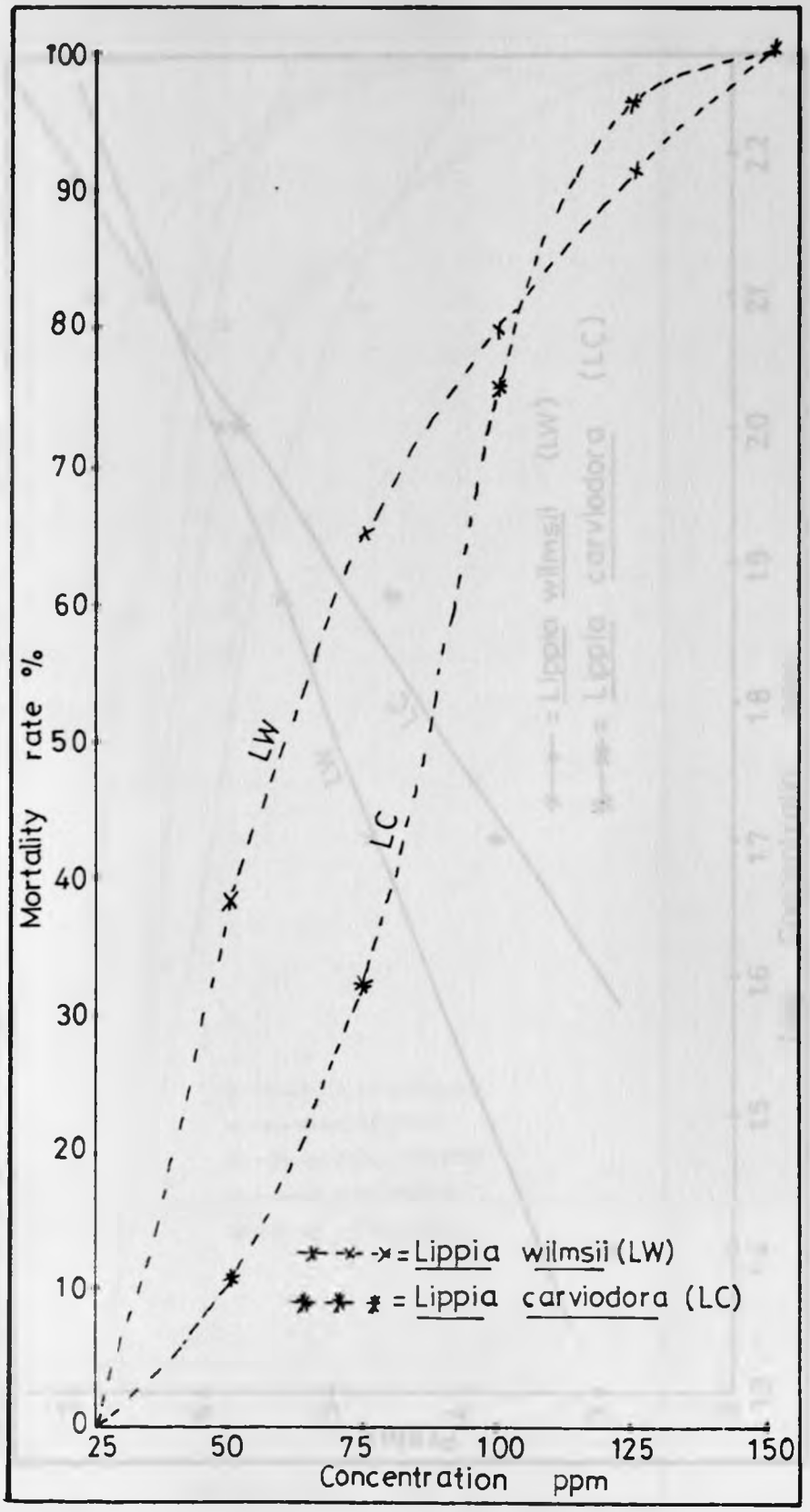


Fig 34 Larvicidal activity of essential oils of Lippia species

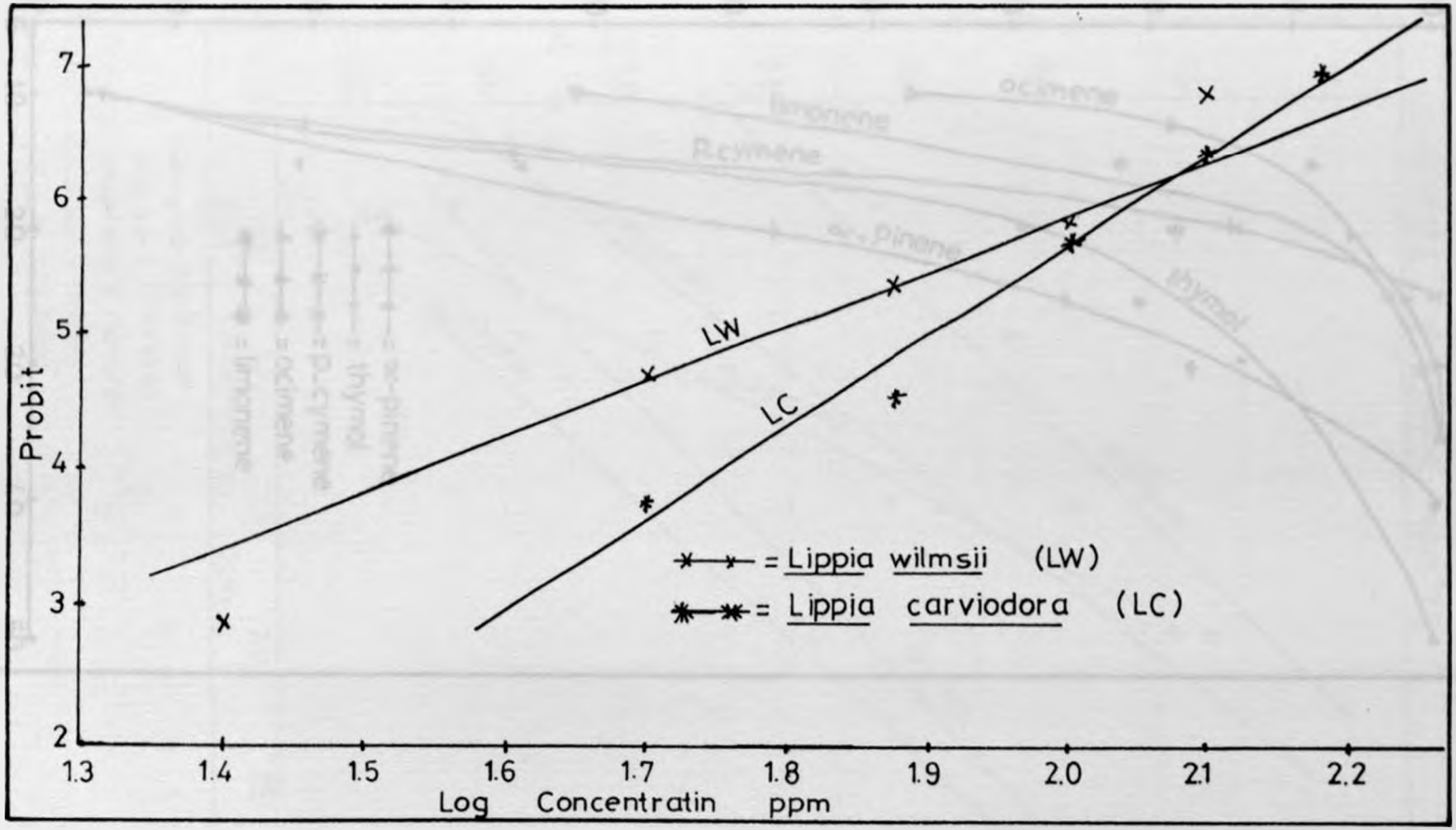


Fig. 35 Larvicidal activity of some essential oil constituents of Lippia species

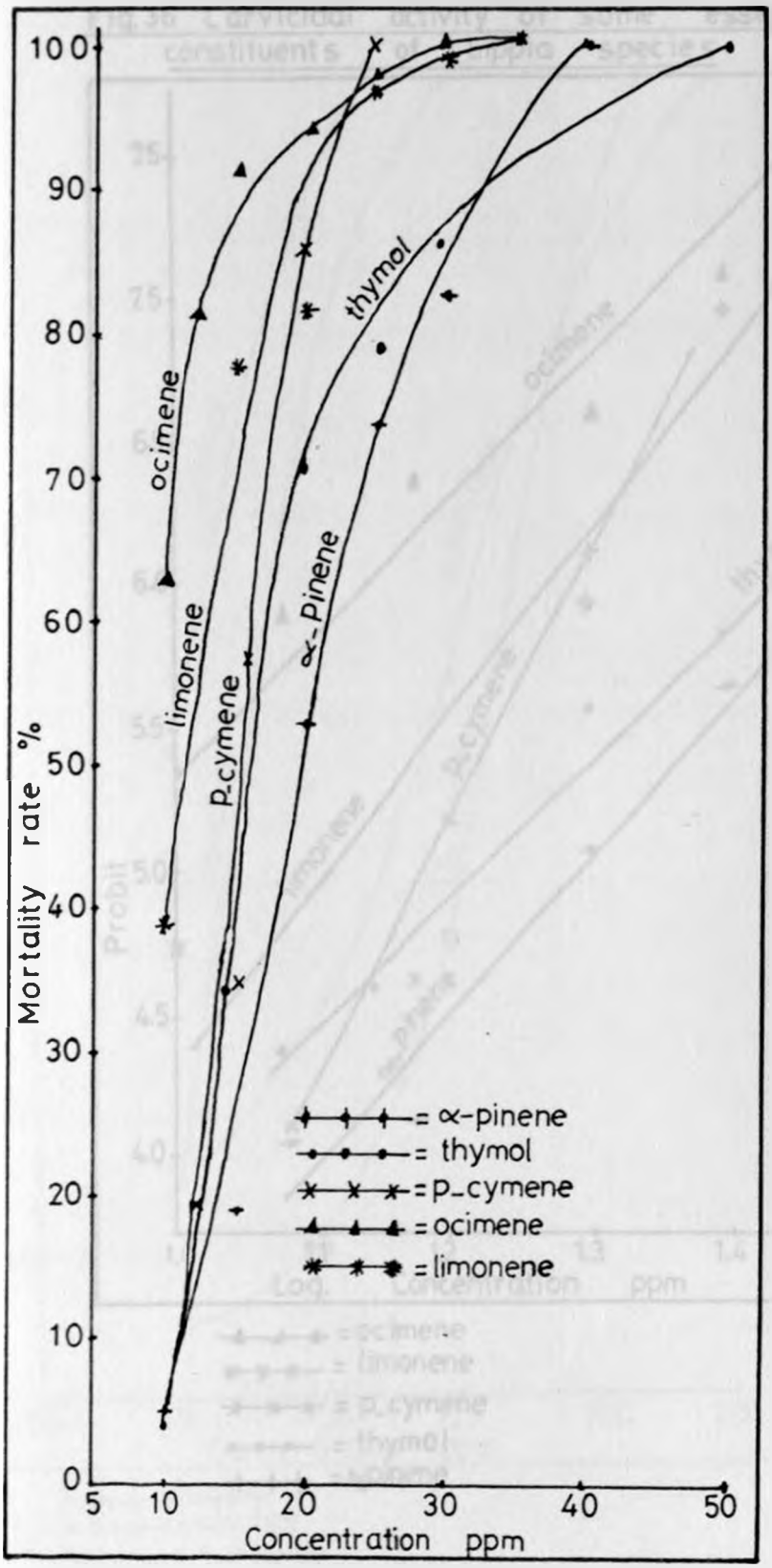


Fig. 36 Larvicidal activity of some essential oil constituents of Lippia species

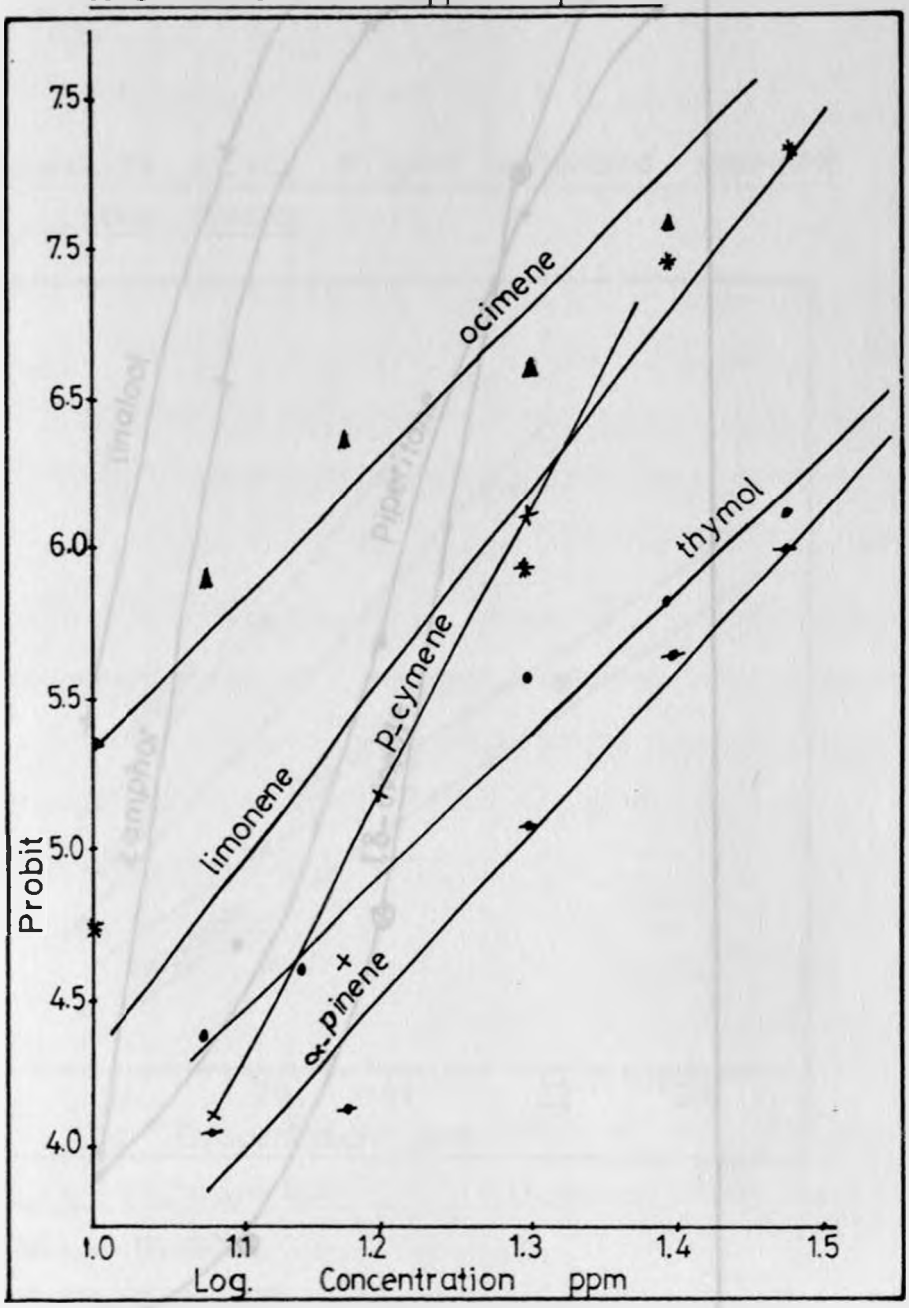


Fig. 37 Larvicidal activity of some oxygenated essential oil constituents of Lippia species

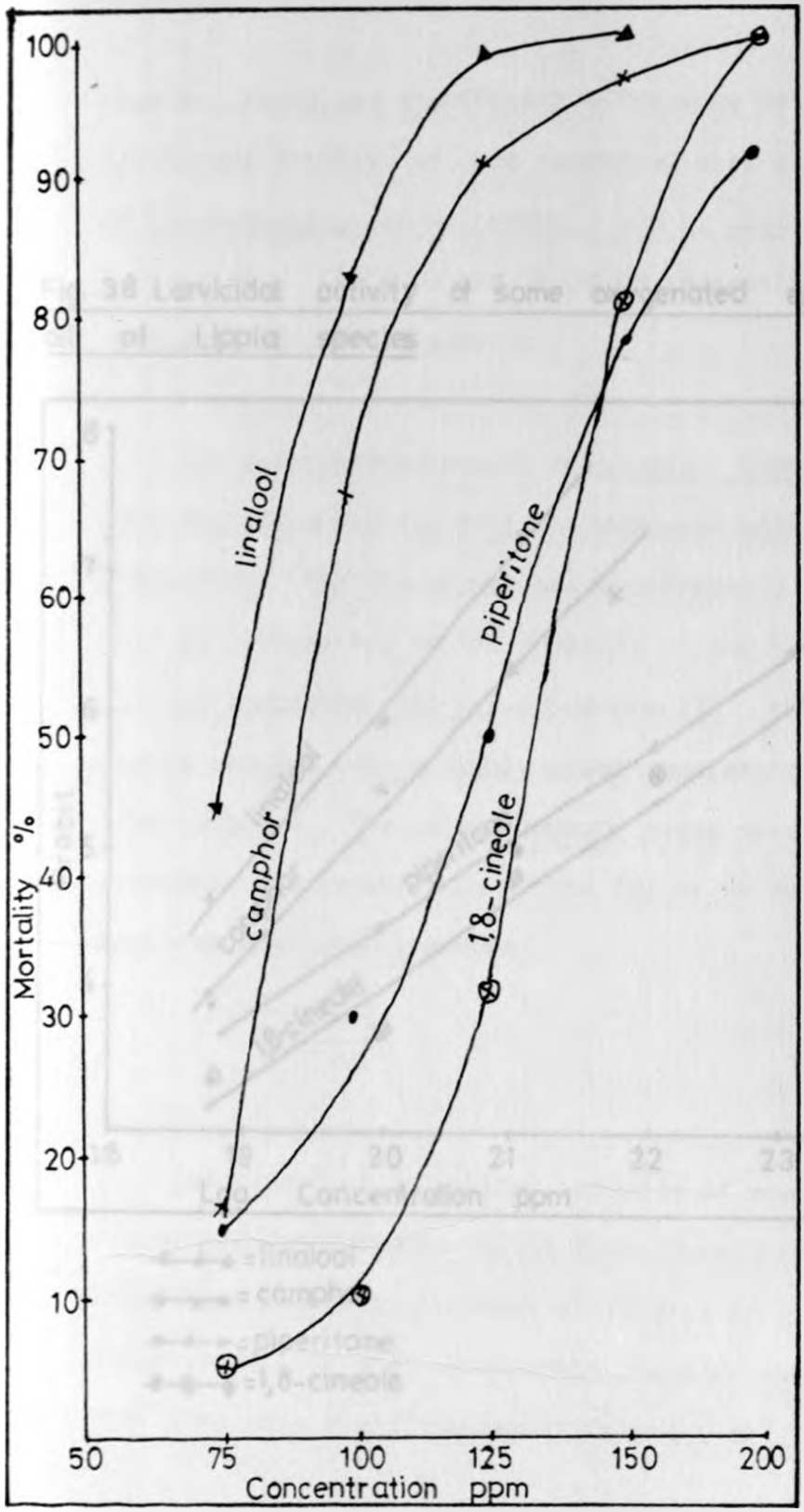
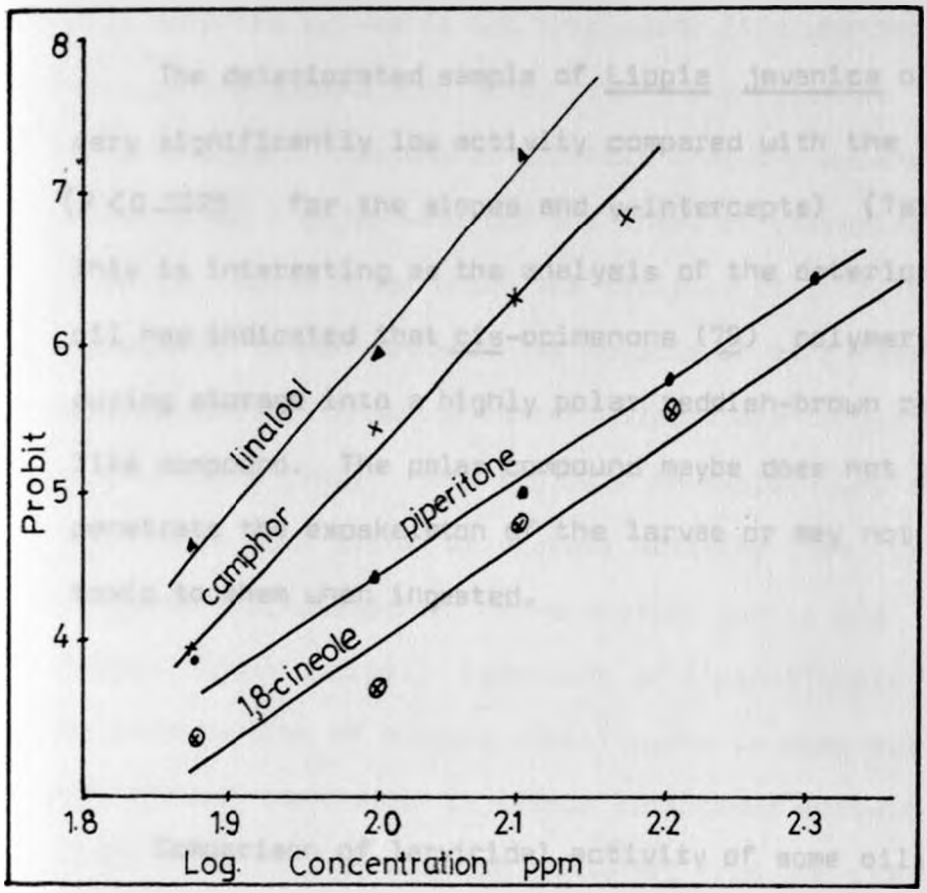


Fig. 38 Larvicidal activity of some oxygenated essential oil of Lippia species



= linalool
= camphor
= piperitone
= 1,8-cineole

However, there was significant difference between the larvicidal activity of some essential oils such as those of L. ukambensis chvar camphor and L. wilmsii and L. carvidora ($P < 0.01$ and $P < 0.05$ respectively for gradient and y-intercepts).

The deteriorated sample of Lippia javanica oil had very significantly low activity compared with the fresh oil ($P < 0.0025$ for the slopes and y-intercepts) (Table 22). This is interesting as the analysis of the deteriorated oil had indicated that cis-ocimenone (79) polymerises during storage into a highly polar reddish-brown resin-like compound. The polar compound maybe does not readily penetrate the exoskeleton of the larvae or may not be toxic to them when ingested.

Comparison of larvicidal activity of some oils and the oxygenated monoterpenes (apart from thymol) indicated that there was no significant difference in activity while comparison with hydrocarbon monoterpenes showed that they were significantly different (Table 22).

However, L. wilmsii oil was as active as limonene which is the major constituent of the oil. Table 21 also shows that all the hydrocarbon monoterpenes were significantly more active than the oxygenated monoterpenes.

The route of entry of the larvicidal essential oils into the larvae is not very clear (i.e whether through the exoskeleton or by taking the poisoned food or water). It may be possible that the exoskeleton route plays a major role for the entry of essential oils into the larvae after which the toxicity of individual components is manifested. The cuticle of most insect exoskeletons is known to contain lipids and long-chain hydrocarbons [20,37]. This may help to explain why thymol (23) had a much higher larvicidal activity than the oxygenated monoterpenes (non-aromatic) due to its higher lipophilicity.. Reduction of lipophilicity by introduction of hydroxy substituents in some quinones has already been shown to reduce larvicidal activity [18,27]. It may therefore be concluded from the present work that although both hydrocarbon and oxygenated monoterpenes contribute to the larvicidal activity of an essential oil, the lipophilicity of the components is probably more important. This is illustrated by the

fact that polymerization of cis-ocimenone (79) into a very polar compound in L. javanica oil drastically reduced the larvicidal activity (Fig 28).

This work was not in agreement with reported larvicidal activity of trans -ocimenone (80) which was reported to be the main larvicidal agent and not cis- ocimenone (79) from Tagetes minuta. The higher mortality reported for trans-ocimenone (LD_{100} at 40 ppm) may actually have been due to the very high acetone concentration (10%) used in the previous experiment [40]. This concentration had been found in a preliminary experiment to have a mortality rate of 100% in 24 hours.

The essential oil of Ocimum sanctum has been reported to show larvicidal mortality rate of 16% at 100 ppm, that of Eucalyptus globulus 16% mortality rate at 20 ppm and that of Ocimum basilicum 8% mortality at 20ppm [39]. Other natural compounds which have been found to have high larvicidal activity are alkamides. In these compounds different amine parts are combined by an amide linkage to various unsaturated acids.

These include fagaramide LD_{100} (15 ppm), piperlongumine LD_{100} (10 ppm), pellitorine LD_{100} (5 ppm), N-isobutyl-2E- 4E-octadienamide LD_{100} (15 ppm) [204], piperine and dihydropiperine LD_{90} (about 25 ppm) [205] and affinin (= spilanthol) [206].

Most of the commercial mosquito larvicides are used in very low concentrations (about 0.01-0.23 ppm) These include organophosphates (e.g malathion, diazinon, chlorthion) and organochlorides (e.g DDT, dieldrin, lindane) while pyrethrins LD_{50} concentration is 0.14 ppm [207] Other mosquito larvicides include Paris green (consists in a complex, copper acetate, copper metarsenite and arsenious oxide) and mineral oils (eg Kerosene, crude oil, diesel etc) applied at concentration of 1-2%.

The present work shows that the larvicidal activity of essential oils of Lippia species was one of the few reports on the larvicidal effects on essential oils. The oils were generally more effective than the few reported essential oils such as Ocimum sanctum but lower than alkamides. The larvicidal activity of the Lippia species oils were however very low as compared with the commerical synthetic larvicides. Organochlorides and organophosphates are poisonous to animals while Paris green is not totally

harmless. Water treated with mineral oils is also unfit for drinking, bathing, washing and dangerous to fish [207]. However, with more research work essential oils of Lippia species may provide a means for small-scale control strategies based on insecticidal plants grown by rural communities themselves. Anopheles gambiae, the most important vector of malaria, Culex quinquefasciatus the vector of Bancroftian filariasis and Aedes aegypti, the vector of yellow fever all breed in small collections of water such as temporary rain puddles, man-made containers, drains, and so on where the possibility exists of considerably reducing the multiplication of mosquitoes by periodic treatment with materials derived from such plants [208]. The larvicidal effect of combining these Lippia species oils with natural pyrethrins should also be investigated.

4.5.3. Maize weevils repellant activity

The maize weevil repellant activity of the essential oils of Lippia species and the standard, DEET (N,N-diethyltoluamide) is shown in Table 24 and Figs. 39, 40. Figs 39 and 40 which are percentage repellency versus dose curves indicate that a number of essential oils were more active than DEET at different doses. However,

Fig 41 which is a graph of % repellency against the items (i.e DEET and the essential oils) made the comparison of each oil with the standard at the same dose easier and more convenient.

Fig 41 shows that the repellant activity increased with increasing doses. Fig 41 also shows that the essential oil of L. ukambensis chvar cineole had the lowest activity being lower or equal to the standard or any oil tested at all doses tested. At the lowest dose (0.625 µl), L. ukambensis chvar camphor had the highest repellant activity being 1.5 more active that DEET while L. ukambensis chvar cineole oil was least active 0.25 less active than DEET). The essential oils of L. javanica, L. ukambensis chvar camphor and L. somalensis were very active at 1.25 µl dose being 1.6, 1.6 and 1.5 respectively more active than the standard at that dose. At 2.5µl dose, essential oils of L. javanica, L. dauensis, L. grandifolia, L. ukambensis chvar camphor and L. somalensis were at least 1.5 times more active than the standard. Indeed, all of them at this dose (2.5 µl) had the same repellant activity as DEET at twice the dose (5.0 µl). The essential oil of L. javanica was 1.2 more active than DEET at 5.0 µl dose while those of L. grandifolia, L. ukambensis

Table 24 Repellant activity of Essential oils of Lippia species on maize weevils (Sitophilus zeamais)

	% Repellency								
Dose µl	DEET	LJ	LD	LC	LW	LG	LU	LUH	LS
0.625	18.5	15	22.1	11.4	16	19.3	27.9	4.6	18.8
1.25	30	47.8	38.3	26.0	33.6	40.0	45.7	27.2	46.1
2.5	41.5	63.8	65.2	45.2	54.2	60.5	60.3	43.3	66.8
5.0	63.7	77.9	80.5	72.7	66.8	81.6	81.8	63.5	79.7

DEET - Standard, N, N-diethyltoluamide

LJ - Lippia javanica oil

LD - L. dauensis oil

LC - L. carviodora oil

LW - L. wilmsii oil

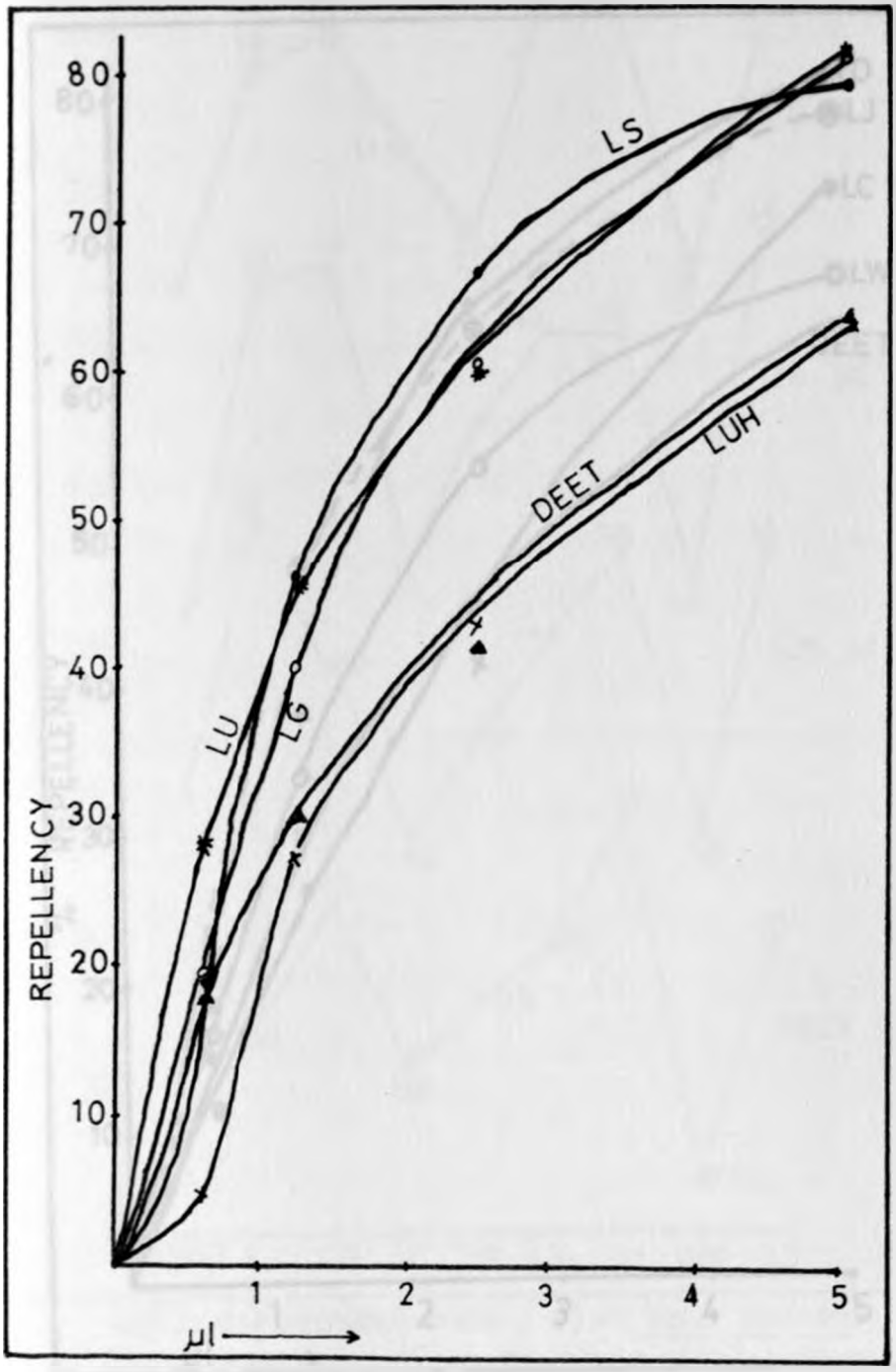
LG - L. grandifolia oil

LU - L. ukambensis chvar camphor oil

LUH - L. ukambensis chvar cineole oil

LS - L. somalensis oil

Fig 39 Dose response curves for the repellency of DEET and essential oils of Lippia species to maize weevils



DEET = N N-Diethyltoluamide

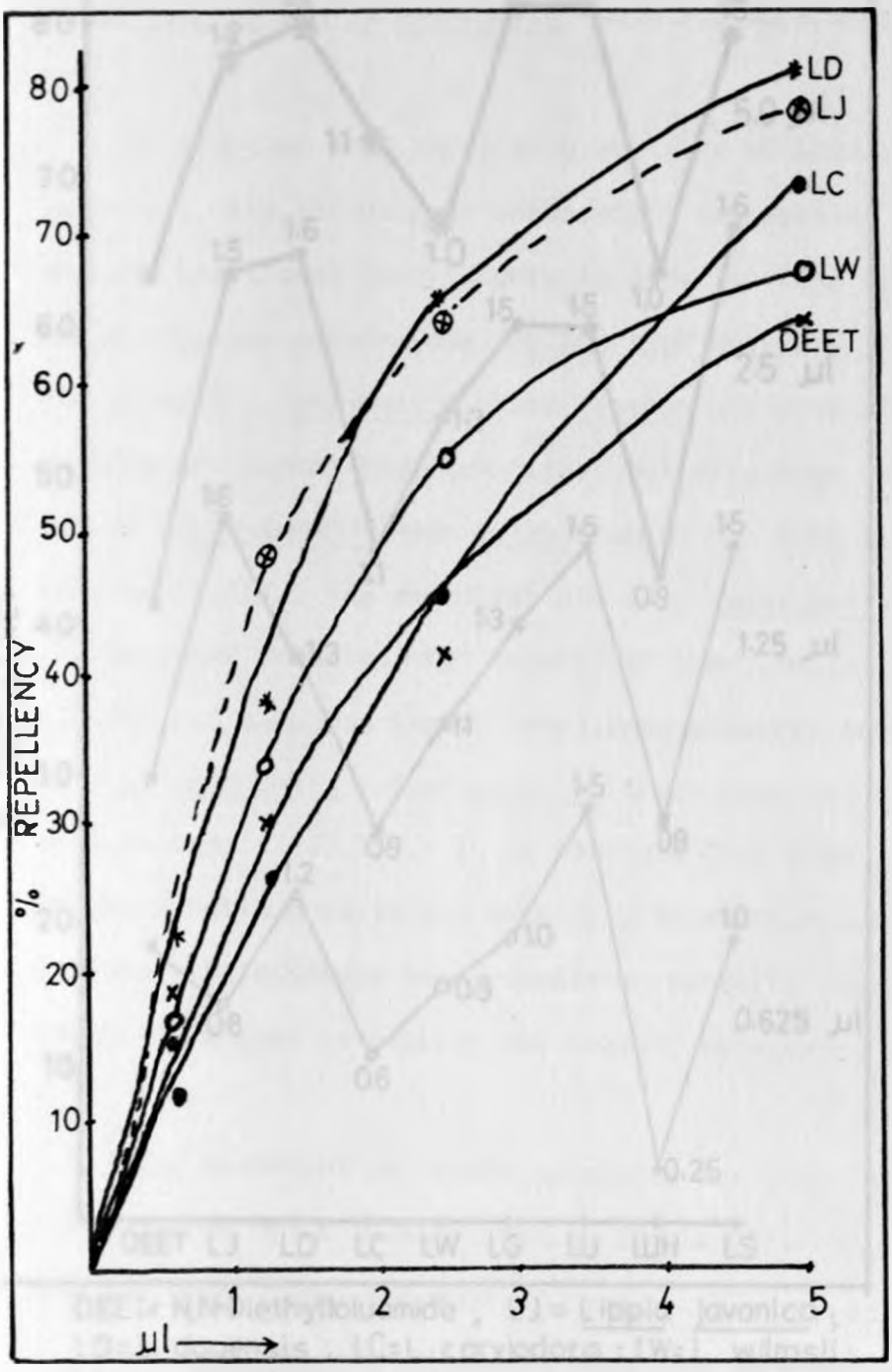
LU = Lippia ukambensis chvar camphor oil

LUH = Lippia ukambensis chvar cineole oil

LS = L. somalensis oil

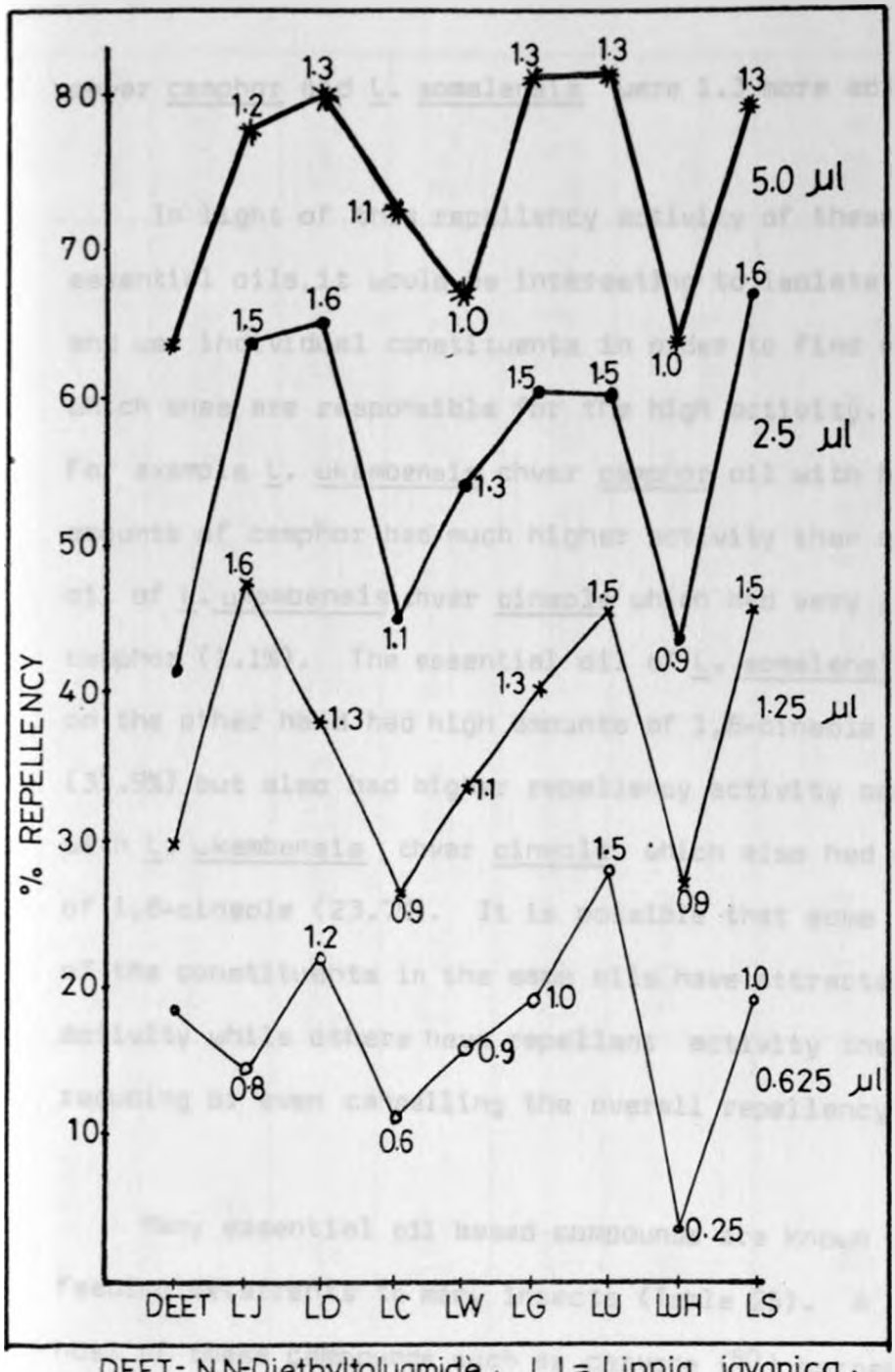
LG = L. grandifolia oil

Fig 40 Dose response curves for the repellency of DEET and essential oils of Lippia species to maize weevils



LD = Lippia dauensis oil
LC = L. carviodora oil
DEET = N,N-Diethyltoluamide
LW = L. wilmsii oil
LJ = L. javanica oil

Fig 41 Comparison of the maize weevils repellency of essential oils Lippia species with DEET



DEET= N,N-Diethyltoluamide ; LJ = Lippia javanica ;
LD= L. dauensis ; LC= L. carviodora ; LW= L. wilmsii ;
LG= L. grandifolia ; LU= L. ukambensis chvar camphor ;
LUH= L. ukambensis chvar cineole ; LS= L. somalensis.

NB: Numbers at the same dose level eg 0.8, 1.2, 0.6, 0.9, 1.0 etc at 0.625 µl indicate relative repellecy of different oils as compared with DEET.

chvar camphor and L. somalensis were 1.3 more active.

In light of this repellency activity of these essential oils, it would be interesting to isolate and use individual constituents in order to find out which ones are responsible for the high activity. For example L. ukambensis chvar camphor oil with high amounts of camphor had much higher activity than essential oil of L. ukambensis chvar cineole which had very little camphor (1.1%). The essential oil of L. somalensis on the other hand had high amounts of 1,8-cineole (31.9%) but also had higher repellency activity compared with L. ukambensis chvar cineole which also had a lot of 1,8-cineole (23.7%). It is possible that some of the constituents in the same oils have attractant activity while others have repellant activity thereby reducing or even cancelling the overall repellency activity.

Many essential oil based compounds are known to be feeding deterrents to many insects (Table 25). A host of these compounds such as carvone (50) borneol (15), p-cymene (34), limonene (42), myrcene (84), α-terpineol (14), terpinen-4-ol (19) among others are constituents of essential oils of Lippia species. It is only with more research work that it can be known whether these compounds have both

Table 25 Insect Feeding Deterrents (Essential oil based)

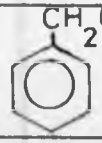
Feeding deterrent	Structure Compound No.	Compound source	Insect name / concentration	Reference
Benzyl alcohol	 (102)	Small grains	Schizaphis graminum 100 ppm	[62,63]
Borneol	(15)	Pinus silvestris	Dendrolimus pini 0.3 %	[64]
Bornyl acetate	(35)	Pinus silvestris	Dendrolimus pini 0.3 %	[64]
Δ^4-Carene	(17)	Pinus silvestris	Dendrolimus pini 0.3 %	[64]
Carvone	(50)	Various	Locusta migratoria migratorioides 0.01 %	[65]
β-caryophyllene	(39)	Various	Locusta migratoria migratorioides 0.01 %	[65]

Table 25 continued

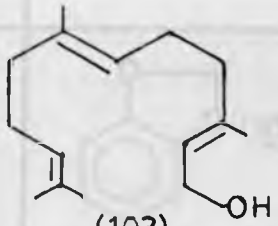
1,8-Cineole	(4)	Eucalyptus spp	Locusta migratoria 0.01% L. migratoria migratorioides 0.05 %. Musca domestica. "mosquitoes", no sp specified	[65, 66]
Citral Citronellal	(29) (25)	Various plants	Locusta migratoria migratorioides 0.001 %.	[65]
Citronellol	(51)	Various plants	Reticulitermes lucifugus santonensis 0.1 ng/insect.	[59]
p-Cymene	(34)	Amorpha fruticosa	Leptinotarsa decemlineata. Locusta migratoria migratorioides. Pieris brassicae.	[67, 68]
Farnesol	 (103)	Various plants	Locusta migratoria 0.1 % Lymantria dispar 3.75 mg/ml	[69, 70]

Table 25 continued

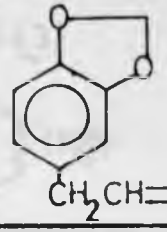
Geranial	(46)	Various plants	Locusta migratoria migratorioides 0.05% Lymantria dispar 375 mg/l Reticulitermes lucifugus santonensis 0.1/ml	[67, 70]
Limonene	(42)	Pinus silvestris	Dendrolimus pini 0.3% Locusta migratoria migratorioides 0.005%	[65]
Myrcene	(84)	Various plants	Reticulitermes lucifugus santonensis 0.1 ng/insect	[59]
Myristicin	 (104)	Pastinaca sativa	"no sp specified"	[66]

Table 25 continued

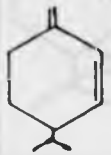
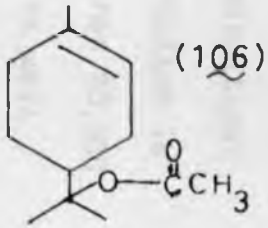
Nerol	(6)	Various plants	Reticulitermes lucifugus santonensis 0.1 ng/insect	[59]
Nerolidol	(31)	Melaleuca leucadendron	Lymantria dispar 3.75mg/ml	[70]
Penyroyal oil (85% pulegone)	(56)	Mentha pulegium	Spodoptera frugiperda	[71]
α-phellandrene β-Phellandrene	(43)  (105)	Various plants	Reticulitermes lucifugus santonensis 0.1 ng/insect	[59]

Table 25 continued

∝-Pinene	(16)	Picea abies Pinus silvestris	Dendrolimus pini. Locusta migratoria migratorioides.	[65, 64]
Terpinen-4-ol	(19)	Amorpha fruticosa	Leptinotarsa decemlineata. Locusta migratoria migratorioides Pieris brassicae.	[68, 67]
∝-Terpineol	(14)	Amorpha fruticosa Pinus silvestris	Dendrolimus pini. Locusta migratoria migratorioides. Pieris brassicae.	[64, 67, 68]
Terpinyl acetate	 (106)	Amorpha fruticosa Pinus silvestris	Dendrolimus pini	[64]

repellent and/or antifeeding activities.

In general therefore, some of the oils tested such as that of L. ukambensis chvar camphor could be very useful since it has a high repellant activity even at low doses. This means that ground leaves of such a plant could turn out to be useful even in rural areas where leaves of such plants are abundant while the pure distilled oil may not be readily available. Of interest also is that L. ukambensis chvar camphor is one of the most widely distributed Lippia species in Kenya. Other essential oils such as those from L. javanica, L. dauensis, L. grandifolia and L. somalensis also showed very promising maize weevils repellant activity.

Approximately 1/3 of the global agricultural production valued at more than US $ 1.0 billion is reportedly destroyed every year by more than 20,000 species of insects, mites and nematodes, as well as plant diseases, weeds, rodents and other plant pests. Losses are higher in developing countries. Synthetic broad-spectrum pesticides used for control are not without problems; toxicity to non-target organisms including human beings, development of pest resistance and environmental degradation are some of these problems. Interest

in alternative pest control has therefore been revived so that the unlimited pest control potential of plants could be exploited [61].

The most important food crop in Kenya is maize and the most common grain storage pest is Sitophilus zeamais. This species is a very serious major (primary) pest of stored grain throughout the warmer parts of the world [209]. Infestation often starts in the field and is later carried into the grain stores. Both larval and adult stages cause heavy damage. The infested grains become very light showing the well known and much detested holes on the seeds. They also attain bad smell and taste and their maize flour goes bad in only a few days. All these together with the ugly sight of floating larva and adult weevils on cooked food makes such maize grains unfit for human consumption [210].

Lippia germinata (wild sage) leaves have been reported to be useful in control of grain pests (species not specified). The leaves and essential oil of Ocimum basilicum have also been reported to be insecticidal to rice weevils (Sitophilus oryzae)

which also attack maize grains /210/. It would therefore be very useful to conduct field experiments with the leaves and essential oils of Lippia species in Kenya especially with those that have been found to show high repellent activity on maize weevils. If proved successful this investigation could be extended to other major storage pests in Kenya such as Angoumois grain moth (Sitotroga cerealella) rice weevils (Sitophilus oryzae), Red flour beetle (Tribolium castaneum) and bean beetle also known as bruchid (Acanthoscelides obtectus) /211/. Apart from the toxicity associated with the commonly used pesticides in grain storage in Kenya (eg Lindane, malathion, Malathion + tetraethyl pyrophosphate and others), resistance to these pesticides by the pests has been recorded in many parts of the world hence strengthening the need for more research on alternative methods for the control of these pests /211/.

4.5.4. PHARMACOLOGY

Effect of Essential oils of Lippia species on Smooth muscles

At the dose of 125 µg/ml bath concentration all the essential oil of Lippia species tested (excluding L. carviodora var minor oil due to low quantity) induced

a 70% inhibition of gastrointestinal smooth muscle contractile activity. The spasms induced by acetylcholine or histamine in guinea pig ileum were also greatly inhibited by over 85% and 45% respectively (Fig 42). This indicates that due to this antispasmodic activity of these oils they may be useful in some gastrointestinal ailments associated with spasms. This would be an added advantage since these oils may also be used in foods as flavouring agents.

The essential oils also had profound spasmolytic effect on the isolated intact guinea pig trachea at 125µg/ml final concentration. In presence of histamine, the essentials of Lippia species did not only completely abolish the constrictor effect of histamines but also caused relaxation of the trachea. This indicates that with more research work these essential oils may prove useful in alleviating asthmatic conditions due to their spasmolytic effects on the trachea. Indeed, piperitone which is one of the major components in Lippia wilmsii had been reported to be asthmolytic [212].

The mechanism of action of essential oils in producing relaxation of respiratory smooth muscles is not known. Theophylline is one of the drugs used in the treatment of asthma. It is an inhibitor of cyclic nucleotide phosphodiesterase and so causes accumulation of

cyclic AMP (anedosine -3,5-monophosphate). It is believed that an increased intracellular cyclic AMP content may influence movements of calcium ions involved in smooth muscle contraction, with the result that relaxation occurs. The brochodilator action of sympathomimetic amines (eg salbutamol) is due to stimulation of adenylate cylase resulting in increased intracellular cyclic AMP [87]. Corticosteroids (eg prednisolone), among other effects, increase the stability of membranes thereby reducing the release of histamine and other broncho-constrictor substances. Sodium cromoglycate as a drug used in asthma treatment also acts as a mast cell stabilizer. It does this by inhibiting cyclic nucleotide phosphodiesterase in mast cell, thereby preventing the increase in calcium permeability by raising the intracellular concentration of cyclic AMP [87]. It is not clear which of these mechanisms is applicable to essential oils of Lippia species but it may be possible that it is due to the inhibition of histamine release from tissues and mast cells which has been associated with the antiinflammatory activity of Curcuma longa oil [85].

Effect on the isolated rabbit heart

The essential oils of Lippia species (L. carviodora var minor not tested) had a negative inotropic effect (decrease in the force of heart contraction) by more than 45% at 200 µg (Fig 42) and 25% at 100µg doses. With more research these essential oils may therefore find use as antiarrhythymic drugs [213]. Rosemary oil, 1,8-cineole and bornyl acetate (35) have been shown to depress contractility of the cardiac muscle and also inhibit acetylcholine-induced contraction of guinea pig ileum. The heart was more sensitive to rosemary oil and 1,8-cineole (4) than the smooth muscle of the ileum [79]. 1,8-cineole was one of the major ingredients in Lippia ukambensis chvar cineole oil and L. somalensis oil. Significant amounts of borneol (15) were also present in L. ukambensis chvar camphor. It would be easy to convert borneol into bornyl acetate chemically if more useful pharmacological effects would be found in the ester form.

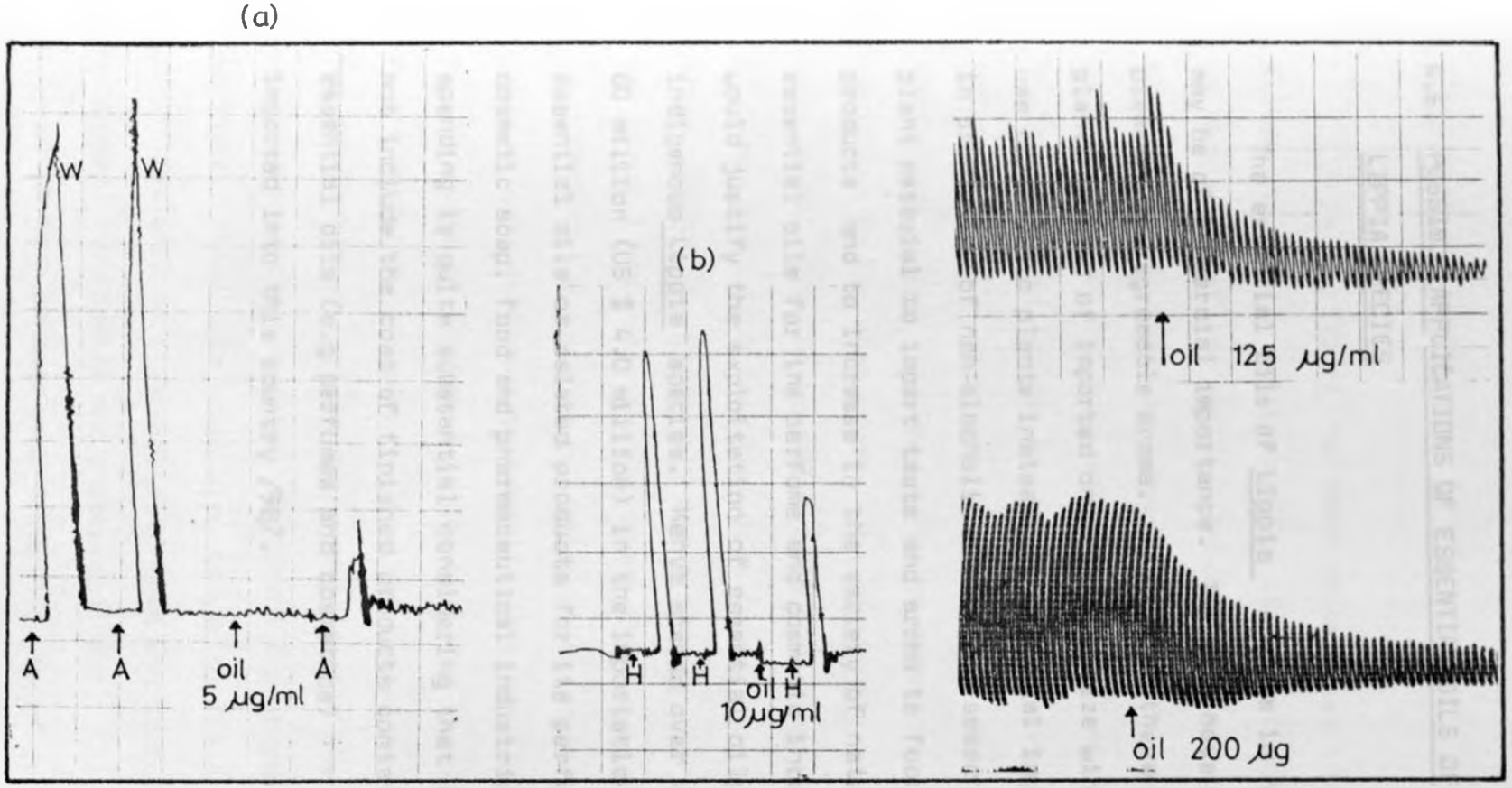


Fig 42 Effect of essential oils of Lippia species on (a) and (b) guinea pig ileum (c) rabbit ileum (d) rabbit heart
A = Acetylcholine 0.05 µg/ml H = Histamine 0.3 µg/ml
W = Wash

4.6. POSSIBLE APPLICATIONS OF ESSENTIAL OILS OF LIPPIA SPECIES

The essential oils of *Lippia* species in Kenya may be of commercial importance. The oils have a pleasant and agreeable aroma. The use of these local plants instead of imported ones to aromatize wine; use of aromatic plants instead of artificial ingredients in production of non-alcoholic beverages; search for plant material to impart taste and aroma to food products and to increase in the variety of natural essential oils for the perfume and cosmetic industry would justify the exploitation of essential oils from indigenous *Lippia* species. Kenya spends over Ksh 80 million (US $ 4.0 million) in the importation of essential oils or related products for its perfume, cosmetic soap, food and pharmaceutical industries. This spending is quite substantial considering that this does not include the cost of finished products containing essential oils (e.g perfumes and cosmetics) imported into this country /98/.

Industrial exploitation

Lippia citriodora has been naturalized in Southern Europe and is also cultivated for its essential oil in many parts of the world. The oil imparts refreshing odour to toilet water perfumes and Eau de Cologne; it is used for scenting bath salts and also blends well with various perfumes and may be used for flavouring liqueurs and non-alcoholic beverages. There is no reason why the indigenous Lippia species cannot be commercially exploited in the same manner. L. somalensis has a mango-like aroma. All the essential oils from Lippia species studied (apart from essential oils from Lippia ukambensis chvar camphor, L. ukambensis chvar cineole and L. carviodora var minor) had very alluring odour that would give perfumes or cosmetics a rounding out effect, the origin of which would be very difficult to identify (The non-identification of the source of fragrance is the base for the success of the perfumery industry). They can therefore be used for manufacturing perfumes and cosmetics with their own unique and particular scent, different from other perfumes. These oils would also be useful (in low concentrations) as flavourings in food industries, for beverages, toothpastes and pharmaceuticals. These essential oils would also enhance user acceptability of certain preparations like insecticidal sprays, varnishes, lubricants and paints.

Uses of some components of essential oils in Lippia species

Some of the components of the essential oils of Lippia species are already well-known for their uses. Thymol (23), which occurs in L. grandifolia is employed in many antiseptic mixtures intended for use on the membrane cavities, especially in gargles, mouth washes and other oral preparations. Linalool also found in the same plant, is one of the most important aromatic isolates, used widely in the perfume, soap and flavour industry. 1,8-cineole (4) (present in L. ukambensis chvar cineole and L. somalensis) is used very widely in pharmaceutical preparations applied locally or taken internally. Internally it serves as a stimulant expectorant in treatment of chronic bronchitis and other respiratory diseases. 1,8-cineole is also a mild local anaesthetic. It is also used in room sprays, lotions and in all kinds of cosmetic preparations.

∝-Terpineol (14) found in L.ukambensis chvar cineole oil is one of the most important compounds used in the perfume, cosmetic and soap industries due to its lilac odour. Camphor (2) found in L.ukambensis chvar camphor oil is used in countless medicinal

preparations as a local anaesthetic and remedy for rheumatic conditions, muscular strain and similar inflammation. Internally it serves as a circulatory and central nervous system stimulant and a carminative. It is included in some pharmaceutical products to ease respiratory disease conditions. Carvone (50) is also the main ingredient in dill and caraway essential oils which are mostly used in children's gripe medicine and in food flavours, Piperitone (45) found in L. wilmsii is used for scenting of many preparations such as insecticidal spray, lubricating agents, varnishes and paints [45]. Piperitone has also been reported to have asthmolytic effect [212]. It should also be noted that rosemary oil (from Rosmarinus officinalis) L. [45] which is extensively used in soaps, room sprays, inhalants, food products, meats, sausages, soups and sauces contains 1,8-cineole (4) and borneol (15) as the major ingredients. These compounds are found in essential oils of either L. ukambensis chemical varieties or in that of L. somalensis. Although some of these constituents (eg carvone, camphor, 1,8-cineole and linalool) in the essential oils of Lippia species could be commercially separated, the total oil is mostly used in many preparations even when a certain property is known to be due to a certain component in a given essential oil.

Medicinal uses of essential oils of Lippia species

The chemical composition of the essential oils would indicate that L. ukambensis chvar camphor, L. ukambensis chvar cineole and L. somalensis (due to high 1,8-cineole (4) and camphor (2) content) would serve mainly as medicinal oils. The finding that the essential oils of Lippia species abolished the bronchospasms produced by histamine may find some clinical application in asthma management. The others apart from L. carviodora var minor would mainly be utilized as has already been suggested in perfume, cosmetic, soap industries, in food industries, beverages and to flavour pharmaceutical products. Although L. carviodora var minor had very little essential oil containing sesquiterpenes, the high amount of salicylic acid (98) found in the ether fraction of the non-volatile portion could be used for its bacteriostatic and fungicidal activity. Since salicylic acid can be used for treatment of a host of other skin ailments it may be possible to formulate the crude samples for treatment of these diseases. This plant could also serve as a source of raw material for the manufacture of aspirin. It is not difficult to imagine the possibility in future, of the use of genetic engineering

to induce *in vivo* conversion of this salicylic acid into aspirin.

Antimicrobial use

Since some of the essential oils studied had considerable antimicrobial activity at low levels, the external preparation (e.g. cosmetics, soaps, mouth preparations and toothpastes) would also have an added advantage of reducing infection by fungi such as *Candida albicans*, *Microsporum canis* and *M. audouinii* or bacteria such as *Staphylococcus aureus*. A number of essential oils of *Lippia* species had antimicrobial activity on these microorganisms at MIC 1000 µg/ml or lower. The commonly used strong antifungal medications for local application in Kenya include clotrimazole "Canesten", econazole "Pevaryl" and bifonazole "Mycospor". They are all used at 1% i.e 10,000 µg/ml final concentration. This indicates that some of these oils may find a role to play as skin antifungal agents. The essential oils of *Satureja montana* and *Lippia citriodora* have already been shown to be effective in prevention of dental caries [214].

The fungitoxic effect of the essential oils on phytopathogenic fungi especially to Colletotrichum coffeanum is of immense potential economic importance. This fungus is the causative agent of Coffee Berry Disease (CBD) in Kenya. This fungal disease affects different parts of the coffee plant. When flowers are attacked, they develop dark brown blotches or streaks and the whole flower soon becomes invaded and dies. However, of more importance is the attack on green and ripe berries. The green berries attain small sunken patches which spread rapidly and the berry soon becomes blackened and mummified and beans fail to develop inside such berries. The ripe berries when attacked yield low quality coffee. The same fungus is also responsible for Elgon die-back, a disease where the coffee plants progressively die from the tip to the roots [215]. It is therefore apparent that a lot of crop is lost through this disease every year.

The most recommended antifungal agents for CBD treatment are the copper based compounds (e.g Copper oxychloride) at a concentration of about 7000 µg/ml. The other recommended but more expensive and sometimes less effective antifungal agents for the disease include

Captafol (4000 µg/ml), Chlorothalonil and Anilazine (4000 µg/ml) and Dithianon (3000 µg/ml) [216]. All the essential oils of Lippia species tested had a fungicidal effect on this fungus at MIC 2000 µg/ml or lower. For example Lippia grandifolia and L. javanica were fungicidal to Colletotrichum coffeanum at MIC 50 and 500 µg/ml respectively. These are much lower concentrations than any of the recommended chemicals. The toxicity of all the essential oils to human beings is very low in general and no such toxic effects as those of copper are reported [217]. The fact that low doses of essential oils of Lippia were effective against C. coffeanum and some of these oils had been shown to have larvicidal activity at low concentrations (e.g Lippia javanica LD_{50} 74.1ppm) may be a good enough reason to test these oils either alone or in combination with other pesticides for the control of CBD and aphids. Lippia citriodora oil (1%) spray has already been shown to cause 93% mortality to aphids [41] while certain essential oil ingredients or precursors in Lippia species have exhibited alarm pheromone activity [47]. Intercropping of Lippia species with coffee plants could be experimented on to see what effect they could have on the fungi and aphids.

Traditional uses

The Lippia species in Kenya especially L. ukambensis chvar camphor and L. javanica have traditionally been used to treat respiratory conditions. The scientific basis for this has been demonstrated by the spasmolytic effect of the essential oils of Lippia species on the respiratory system. Some of the components identified are also known to have expectorant activity.

The traditional scenting of cooking animal fat by macerating the leaves of Lippia species (especially L. carviodora and L. dauensis) in molten fat is justified considering the high essential oil content of these plants and the agreeable aroma due to their favourable chemical composition.

CHAPTER 5

5. CONCLUSION AND RECOMMENDATIONS

5.1. CONCLUSION

1. The presence of essential oils from *Lippia wilmsii*, *L. somalensis* and *L. carviodora* var *minor* and the chemical composition of these oils has been reported for the first time in the present work.

2. Based on essential oil composition two chemical varieties (chvar) of *L. ukambensis* have been discovered in Kenya. These varieties exhibit similar morphological and microscopic features but different chemical composition in their essential oils. These have been designated *Lippia ukambensis* chvar *camphor* and *Lippia ukambensis* chvar *cineole*. The Tanzanian *L. ukambensis* has been designated as *L. ukambensis* chvar *camphor-cineole*. This is an important finding in the chemotaxonomy of *Lippia ukambensis* varieties.

3. The essential oil of *L. somalensis* has been shown to have a lot of qualitative similarities and quantitative differences with the oils from *L. ukambensis* chemical varieties in 15 constituents.

4. The essential oil of L. carviodora contains mainly monoterpenes (carvone and limonene) while L. carviodora var minor contains mainly sesquiterpene hydrocarbons. So apart from their differences in morphological features, there are major differences in their essential oil composition. A substantial amount of salicylic acid was also isolated from the non-volatile portion of L. carviodora var minor.

5. The composition of the essential oil of L. dauensis has been reported in the present work for the first time. The actual composition of Lippia javanica oil especially the contribution of myrcenone, cis- and trans-ocimenone was also reported for the first time in the present work. A compound (2-methyl-6-methylene-2, 7-octadien-4-ol) identified in the essential oil of L. dauensis is a known sex attractant pheromone produced by male Ips species (Pine bark bettles). This is the first reported work on the presence of this compound in plants. There were also other compounds in the essential oils of L. javanica and L. dauensis that could easily be converted into important sex pheromones of this pine bark bettles.

6. The essential oil of L. grandifolia has shown the presence of useful components.

7. The antimicrobial assay indicates that some essential oils for example those of L. javanica and L. grandifolia were most active especially against both the human and plant pathogenic fungi. Indeed, the essential oil of L. grandifolia was fungicidal to Colletotrichum coffeanum at a minimum inhibition concentration (MIC) of 50 µg/ml. This is the causative agent of Coffee Berry Disease (CBD) which is one of the most prevalent and feared diseases in coffee farming in Kenya. The most recommended antifungal agent in CBD treatment are the copper based compounds (e.g copper oxychloride) at a concentration of about 7000 µg/ml.

8. Results obtained from the bioassay of larvicidal activity of essential oils from Lippia species indicate that these oils may provide a means for small-scale control of mosquitoes.

9. The bioassay on the repellant activity of essential oils of Lippia species to maize weevils (Sitophilus zeamais) shows that most of the oils are more active

than the standard synthetic insect repellant (N,N-diethyltoluamide). This activity may be very useful in maize grain stores. Organophosphate and carbamate based insecticides are the commonly used compounds in maize stores. These compounds are relatively more toxic than the essential oils.

5.2. RECOMMENDATIONS

Kenya imports all the essential oils or their ingredients for its domestic use either in perfume, cosmetic, soap, food or pharmaceutical industry. A substantial amount of foreign exchange is spent for this purpose. The country would save a reasonable amount of foreign exchange if essential oils of *Lippia* species as well as other possible rich sources of these oils, were exploited.

From the work already carried out in the present project, the following could be recommended:-

(a) Conservation of these *Lippia* species should be encouraged. This is particularly so for species such as *Lippia grandifolia*, *Lippia wilmsii*, *Lippia dauensis* and *Lippia carviodora* var *minor* which might be endangered species soon. The areas they now occur are being economically and ecologically exploited. This is taking place through cultivation of agricultural crops, grazing and competition with introduced trees and shrubs. As already mentioned, a number of these *Lippia* species are considered as obnoxious weeds which should be eradicated even in rangelands.

(b) Cultivation of various Lippia species so that the materials are readily available for commercial exploitation of the essential oils or any other scientific work and for preservation of species and varieties such as Lippia ukambensis chvar cineole. Some countries are already cultivating Lippia citriodora on commercial scale [218].

(c) Commercial exploitation of the already available materials as sources of essential oils especially from those species which occur in abundance such as Lippia carviodora and Lippia ukambensis chvar camphor. Local industries especially those dealing in cosmetics, perfumes and soaps should be encouraged to use the locally available essential oils such as these of Lippia species instead of relying on imported ones.

(d) Field experiments on antifungal activity of Lippia grandifolia oil or its constituents should be carried out on Colletotrichum coffeanum, the causative agent of Coffee Berry Disease (CBD), Hemileia vastaria, the causative agent of leaf rust (both fungal diseases) and aphids. If

L. grandifolia oil or any other oil from *Lippia* species is found to have significant effect and at economic rates, this would be a very useful finding.

(e) Research work using isolated constituents should be carried out to find out which constituents are responsible for the maize weevils repellant activity.

Field experiments should also be initiated to establish the effectiveness of the essential oils of *Lippia* species in Kenya in maize protection from weevils (*Sitophilus zeamais*) during storage. For the plants whose essential oil has been shown to be highly repellent, for example *L. ukambensis* chvar *camphor*, *L. javanica*, *L. dauensis*, *L. somalensis* and *L. grandifolia*, ground leaves should also be tested on their repellency to maize weevils and other common grain storage pests. If proved to be potent, individual farmers could be encouraged to grow the specific species for domestic storage of the grains.

Other biological assays on the essential oils of *Lippia* or their constituents should be carried out to find out, for example, whether they could serve as pheromones. The finding that some ingredients of *Lippia*

dauensis oil and Lippia javanica oil are either sex attractants of some beetles or intermediate precursors of these pheromones is very interesting [177]. Maybe with enough research work some of these oils may not only be found to be sex attractants but also alarm and trail pheromones to insect pests. They could therefore be utilized in biological control techniques against pests.

Some of the ingredients found in Lippia species such as camphor (2), α-terpineol (14), 1,8-cineole (4), linalool (30), myrcene (84) terpine-4-ol (19), limonene(42) and borneol (15) among others have already been reported to have either insect repellent and/or antifeeding activities [56, 59, 64, 65]. More research work on essential oils of Lippia species or their constituents would reveal the full potential of these oils as insect antifeedants. As pheromones or antifeedants, the essential oils of Lippia species would be very useful in various aspects of agriculture.

The full pharmacological and toxicological profile of these essential oils including the effect on central nervous system neurotransmitter metabolism should be established.

(f) Further research work should be carried out on the non-volatile fractions of these Lippia species in order to establish their chemical composition and the biological /pharmacological activities. It is known for example that some of these plants are used as tea substitutes (e.g Lippia javanica, L. carviodora and L. grandifolia). Apart from the essential oils which may provide the flavour, there may be other non-volatile components in these "teas" whose pharmacological and/or toxicological profiles are still unknown.

The fact that pharmacological investigations of the aqueous extract of the Lippia grandifolia leaves have demonstrated a muscle relaxant property bordering on tranquillizing action and antihypertensive effect in both man and animals /154/, indicates the need for further investigations on the non-volatile constituents of the same plant and other indigenous Lippia species. The presence of salicylic acid from Lippia carviodora var minor in the present work further supports the need for more investigations on non-volatile constituents of Lippia species.

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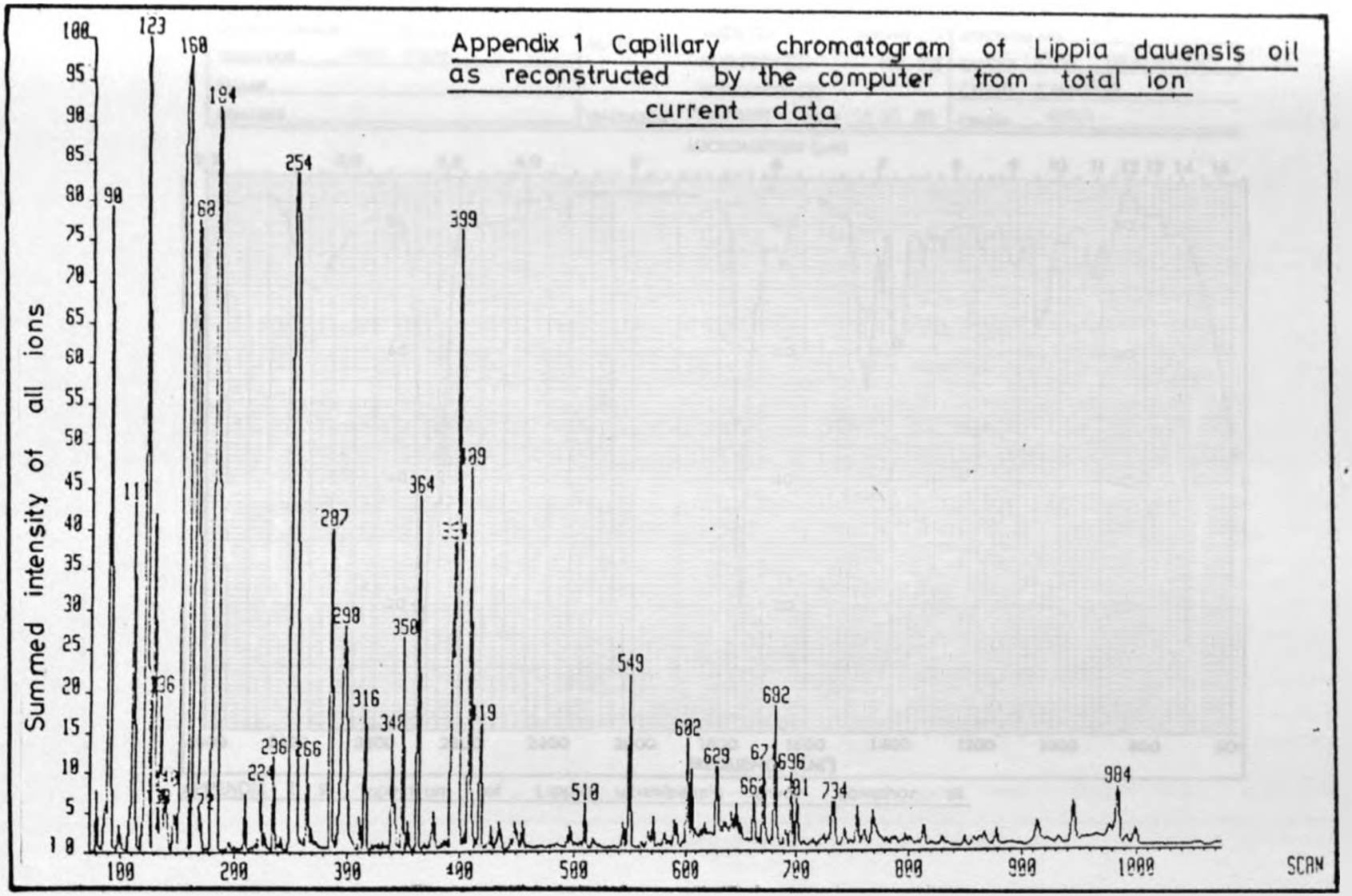
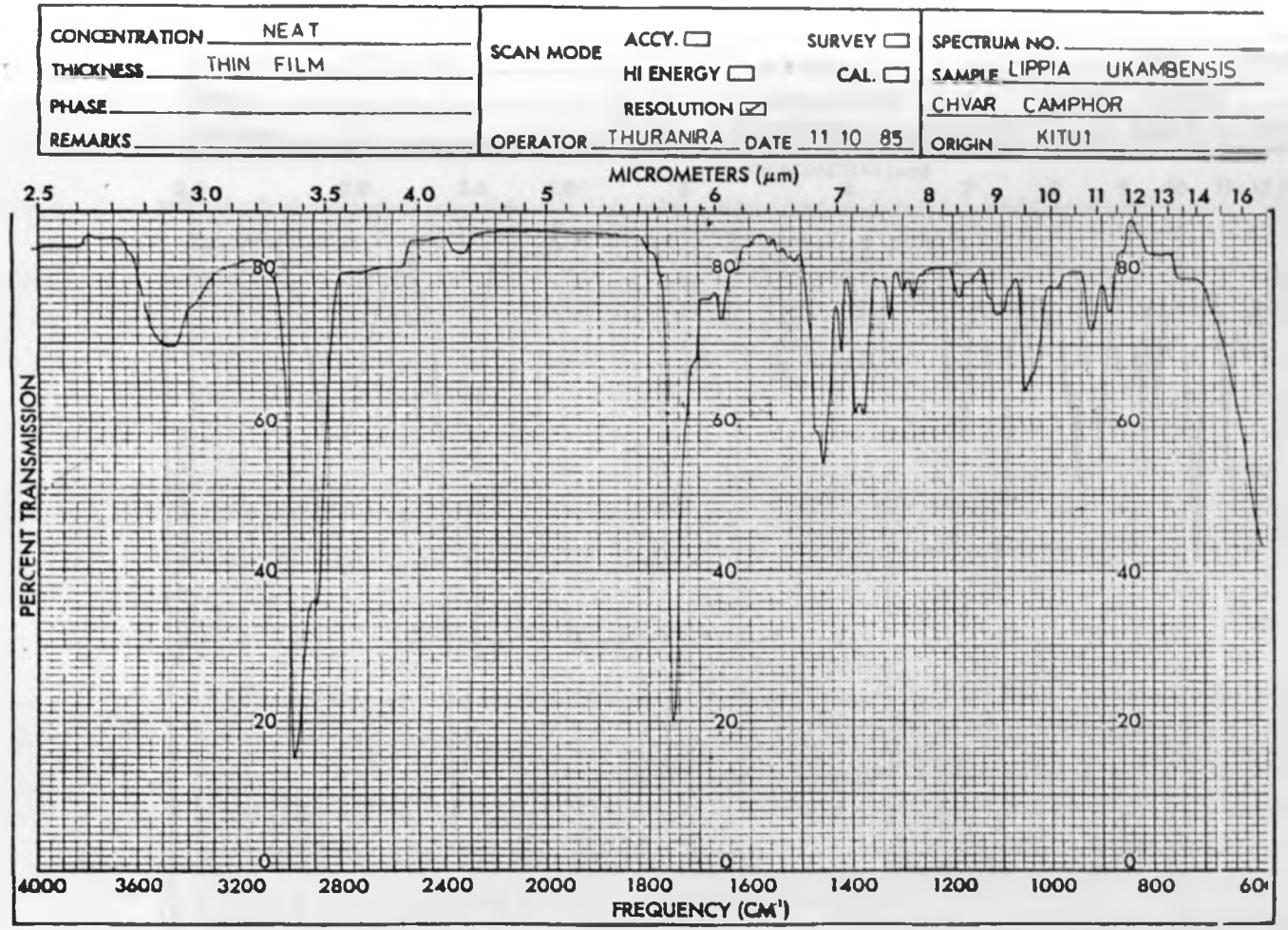

Appendix 1 Capillary chromatogram of Lippia dauensis oil as reconstructed by the computer from total ion current data
Summed intensity of all ions
SCAN
123
168
194
254
98
68
399
364
287
298
350
316
549
682
602
629
696
734
984
510
100
200
300
400
500
600
700
800
900
1000

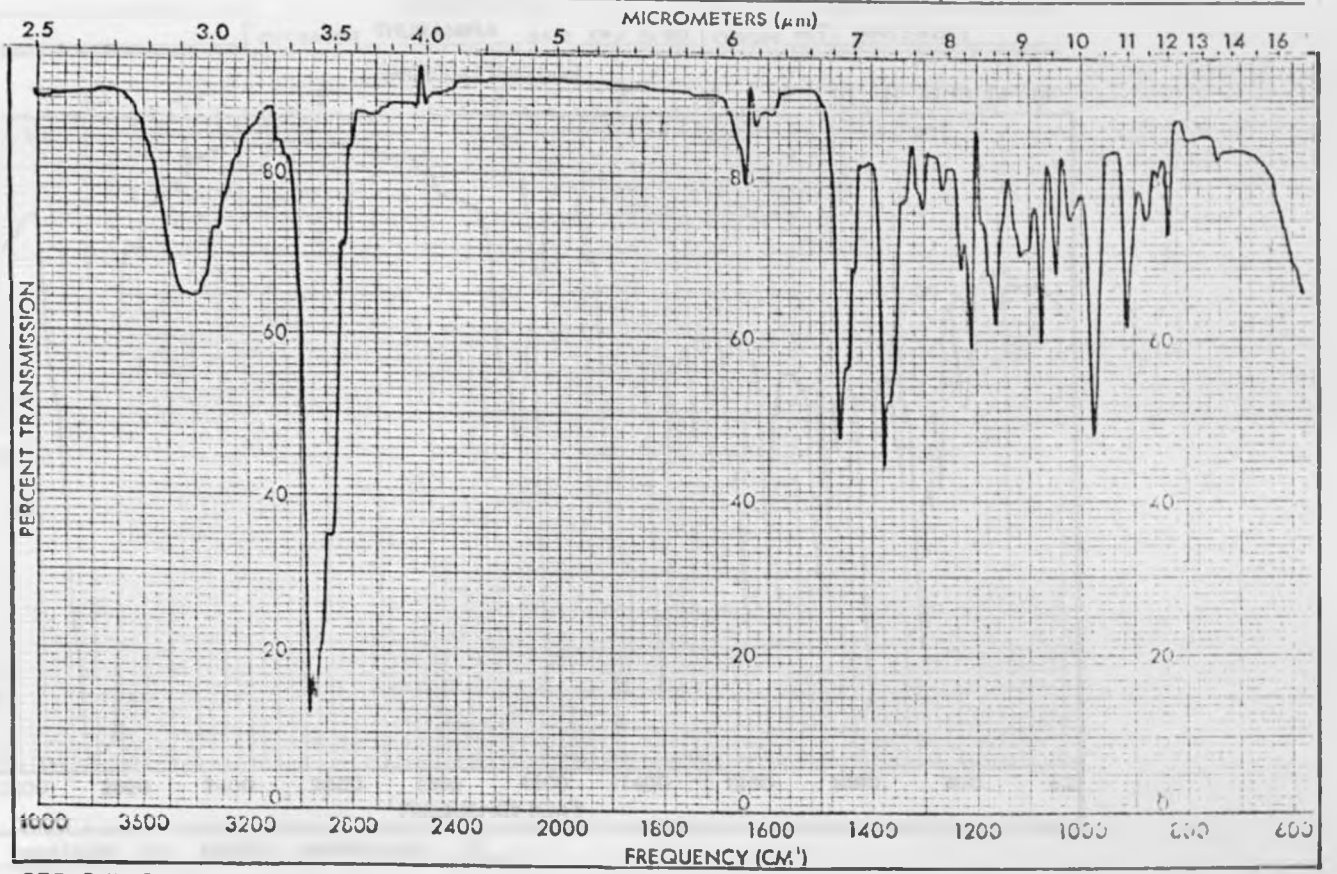


APPENDIX 2 IR spectrum of Lippia ukambensis chvar camphor oil

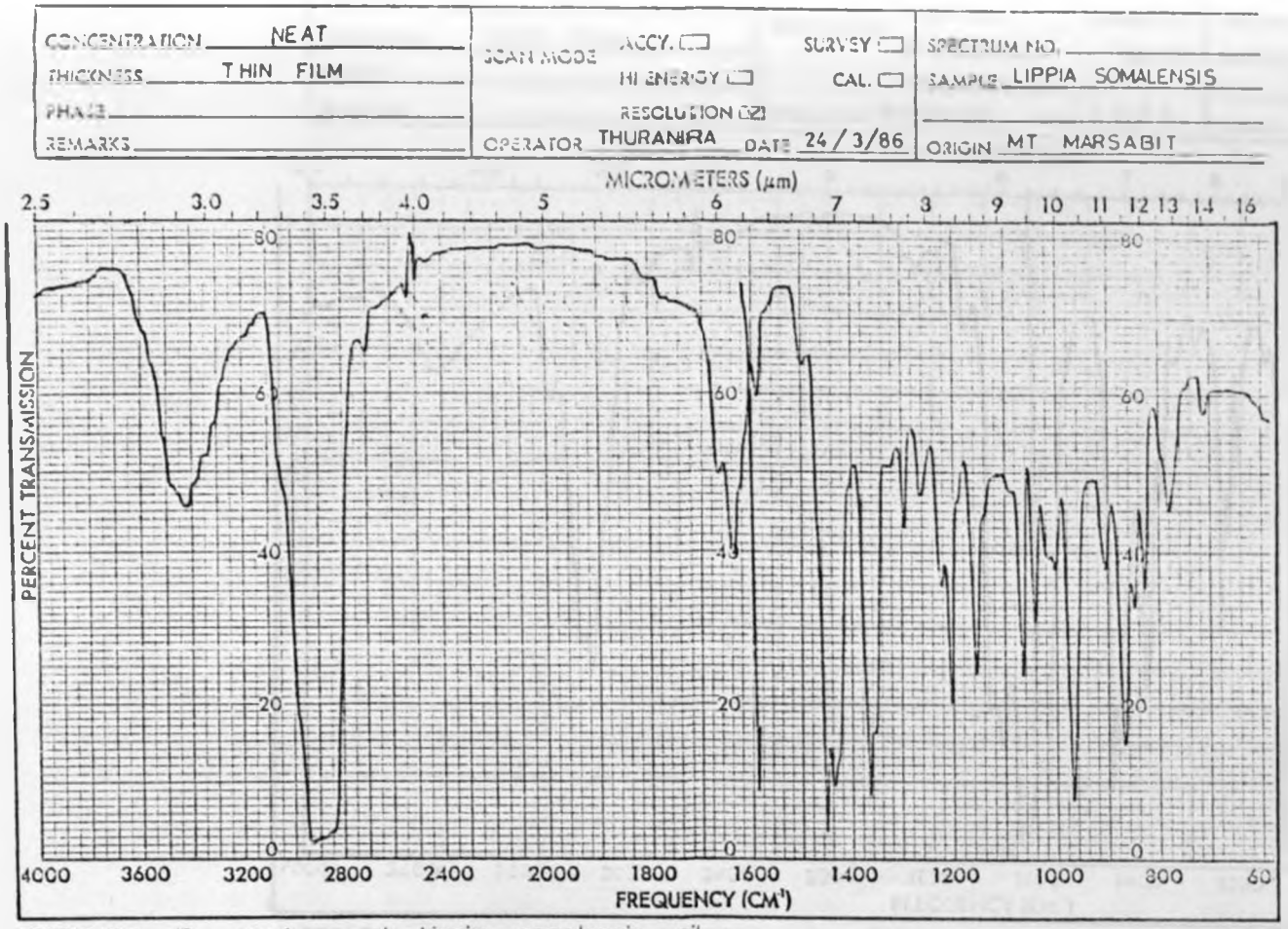
CONCENTRATION NEAT
THICKNESS THIN FILM
PHASE
REMARKS

SCAN MODE ACCY. ☐ SURVEY ☐
HI ENERGY ☐ CAL. ☐
RESOLUTION ☑
OPERATOR THURANIRA DATE 11/10/85

SPECTRUM NO.
SAMPLE LIPPIA UKAMBENSIS
CHVAR CINEOLE
ORIGIN KABETE ex KIRINYAGA



APPENDIX 3 IR spectrum of Lippia ukambensis chvar cineole oil

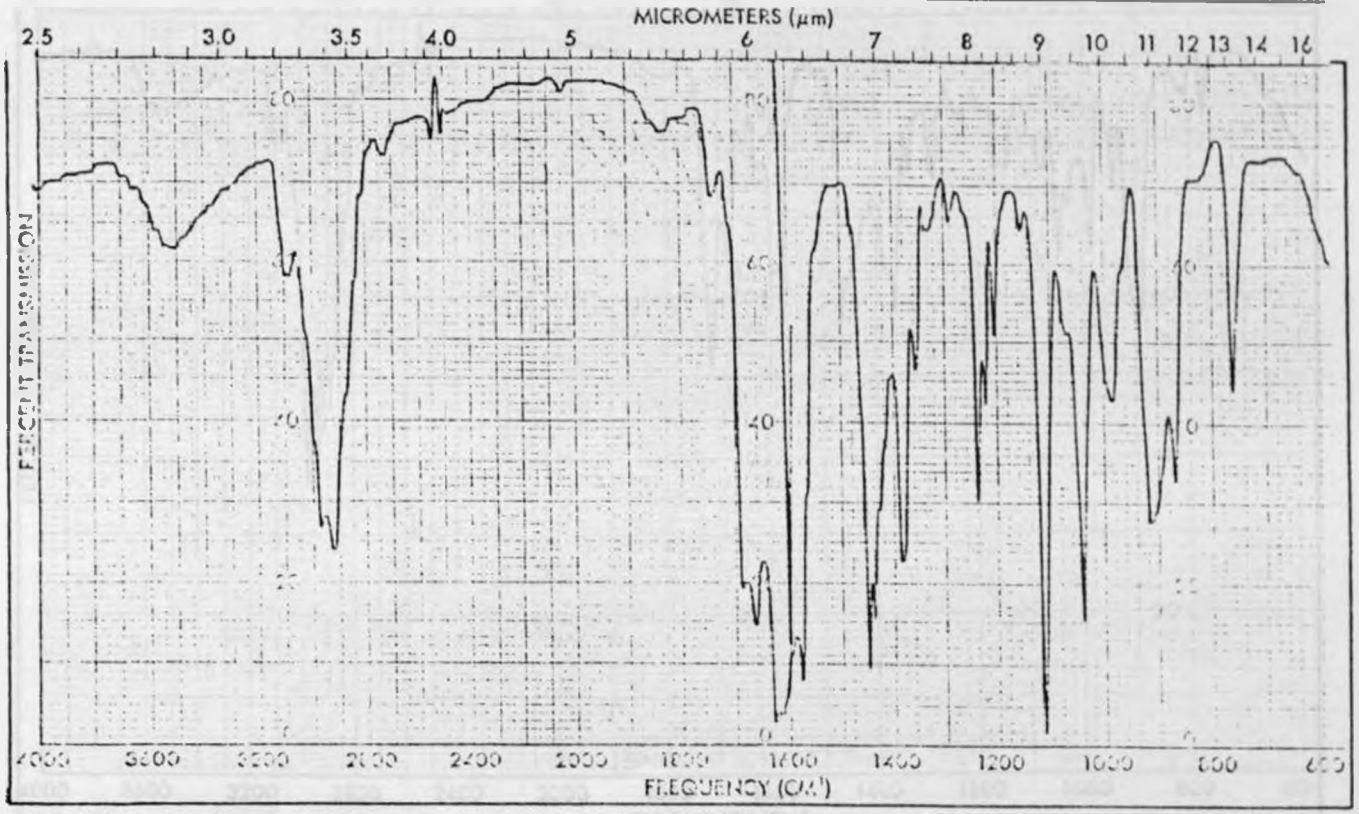


APPENDIX 4 IR spectrum of Lippia somalensis oil

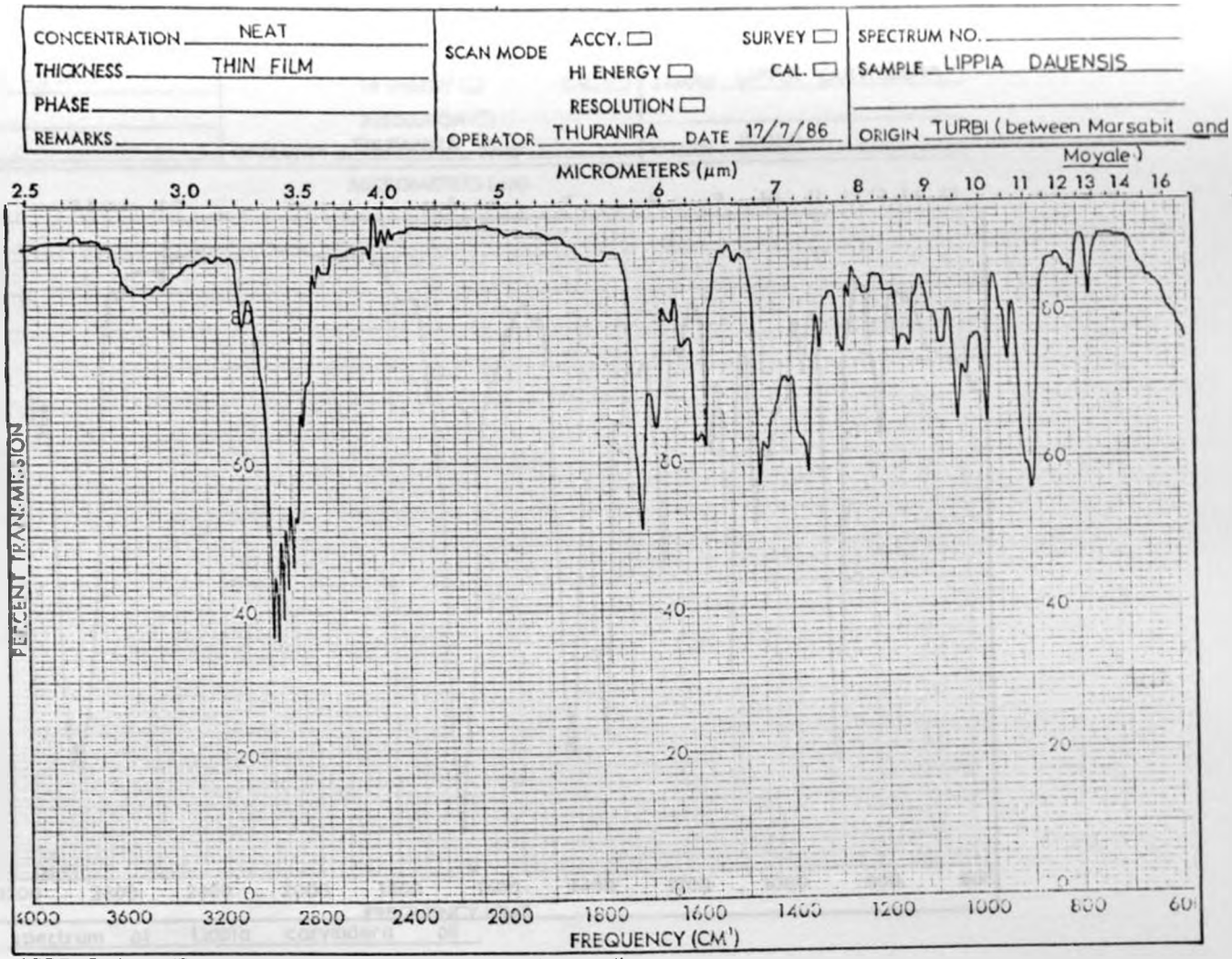
CONCENTRATION NEAT
THICKNESS THIN FILM
PHASE
REMARKS

SCAN MODE ACCY. ☐ SURVEY ☐
HI ENERGY ☐ CAL. ☐
RESOLUTION ☑
OPERATOR THURANIRA DATE 4/5/84

SPECTRUM NO.
SAMPLE LIPPIA JAVANICA
(fresh)
ORIGIN NAIROBI



APPENDIX 5 IR spectrum of Lippia javanica oil

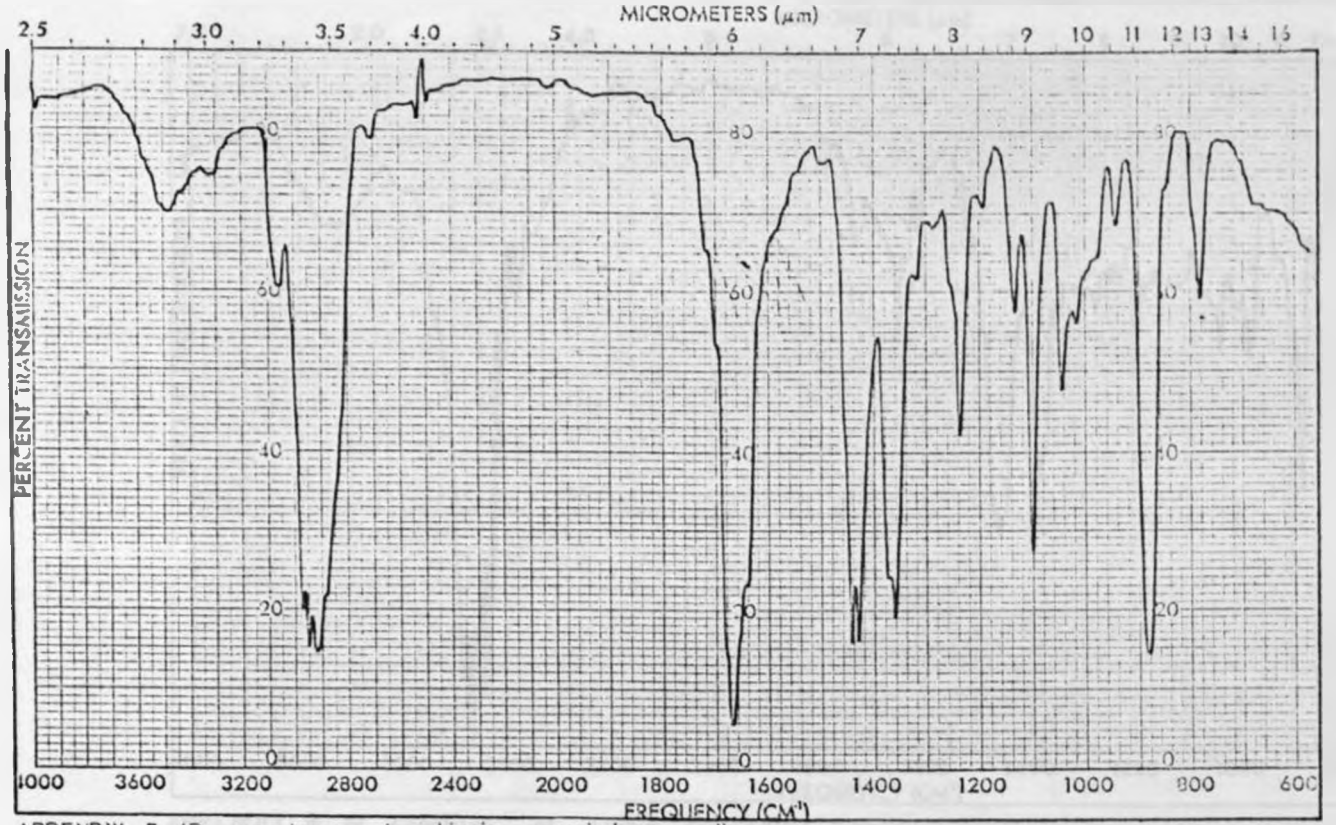


APPENDIX 6 IR spectrum of Lippia dauensis oil

CONCENTRATION NEAT
THICKNESS THIN FILM
PHASE
REMARKS

SCAN MODE ACCY. ☐ SURVEY ☐
HI ENERGY ☐ CAL. ☐
RESOLUTION ☐
OPERATOR THURANIRA DATE

SPECTRUM NO.
SAMPLE LIPPIA CARVIODORA
ORIGIN ISIOLO

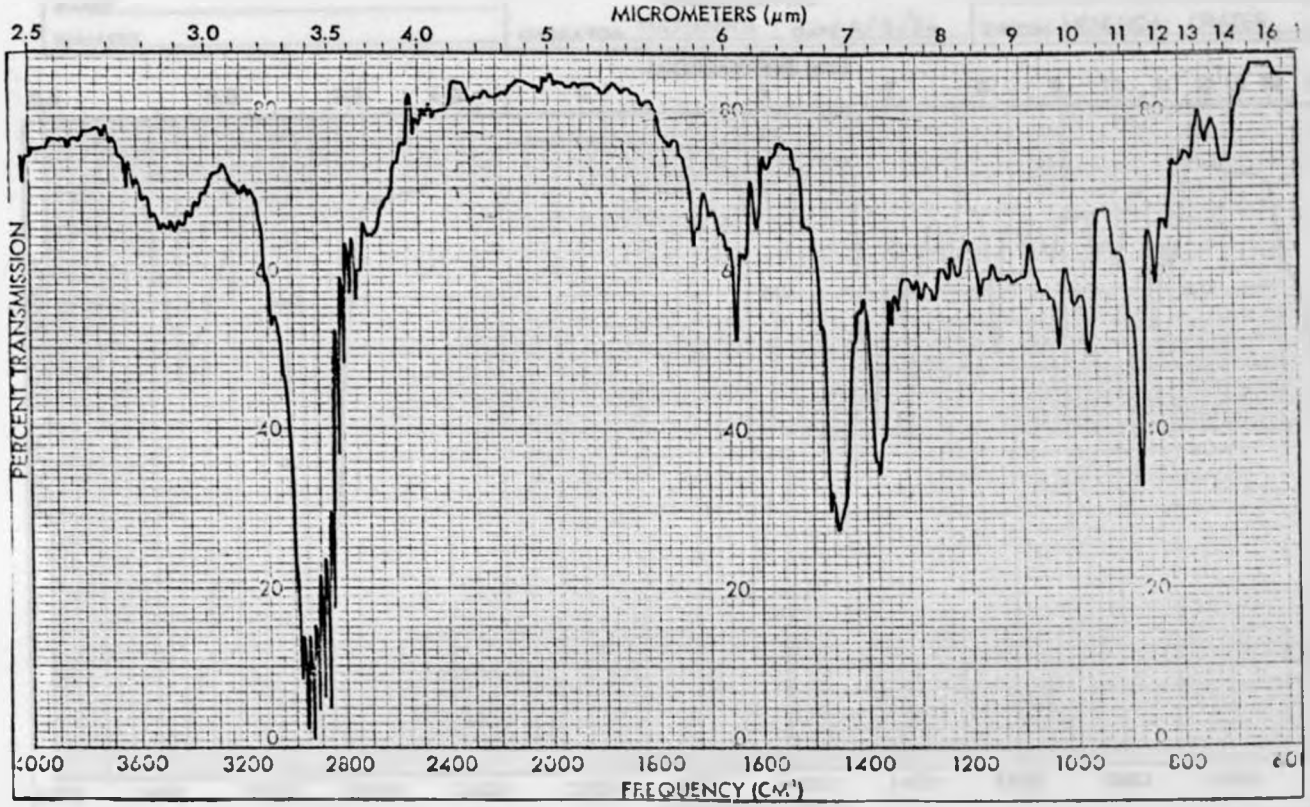


APPENDIX 7 IR spectrum of Lippia carviodora oil

CONCENTRATION NEAT
THICKNESS THIN FILM
PHASE
REMARKS Noise at high frequecy

SCAN MODE ACCY. ☐ SURVEY ☐
HI ENERGY ☐ CAL. ☐
RESOLUTION ☑
OFERATOR THURANIRA DATE 17/12/86

SPECTRUM NO.
SAMPLE LIPPIA CARVIODORA
VAR MINOR
ORIGIN TSAVO WEST NATIONAL PARK

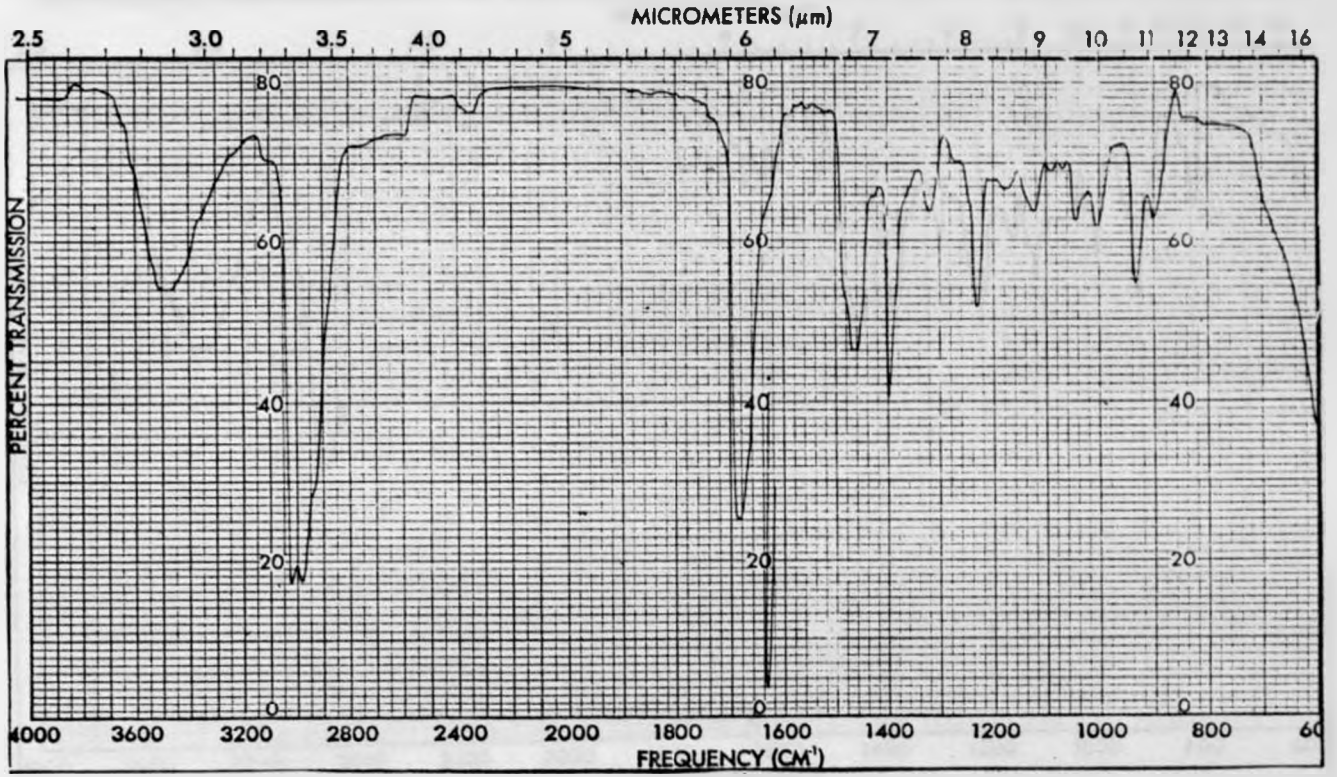


APPENDIX 8 IR spectrum of Lippia carviodora var minor oil

CONCENTRATION NEAT
THICKNESS THIN FILM
PHASE
REMARKS

SCAN MODE ACCY. ☐ SURVEY ☐
HI ENERGY ☐ CAL. ☐
RESOLUTION ☑
OPERATOR THURANIRA DATE 4/5/84

SPECTRUM NO.
SAMPLE LIPPIA WILMSII
ORIGIN MENENGAI CRATER

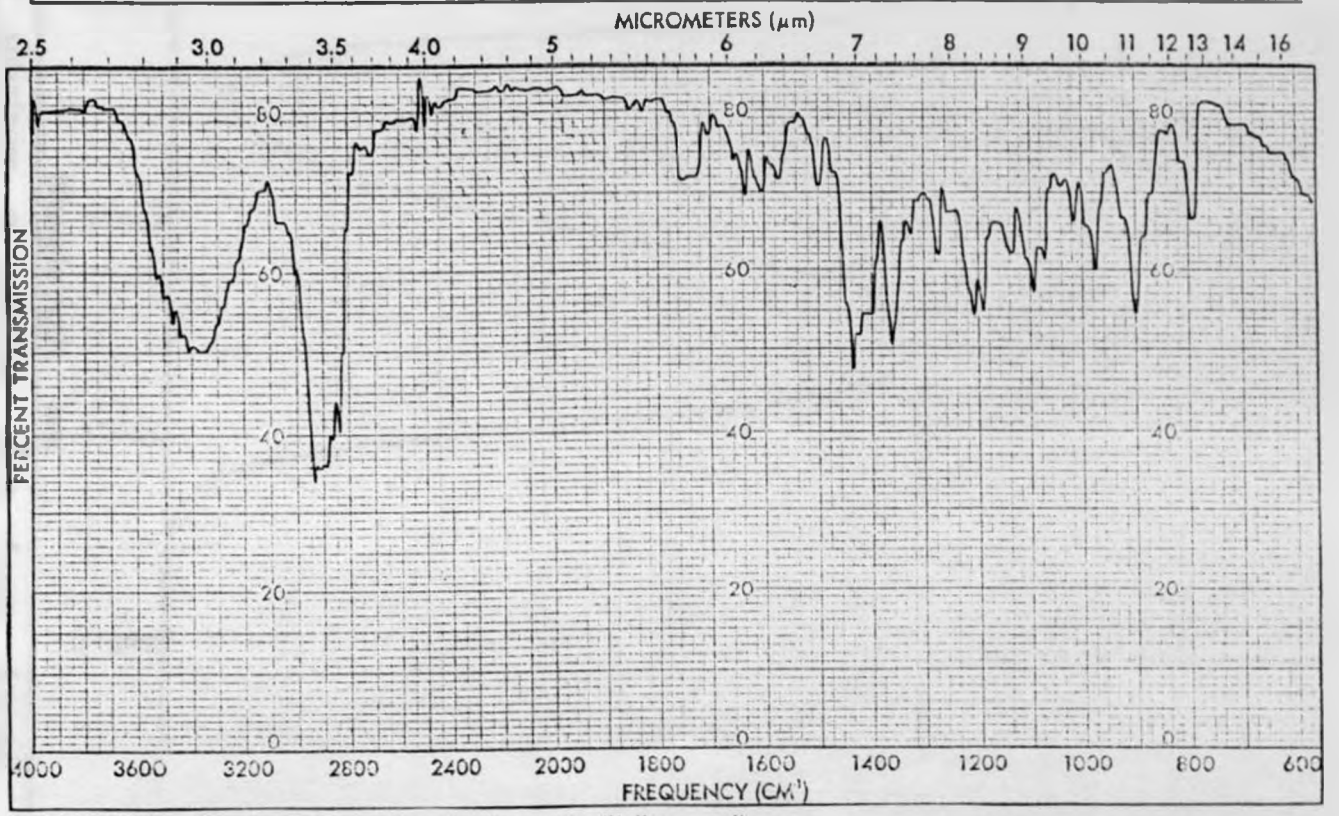


APPENDIX 9 IR spectrum of Lippia wilmsii oil

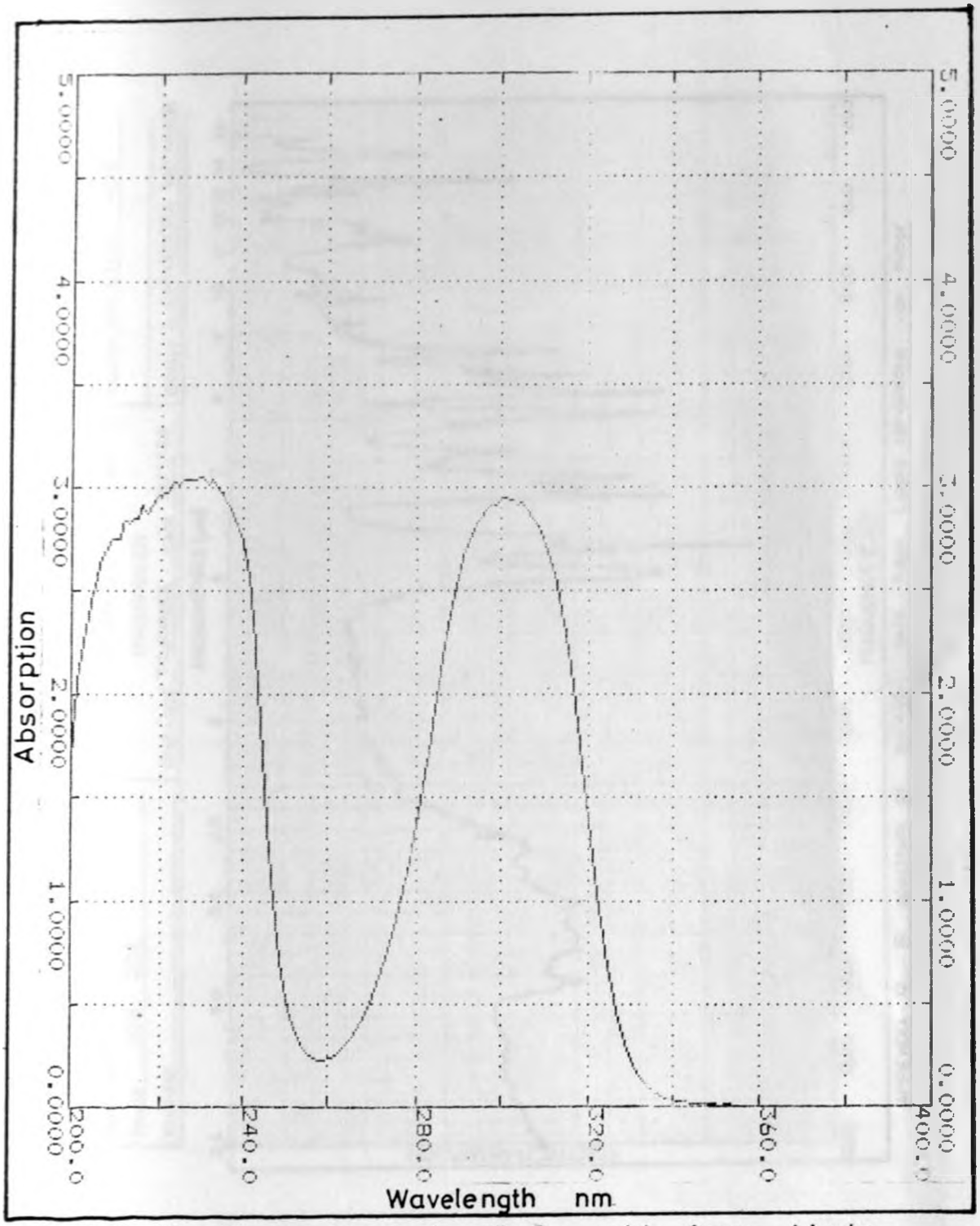
CONCENTRATION NEAT
THICKNESS THIN FILM
PHASE
REMARKS

SCAN MODE ACCY. ☐ SURVEY ☐
HI ENERGY ☐ CAL. ☐
RESOLUTION ☑
OPERATOR THURANIRA DATE 6/3/86

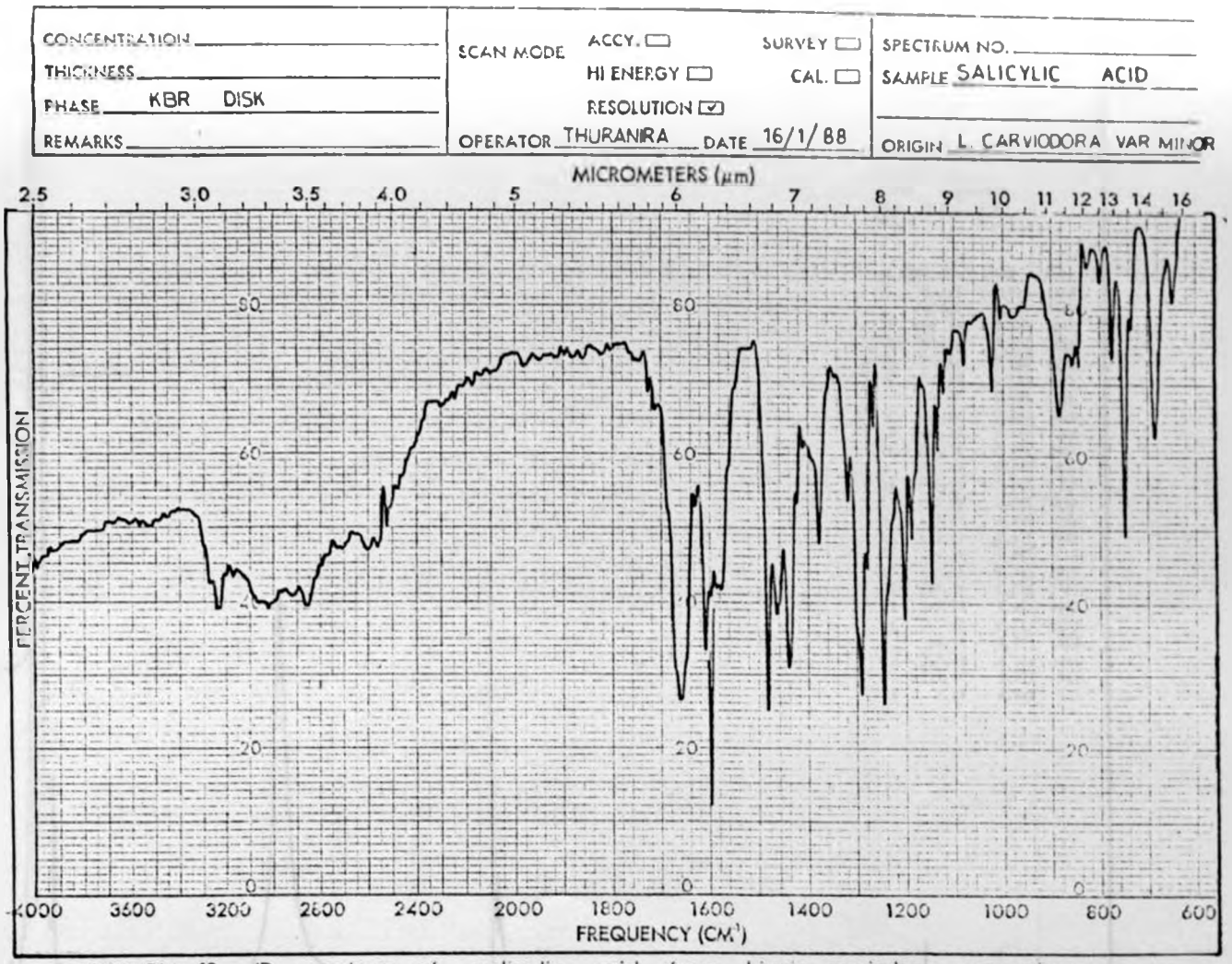
SPECTRUM NO.
SAMPLE LIPPIA GRANDIFOLIA
ORIGIN CHERANGANI HILLS



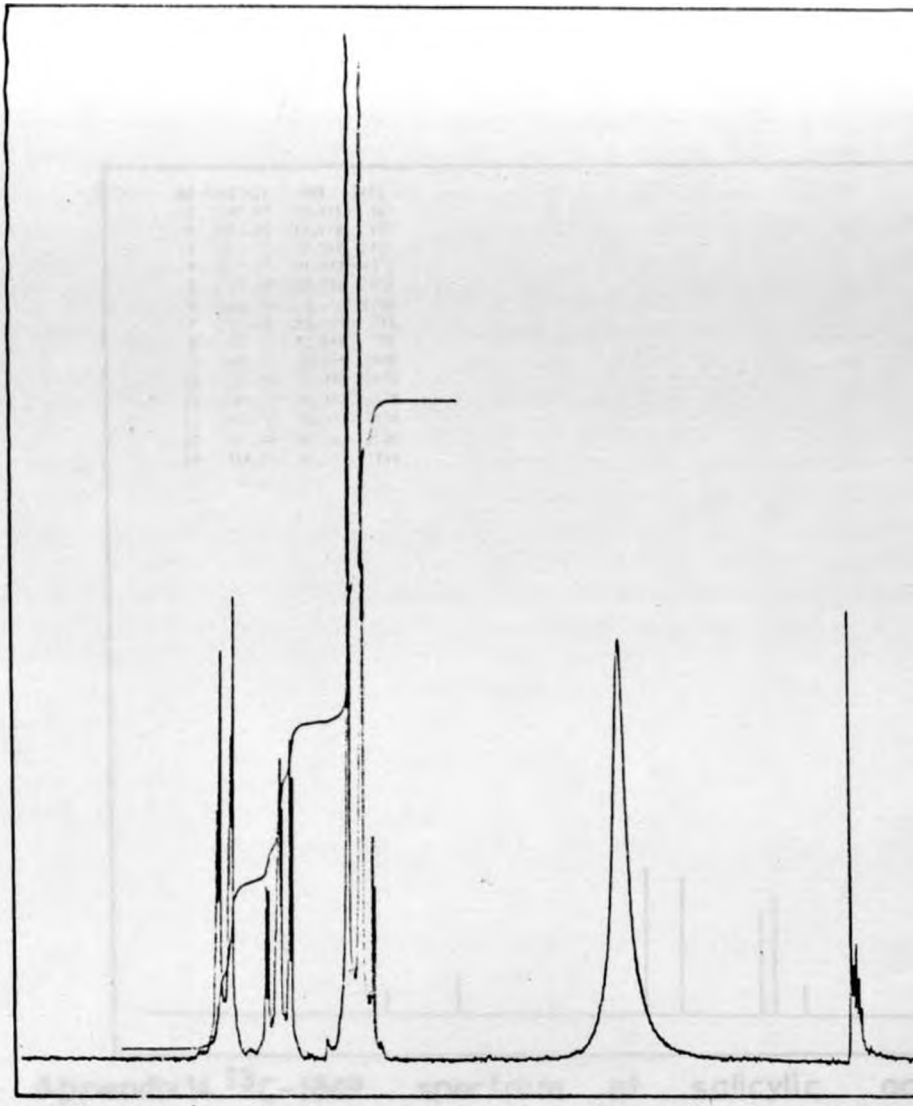
APPENDIX 10 IR spectrum of Lippia grandifolia oil



APPENDIX 11 UV spectrum of salicylic acid from Lippia carviodora variety minor



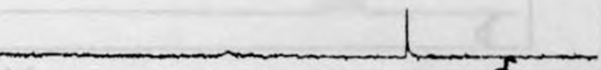
APPENDIX 12 IR spectrum of salicylic acid from Lippia carviodora var minor



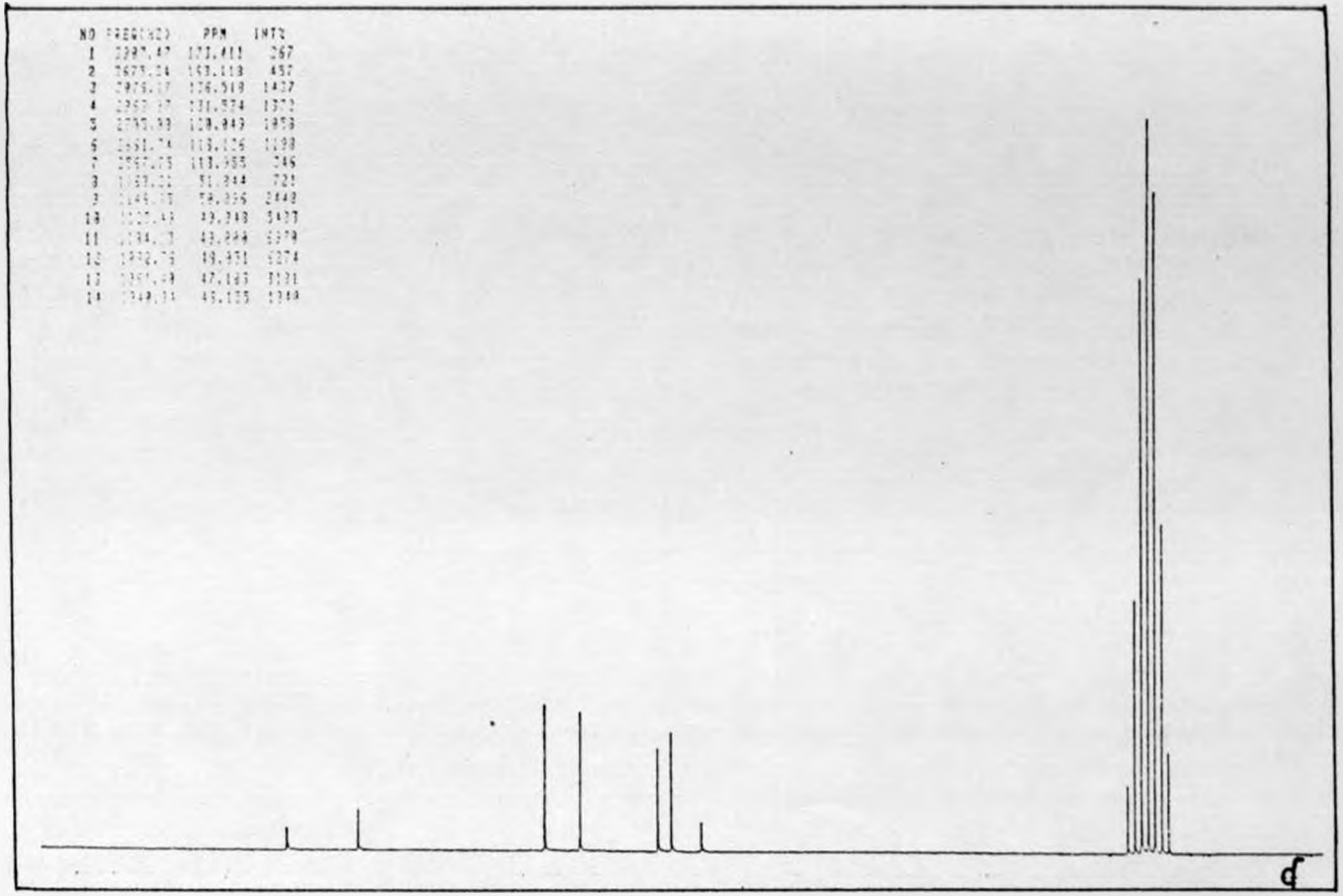
Appendix 13 ^{1}H-NMR spectrum of salicylic acid

TOTAL 24
RESOLU[illegible] -5 HZ
CHREF 0.000PPM
OBS 379.2704 HZ
NGAIN 4

NO	FREQ(HZ)	PPM	INT%
1	793.22	7.915	2410
2	787.33	7.894	3901
3	781.90	7.833	2894
4	780.30	7.821	3107
5	759.46	7.806	4390
6	577.00	7.555	1694
7	575.17	7.535	1661
8	655.67	7.473	2819
9	663.07	7.456	2383
10	655.74	7.441	2434
11	651.43	7.302	3472
12	653.55	7.362	2715
13	627.39	6.962	10000
14	623.41	6.957	7269
15	615.05	6.875	9443
16	614.36	6.862	4713
17	592.00	6.796	2165
18	607.79	6.783	1781
19	443.35	5.921	4002
20	[illegible]	3.359	4273
21	332.13	3.339	1400
22	327.60	3.321	1293
23	233.42	3.301	963
24	0.00	0.000	525



d



Appendix 14. ^{13}C-NMR spectrum of salicylic acid