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THE ANTIMICROBIAL PROPERTIES AND CHEMICAL COMPOSITION OF LEAF ESSENTIAL OILS OF SELECTED LAMIACEAE SPECIES IN SOUTH AFRICA

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A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the degree of Master of Science in Medicine (Pharmacotherapy).

Johannesburg 2003

DECLARATION

I, Jeeteen Vallabh, declare that this research report is my own work. It is being submitted in partial fulfilment for the degree, Master of Science in Medicine (Pharmacotherapy), in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

Vallabh

10 th day of November, 2003.

I would like to thank GOD Almighty for giving me the strength, courage and ability to complete this research report.

I dedicate this research report to my loving and amazing parents and family. Thank you for always being there and for the continued love and support that you showed me.

To the most amazing person in the world, Kesheni, thank you for all your love and support.

ABSTRACT

Many species belonging to the Lamiaceae (mint family) are used extensively in traditional healing. Indigenous Lamiaceae species such as *Ballota africana, Leonotis loenurus* and *Salvia runcinata* have a distinct common aromatic property. Although many members of the Lamiaceae such as lavender and rosemary have become household names with proven antibacterial properties, the South African representatives of this family of plants remain largely unexplored. South Africa has a cultural diversity with traditional healing being integral to each ethnic group. Indigenous medicinal plant use is well recorded in the readily available local ethnobotanical literature. Despite the well-documented ethnobotanical data, very little scientific information is available on our indigenous medicinal plants. A concerted and organised effort is required to explore the chemistry and biological activity of our indigenous medicinal plants with the aim of finding a scientific rationale for the many "muthi medicines" used by the people of South Africa.

Given the current problem with the development of bacterial resistance there is a great urge to discover new potent antibiotics. Worldwide spending on finding new anti-infective agents is expected to increase 60% from the spending levels in 1993, and it is predicted that phytochemicals will find their way into the arsenal of antimicrobial drugs prescribed by physicians. Many plants have been reported to be used in the treatment of cuts, wounds, sores, ear infections etc. indicating possible antimicrobial properties. Due to the known antimicrobial properties of essential oils, an interest has developed in aromatic plants in general, in the continued search for new natural molecules to be used in phytopharmaceuticals.

Ten Lamiaceae species commonly found in South Africa were selected and leaf material collected. The leaves were hydro-distilled to extract the essential oils. The oils were analysed by gas chromatography (GC) and GC coupled to mass spectrometry (GC-MS). Antimicrobial assays incorporating four bacteria, two yeasts and one fungus were performed on the essential oils.

Table 1: Species that were active against either the bacteria or the yeasts or the mould.

Species	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Cryptococcus neoformans*	*Candida albicans*	*Aspergillus niger*
Hemizygia modesta	Inactive	Active	Active	Inactive	Inactive
Mentha longifolia	Active	Inactive	Active	Active	Inactive
Ocimum americanum	Inactive	Inactive	Active	Inactive	Slightly active
Salvia africana-lutea	Inactive	Active	Inactive	Active	Active
Salvia disermas	Inactive	Inactive	Active	Active	Inactive
Salvia namaensis	Active	Inactive	Inactive	Active	Inactive
Salvia runcinata	Active	Inactive	Active	Active	Active

Seven of the ten oils that were tested showed some antimicrobial activity. The antimicrobial activities of the oils were shown to differ depending on the type of compounds found within the essential oil. Results from previous studies implicate compounds such as linalool, terpenin-4-ol and carvone to possibly contribute to the antimicrobial properties exhibited by some of the active species in this study.

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1. INTRODUCTION

Since the beginning of time, man has used plants for medicinal purposes. People all over the world have long applied poultices and infusions of indigenous plants for various ailments. There are close to 250 000-500 000 species of plants, only a small percentage have been investigated and even a smaller percentage have been properly studied in terms of their pharmacological properties. About 25% of drugs prescribed today are of plant origin. It is also important to note that plants can be used as therapeutic resources in several ways. They can be used as crude extracts, as tinctures, fluid extracts, powders, pills, and capsules. With so many species available, one can only imagine what therapeutic properties are still waiting to be discovered.

In recent years there has been a growing interest in complementary therapies and the therapeutic use of natural products, especially those derived from plants. There is particular interest in the role of plants to be used as antimicrobials. The main reason for this is that the organisms (bacteria) are becoming resistant to conventional antimicrobials. The food industry has tended to reduce the use of chemical preservatives in their products due to increasing pressure from consumers or legal authorities, to either completely remove or to adopt more "natural" alternatives for the maintenance or extension of product shelf life (Tassou *et al.*, 2000). Many spices and herbs may serve as potential alternatives since their essential oils possess antimicrobial activity (Tassou *et al.*, 2000). There is considerable interest in the possible use of these compounds as food additives to delay the onset of food spoilage or to prevent the growth of food-borne pathogens.

Koga *et al.* (1999) performed studies on the bactericidal activities of essential oils of basil and sage against a range of bacteria and the effect of these essential oils on *Vibrio parahaemolyticus*. They found that Gram-positive bacteria showed a higher resistance to basil and sage essential oils than Gram-negative bacteria, while *Vibrio* species showed a high sensitivity to both the oils.

In a study done by Youdim and Deans (1999), it was found that adding a dietary supplementation of thyme (*Thymus vulgaris*) essential oil to a rat's diet, resulted in the rat maintaining a more favourable antioxidant capacity during its life span. This study was done

to identify age-related changes in certain antioxidant systems and to assess whether dietary supplementation of thyme oil could address the unfavourable antioxidant-pro-oxidant balance that occurs with age.

Essential oil molecules (terpenoids) are made up primarily of carbon, hydrogen and oxygen. The monomer of terpenoids is a five-carbon molecule referred to as an isoprene unit. When two of these units are joined together they form a monoterpene, when three join they create a sesquiterpene, and so forth. Different molecules in the same oil can exert different effects because the chemical composition of the specific essential oil determines its function.

A wide variety of essential oils and many of their components, mainly mono- and sesquiterpenes, have been shown to have antimicrobial activity. The type of antimicrobial activity shown by essential oils and their components varies from bacteriostatic to bactericidal activity. The spectrum of the antimicrobial activity seems to be quite broad showing activity against oral bacteria, micro-organisms causing dermatological problems, commonly studied pathogens such as *E. coli* and *S. aureus* and some antibiotic-resistant micro-organisms (Griffin *et al.,* 1999). Some studies have shown that specific essential oil components have a similar broad spectrum of activity. Other research has focused on demonstrating the activity of essential oil components against certain types of micro-organisms, such as food-borne pathogens, with a view to their use as food preservatives. Correlations have also been made between the chemical composition of essential oils and their antimicrobial activity (Griffin *et al.,* 1999).

There have been a few studies conducted on various essential oils found in the Lamiaceae family. The Department of Food Science, University of Udine, Italy has done a study to determine the antimicrobial activity of essential oils from Lamiaceae and Asteraceae. In this study a wide range of essential oils from sage, mint, hyssop, chamomile, and oregano were tested for their inhibitory effects against nine strains of Gram-negative bacteria and six strains of Gram-positive bacteria. The data obtained indicated that while the essential oils from sage, mint, hyssop and chamomile generally had bacteriostatic activity, the essential oil from oregano appeared to be bactericidal at concentrations above 400 ppm, probably because of high content of phenolic compounds.

Although the antibacterial activity of essential oils from spices has recently been reviewed (Tassou *et al.,* 2000), their mechanism of action against the micro-organisms has not been

studied in great detail. Only a few studies have focused on the mechanism by which spices or their essential oils inhibit micro-organisms. According to Conner and Beuchat (1984a,b) the antimicrobial action of essential oils may be due to impairment of a variety of enzyme systems including those involved in energy production and structural component synthesis. In general the active antimicrobial compounds of essential oils are terpenes e.g. eugenol, thymol, and carvacrol, which are phenolic in nature. In particular, the bactericidal activity of mint essential oil that has recently been shown in broth and in food model systems (Tassou *et al.,* 2000) was due to ketones (pulegone, isomenthone, piperitone, carvone). It has been concluded that essential oils do not all have the same mechanism of action and there may be several targets that can lead to inhibition of bacteria. Much of the research on the mechanism of phenolic compounds centres on their effects on cellular membranes (Davidson, 1997). Indeed, phenolics not only attack the cytoplasmic membrane, thereby destroying its permeability and releasing its intracellular constituents (ribose, sodium glutamate, etc) but could also cause membrane dysfunction in respect of electron transport, nutrient uptake, nucleic acid synthesis and ATPase activity (Denyer and Hugo, 1991). More research is needed in this field in order to elucidate the antibacterial mechanism of action of the essential oils on bacteria.

Many species of the Lamiaceae have been used traditionally for various ailments. Some examples are listed in Table 2.

Table 2: Botanical name, common names and uses of some plants from the Lamiaceae family.

Botanical name	**Common name**	**Uses**
Hyssopus officinalis	Hyssop	Antiviral
Lavandula angustifolia	Lavender	Antimicrobial, analgesic
Leonotis leonurus	Wild dagga	Dysentery, coughs, cold, skin conditions, respiratory conditions.
Melissa officinalis	Lemon balm	Bactericidal, antispasmodic
Mentha aquatica	Water mint, "Kruisemint"	Colds, diarrhoea, dysentery
Mentha longifolia	Wild mint	Coughs, colds, asthma
Ocimum basilicum L	Basil	Bactericidal
Rosmarinus officinalis	Rosemary	Analgesic, antimicrobial
Salvia runcinata	"Kruipsalie"	Purgative
Salvia coccinea	Texas sage	Cough, pulmonary tuberculosis
Thymus vulgaris	Thyme	Antioxidant, antibacterial

Locally, many of the plant species belonging to the mint family have been used in traditional healing. The Khoi and Nama cultures have used *Bollota africana* to treat colds, fevers and arthritis (van Wyk *et al.,* 1997). Leaf concoctions of *Leonotis leonurus* (wild dagga) are used to treat respiratory problems and also applied externally to treat various skin disorders. A species of *Salvia* known as bergsalie is used by the European in the Western Province, usually with *Leonotis leonurus,* for influenza, chest inflammations, pulmonary tuberculosis, rheumatic conditions, gynaecological complaints and skin diseases. A decoction of the herb of *Mentha longifolia* is administered by the Europeans and the African to reduce labour pains. It is also used to relieve pain in the back and to relieve pain during menstruation. Although many members of the Lamiaceae are widely distributed in South Africa and have been used extensively in medicinal herbal preparations, the chemical and biological activities of these plants remain unexplored. Some species have been selected in this study based on recorded ethnobotanical information (*Leonotis*). However, it is often not stated which species is selected and often any aromatic plant is used by the traditional healer. For this reason we also relied on taxonomic criteria (and chemotaxonomic) to select species for this study.

South African Lamiaceae

Table 3: List of Lamiaceae genera, as well as the number of species found in South Africa (Codd, 1985).

Genus	**Total number of species**	**Number of species in South Africa**
Acrocephalus	>70	1
Acrotome	8	6
Aeollanthus	40	6
Ajuga	100	1
Ballota	38	1
Basilicum	2-3	1
Becium	>10	4
Cedronella	1	1
Endostemon	17	3
Englerastrum	5	??
Geniosporum	20	1
Hemizygia	35	28
Holostylon	4	1
Hoslundia	1	1
Hyptis	300	3
Lamium	40	1
Leonotis	10	3

Genus	Total number of species	Number of species in South Africa
Leucas	160	8
Marrubium	30	1
Mentha	20-30	2
Neohyptis	1	0
Ocimum	6	2
Orthosiphon	50	9
Plectranthus	350	44
Prunella	5	1
Pycnstachys	40	3
Rabdosiella	2	1
Salvia	800-900	22
Satureja	>100	4
Scutellaria	150	1
Solenostemon	60	2
Stachys	450	41
Syncolostemon	9	9
Tetradenia	6	3
Teucrium	200	3
Thorncroftia	3	3
Tinnea	19	4

2. STUDY OBJECTIVES

There are three main objectives of this project:

1. To determine the antimicrobial properties of the essential oils distilled from local Lamiaceae species.
2. To chromatographically record, for the first time, the essential oil composition of the selected species.
3. To provide a scientific rationale for the traditional use of some Lamiaceae species in herbal preparations.
4. To complete herbal monographs of South African species. This forms part of a greater project in the Department of Pharmacy and Pharmacology to provide complete monographs for South African medicinal aromatic plants.

3. MATERIALS AND METHODS

3.1 Collecting of plant material

Guided by the availability, chemotaxonomic criteria and traditional use of the plants, a selection of 10 species was made. The plants were collected from various places **(Figure 1 and Table 4).** Botanists at the Moss Herbarium (WITS University) confirmed the identities of the plants.

Table 4: List of the species that were studied and the places from where they were collected.

Species	Place where species was collected
Hemizygia modesta Codd	Stella Mine (Mpumalanga)
Hemizygia pretoriae (Gürke) Ashby	Melville Koppies (Gauteng)
Leonotis leonurus L	Linden, Randburg (Gauteng)
Mentha longifolia (L) Hudds	Witwatersrand Botanical Garden (Gauteng)
Ocimum americanum L	Swaziland
Salvia africana-lutea L	Clanwilliam (Northern Cape)
Salvia disermas L	Kokerboom Woud (Northern Cape)
Salvia dolomitica Codd	Witwatersrand Botanical Garden (Gauteng)
Salvia namaensis Schulz	Swartberg Pass (Eastern Cape)
Salvia runcinata L	Midrand (Gauteng)

3.2 Distilling of plant material

The essential oils were selectively extracted using conventional hydro-distillation (3hrs) in a Clevenger-type apparatus **(Figure 2).** As the essential oils are volatile, they were stored in amber bottles and kept in the fridge.

3.3 Chemical analysis of the essential oils

3.3.1 Thin layer chromatography (TLC)

A 20cm X 20cm TLC plate was divided into two equal parts. The TLC plate that was used was that of Alugram SIL G/UV_{254} made by Macherey-Nagel. Essential oil was diluted 1:8 with hexane and placed onto the TLC plate **(Figure 3).** The TLC plate was then placed into a mobile phase of toluene and ethyl acetate (93:7) and allowed to develop. After development and drying the TLC plate was sprayed with vanillin-sulphuric acid reagent **(Figure 4)** and

heated in the oven at 100°C for 5min. This study was done to get a general impression of the essential oil variation between the selected taxa. Due to the complexity of the essential oils (as seen by the GC-MS profiles), TLC is not an appropriate technique to study the oils and hence the results have not been included.

The essential oils were analysed by gas chromatography (GC) using the following operating conditions; Shimadzu 17A gas chromatograph using the following parameters; Column: J&W-DB1 (30m x 0.25mm id, μm film thickness), Temperatures: injection port 230 °C, column 60 °C for 1min, 5 °C/min to 180 °C, 180 °C for 2min, (total=25min).

3.3.2 Gas chromatography-mass spectroscopy (GC-MS)

The samples were also analysed by gas chromatography coupled to mass spectrometry (GC-MS). This part of the project was completed by myself at The Medicinal and Aromatic Plant and Drug Research Centre (TBAM), Anadolu University, Turkey. For GC-MS analysis the operating conditions were as follows; column: HP-Innowax (60m x 0.25mm id., 0.25μm film thickness), temperatures: injection port 250 °C, column 60 °C for 10min, 4 °C /min to 220 °C, 220 °C for 10min, 1 °C /min to 240 °C (total = 80min). Helium was used as the carrier gas.

0.9μl of hexane with 0.1μl of the essential oil was injected. Identification took place with the use of TBAM's database libraries by matching both retention indices and mass spectral fragmentation patterns.

3.4 Microbiological assays

The antimicrobial component was divided into two separate microbiological assays:

- Disc diffusion assays
- Minimum inhibition concentrations (MIC using the micro titre plate method)

3.5 Disc diffusion assays

Antimicrobial assays were performed on the hydro-distilled oils using the following bacterial test organisms: *Pseudomonas aeruginosa* (ATCC 9027), *Escherichia coli* (ATCC 8739), *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212), *Salmonella typhi* and *Klebsiella pneumoniae* (NCTC 9633). A series of fungal test organisms; *Candida albicans* (ATCC 10231), *Cryptococcus neoformans* (ATCC 90112) and *Aspergillus niger* were also studied. Clinical strains of *Aspergillus niger* and *Salmonella typhi* were used. The microbial species selected represented a broad antimicrobial spectrum, selected based on their pathogenesis. *Staphylococcus aureus* and *Enterococcus faecalis* represent the Gram- positive bacteria while *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Salmonella typhi* represent the Gram-negative bacteria. *Candida albicans, Cryptococcus neoformans* and *Aspergillus niger* are organisms responsible for systemic fungal infections. The disc diffusion method was employed whereby bacterial spore suspensions yielding an inoculum size of $1x10^6$,was incorporated into a suitable agar. Using aseptic manipulation, discs saturated with the plant essential oils were introduced onto the seeded agar plate. Positive and negative controls were included for each test organism. The zones of inhibition were examined after 24hrs to 7 days, depending on the test organism. Tryptone Soya agar (Oxide) was used for the fungi and Mueller Hinton agar (Oxoid) for the bacteria **(Figure 5)**.

3.6 Minimum inhibitory concentration (MIC using the micro titre plate method)

The minimum inhibitory concentration was determined using the p-iodonitrotetrazolium (INT) microplate method. This involved the dilution of essential oils with acetone in a micro titre plate **(Figure 6)**. A fixed bacterial culture yielding an inoculum size of $1x10^6$ was added to all the wells and incubated at 37 °C for 24 hours. p-Iodonitrotetrazolium violet (INT) solution was then added to the wells to determine a colour change in relation to the concentration of microbial growth. Neat essential oils were used and were colourless. The increments between the wells were relatively large and hence it was easier to note colour changes. The concentration of INT was 40µL/well at a volume of 0.2mg/ml. The INT was sourced at SIGMA. The MIC could then be determined by visualizing the first well having no microbial growth. Positive controls were included having bacterium but no essential test oil.

3.7 Scanning electron microscopy (SEM)

Where readily available, plant material was collected and small pieces of leaf material were fixed in 2.5% glutaraldehyde in 0.075M phosphate buffer at pH 7.4 – 7.6 for one hour. The material was rinsed in 0.075M phosphate buffer three times for five minutes each, and then postfixed in 1% aqueous osmium tetroxide for two hours after which it was rinsed three times in distilled water. The material was dehydrated with a series of different methanol concentrations of 30%, 50%, 70%, 90% and 100% followed by critical point drying in liquid carbon dioxide. The material was mounted on aluminium stubs using double-sided adhesive tape, and then gold sputter coated for 40 seconds using a SEM autocoating unit E5200. The prepared stubs were viewed under a JEOL 840 SEM. The examined surfaces included the abaxial and adaxial surfaces of the leaves and stem surfaces of the plant material. This study was undertaken to add anatomical characterisation to the monographs in addition to morphological and chemical data.



Figure 1: Collecting *Leonotis leonurus* in Linden (Randburg).

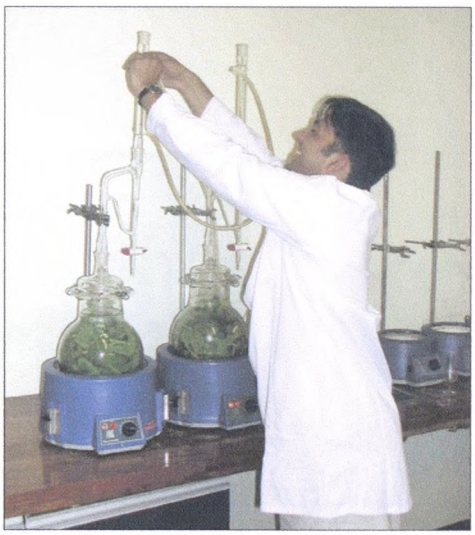


Figure 2: Distilling of plant material using the Clevenger apparatus.



Figure 3: Applying the diluted essential oil samples to the TLC plate.



Figure 4: Spraying the TLC plates with the vanillin-sulphuric acid spray reagent.



Figure 5: Placing of sterile disc onto large agar plate inoculated with test organism.

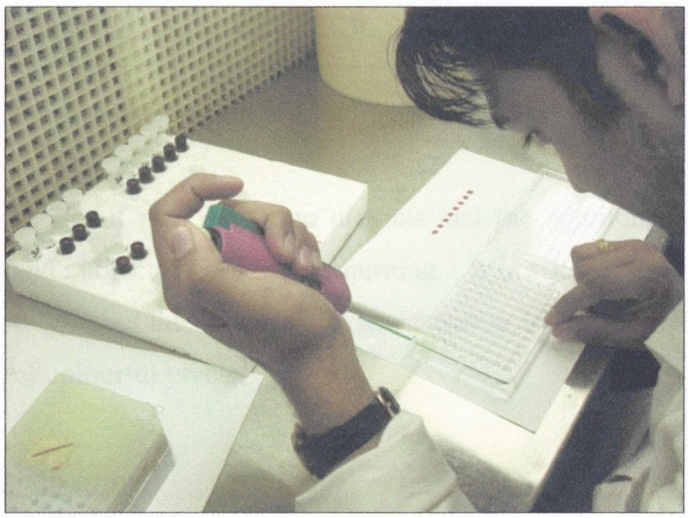


Figure 6: Preparing dilutions of essential oils in the micro titre plate, for the minimum inhibitory concentration assay.

4. SPECIES MONOGRAPHS

4.1 *Hemizygia modesta* Codd

Botanical description

A herb 0.12 - 0.25m tall, with a few to several stems arising annually from a perennial woody rootstock. The stems are slender, sparingly branched, softly woody below, and hispid to villous. The leaves are sessile or subsessile. The calyx is 7-8mm long at maturity. The corolla is white to pale mauve, widening to 4mm at the mouth.

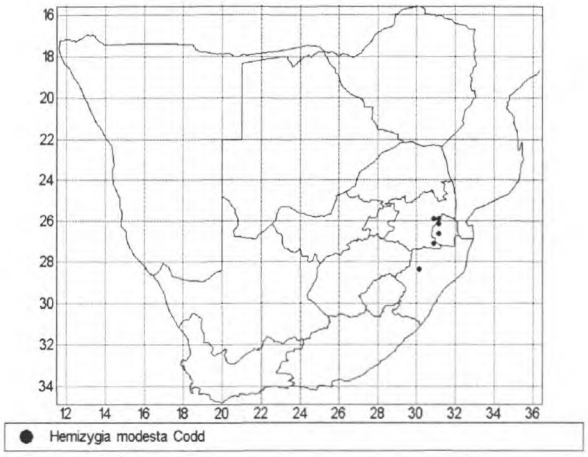


Figure 7: Geographical distribution of *Hemizygia modesta*.

Found in the Piet Retief and Barberton districts and the adjoining parts of Swaziland, in mountain grassland subjected to periodic burning. It is sometimes confused with another species *H. thorncroftii*, but the latter usually has narrower leaves, 4-6 flowered verticils, and a persistent coma of colourful bracts.

Essential oil composition

The essential oil was obtained by hydro-distillation from plants collected near Stella Mine, Mpumalanga.

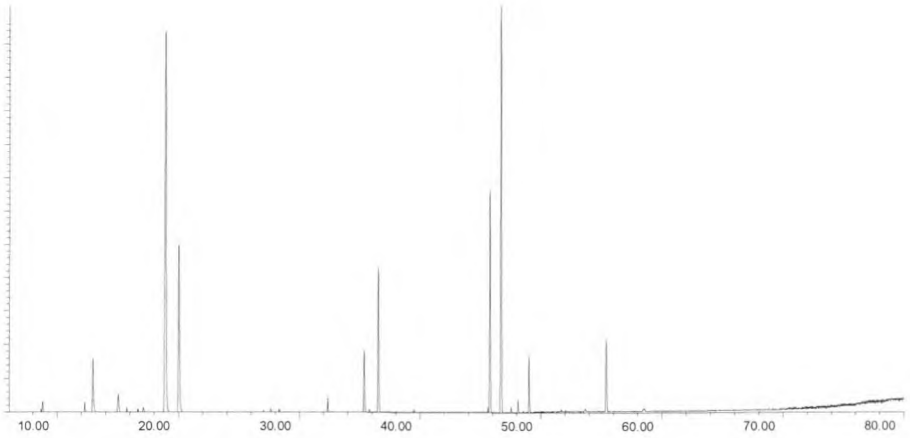


Figure 8: GC-MS profile of the hydro-distilled essential oil obtained from *Hemizygia modesta.*

Table 5: Compounds identified in the essential oil of *Hemizygia modesta.*

RRI	Compound	Area percentage
1132	Sabinene	3.67
1176	α- phellandrene	1.33
1255	**γ-terpinene**	**29.36**
1280	**ρ-cymene**	**11.92**
1719	Borneol	3.20
1755	Bicyclogermacrene	8.44
2104	**Viridiflorol**	**12.10**
2144	**Spathulenol**	**23.09**
2247	*trans*-α-bergamotol	2.38
	8,13-abietadiene	4.50
	Total	**99.99**

The major compound in the oil is γ-terpinene, which contributes 29.36% of the total oil composition. The two sesquiterpenoids, viridiflorol and spathulenol are present at yields of 12.1% and 23.1% respectively.

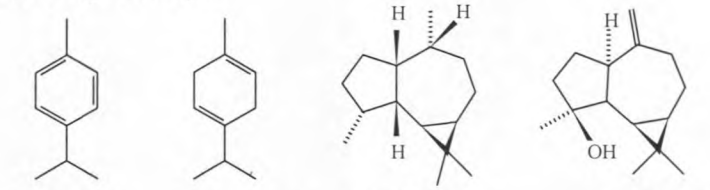


Figure 9: Chemical structures for major compounds detected in the essential oil of *Hemizygia modesta.*

4.2 *Hemizygia pretoriae* (Gürke) Ashby

Botanical description

This plant is a bushy herb, 0.1-0.3m tall with a few to many erect or ascending stems arising annually from a perennial woody rootstock. The stems are simple, slender; hispid to villious, sometimes with branched hairs intermingled. The leaves are subsessile to shortly petiolate. The calyx is usually 9-11mm long. The corolla is whitish to pale mauve, 14-16mm long.

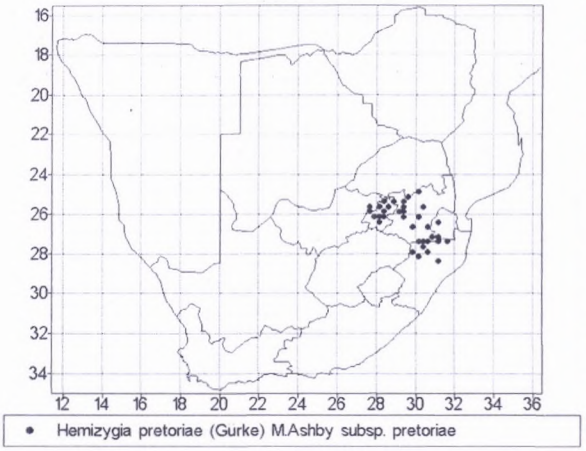
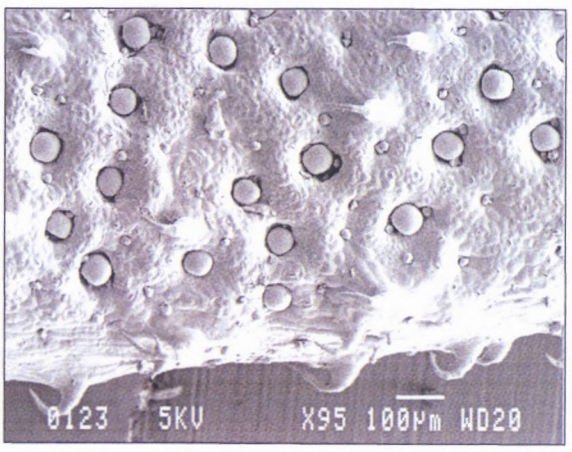


Figure 10: Geographical distribution of *Hemizygia pretoriae*.



Figure11: *Hemizygia pretoriae* in habitat (Melville Koppies, Johannesburg).





Figures 12 & 13: Scanning electron micrographs of the oil glands on the abaxial leaf surface of *H. pretoriae*.

Distribution

Distributed from central to eastern Gauteng, Swaziland and northern Natal. The plant usually grows in dense grasslands subject to periodic burning, often among rocks, at altitudes of 1000 to 1800m.

Essential oil composition

The essential oil was obtained by hydro-distillation from plants collected from Melville Koppies. The essential oil yield was 0.10%.

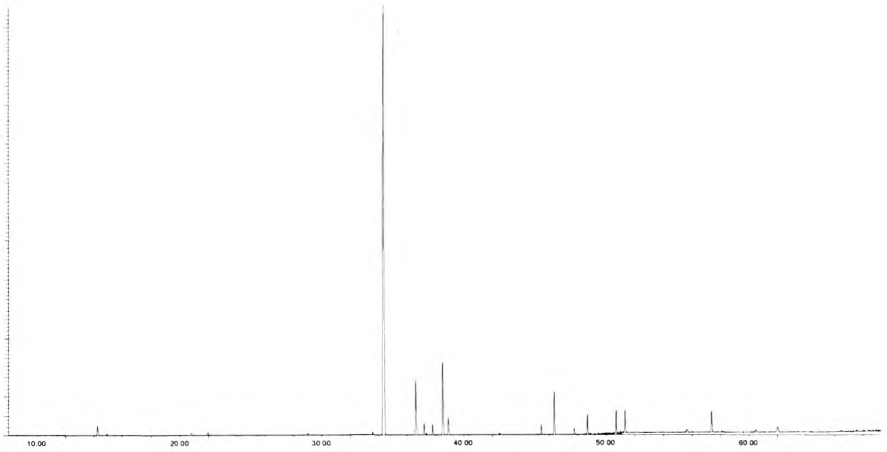
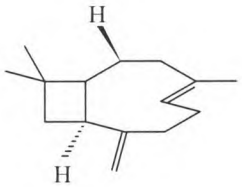


Figure 14: GC-MS profile of the hydro-distilled essential oil obtained from *Hemizygia pretoriae.*

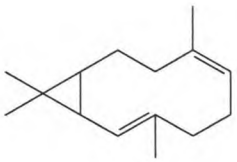
Table 6: Compounds identified in the essential oil of *Hemizygia pretoriae.*

RRI	Compound	Area percentage
1612	**β-caryophyllene**	**69.31**
1687	α-humulene	6.85
1755	**Bicyclogermacrene**	**9.98**
2050	*E*-nerolidol	5.00
2264	Intermedeol	1.95
	8,13-abietadiene	2.67
	Total	**95.76**

β-caryophyllene, a sesquiterpene, is the major compound in the hydro-distilled essential oil representing 69.31% of the total composition. Bicyclogermacrene represents 9.98% of the total composition.



β-caryophyllene



bicyclogermacrene

Figure 15: Chemical structures for major compounds detected in essential oil of *Hemizygia pretoriae*.

4.3 *Leonotis leonurus* L

Botanical description

This shrub is 2-5m tall with stems branching from a thick woody base. The leaves are petiolate, with the upper surface green and rough. The calyx is 12-16mm long and 4mm in diameter. The corolla grows to between 40 and 49mm long, covered with orange-coloured hairs.

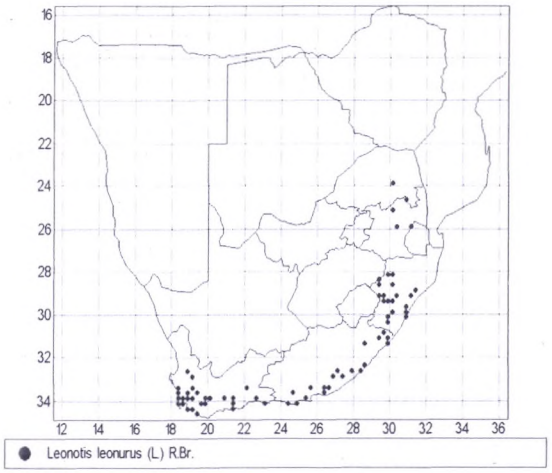


Figure 16: Geographical distribution of *Leonotis leonurus*.



Figure17: *Leonotis leonurus* photographed at the Witwatersrand Botanical Garden.

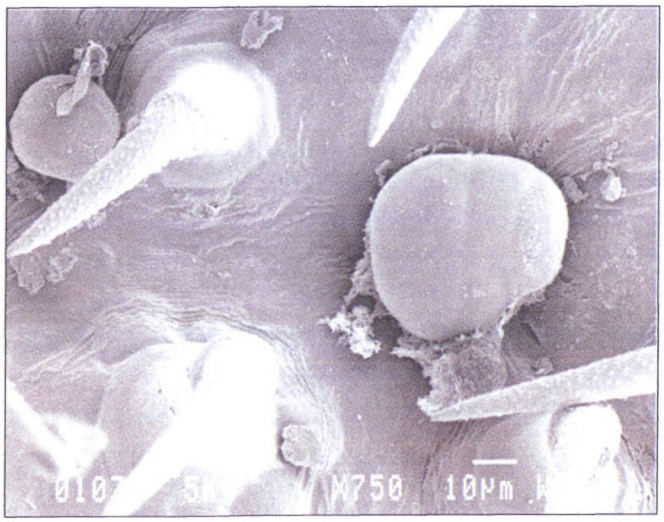


Figure 18: Scanning electron micrograph showing the oil glands and hairs on the abaxial leaf surface of *Leonotis leonurus*.

Distribution

Plants abundant in Gauteng, Natal and the Cape. In Gauteng, the species occurs at altitudes between 900 and 2000m, while in the southern provinces it descends to sea level. It is locally common at forest margins, often on riverbanks, on rocky hillsides or in tall grasslands.

Common names

Cape hemp, Lions ear.

Uses

Used for feverish headaches and coughs and colds. (Codd, 1985, Watt and Breyer-Brandwijk, 1962).

Essential oil composition

The essential oil was obtained by hydro-distillation from plants collected from Linden (Gauteng). The essential oil yield was 0.02%.

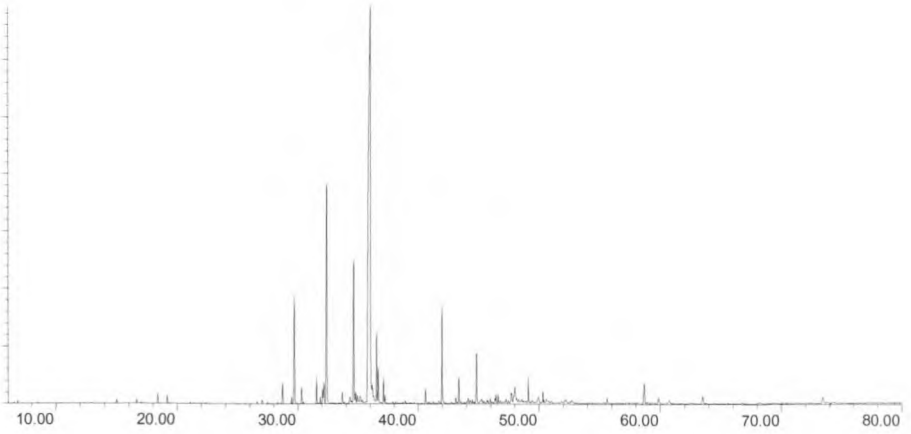


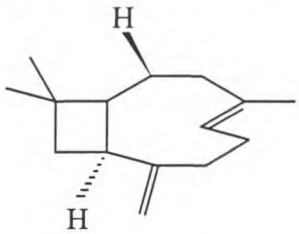
Figure 19: GC-MS profile of the hydro-distilled essential oil obtained from *Leonotis leonurus*.

Table 7: Compounds identified in the essential oil of *Leonotis leonurus*.

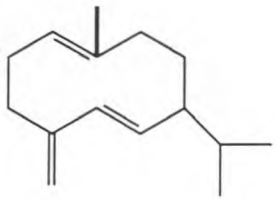
RRI	Compound	Area percentage
1032	α-pinene	0.03
1118	β-pinene	0.01
1132	Sabinene	0.01
1174	Myrcene	0.13
1203	Limonene	0.14
1213	1,8-cineole	0.03
1246	*Z*-β-ocimene	0.31
1266	*E*-β-ocimene	0.26
1452	1-octen-3-ol	0.16
1466	α-cubebene	0.07
1479	δ-elemene	0.01
1755	Bicyclogermacrene	0.08
1497	α-copaene	0.72
1528	α-bourbonene	0.23
1535	β-bourbonene	4.00
1600	β-elemene	0.39
1612	**β-caryophyllene**	**11.30**
1661	Alloaromadendrene	0.05
1668	*Z*-β-farnesene	0.36
1687	α-humulene	5.68
1659	γ-gurjunene	0.45
1704	γ-muurolene	0.45
1726	**Germacrene-D**	**45.29**
1740	Valencene	1.53
1755	Bicyclogermacrene	2.44
1758	*E*, *E*-α-farnesene	0.99
1773	δ- cadinene	0.73
1776	γ-cadinene	0.22
1810	3,7-guaiadiene	0.07
1838	β-damascenone	0.09
1853	*cis*-calamenene	0.05
1868	*E*-geranyl acetone	0.05
1900	Epicubebol	0.47
1941	α-calacorene-I	0.01
1957	Cubebol	3.11
2001	Isocaryophyllene oxide	0.20
2037	Salvial-4 (14)-en-1-one	0.10
	11-norbourbonana-1-one	0.18
2088	1-epi-cubenol	0.36
2100	Heneicosane	0.07
2131	Hexahydrofarnesylacetone	0.23

RRI	Compound	Area percentage
2144	Spathulenol	0.27
2202	Germacrene-D-4-ol	1.90
2209	T-muurolol	1.11
2219	δ-cadinol	0.08
2255	α-cadinol	0.79
2400	Tetracosane	0.27
2500	Pentacosane	0.26
	14-Hydroxy-α-muurolene	0.05
2600	Hexacosane	0.04
	14-hydroxy-δ-cadinene	0.05
2622	Phytol	0.40
	Heptacosane	0.50
	Anthracene	0.01
	Octacosane	0.14
	Nonacosane	0.48
	Total	**88.28**

Leonotis leonurus has two major compounds, Germacrene D representing 45.29% of the oil composition and β-caryophyllene representing 11.38% of the total oil composition.



β-caryophyllene



Germacrene D

Figure 20: Chemical structures for major compounds detected in the essential oil of *Leonotis leonurus*.

4.4 *Mentha longifolia* (L) Huds

Botanical description

A perennial rhizomatous herb. The stems are erect, up to 1.5m tall. The leaves are sessile or subsessile. The calyx is usually tubular-campanulate, 2-2.5 (-3) mm long, dense to sparingly glandular hispid. The corolla is white to mauve, 3-5mm long.

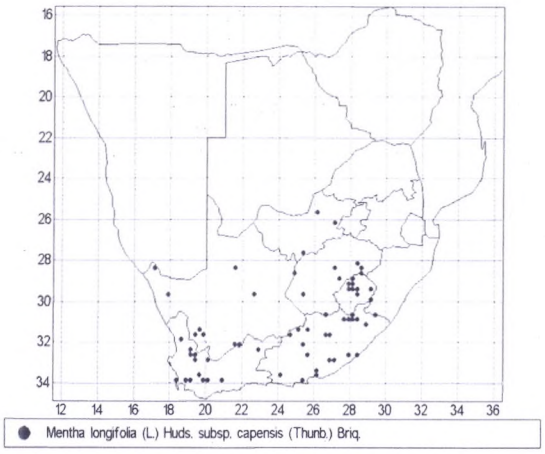


Figure 21: Geographical distribution of *Mentha longifolia*.



Figure 22: *Mentha longifolia* (Witwatersrand Botanical Garden).



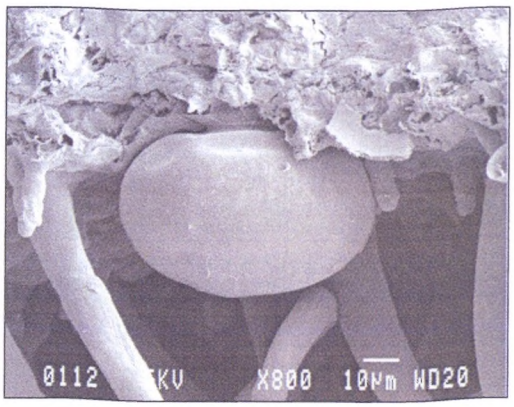


Figure 23 & 24: Scanning electron micrographs of the oil glands on the abaxial leaf surface of *Mentha longifolia*.

Distribution

Mentha longifolia is an extremely polymorphic species, widespread in Europe and the Mediterranean region, eastern Asia and the Canary Island. Also found in Ethiopia, Zimbabwe and Southern Africa. The plants are found on riverbanks and in moist places. Known in England as Horse Mint because the leaves are usually unpleasantly scented.

Common name

Wild Mint, Kruismint, Mint.

Uses

Coughs, colds, asthma. (Watt and Breyer-Brandwijk, 1962).

Essential oil composition

The essential oil was obtained by hydro-distillation from plants collected from the Witwatersrand Botanical Garden.

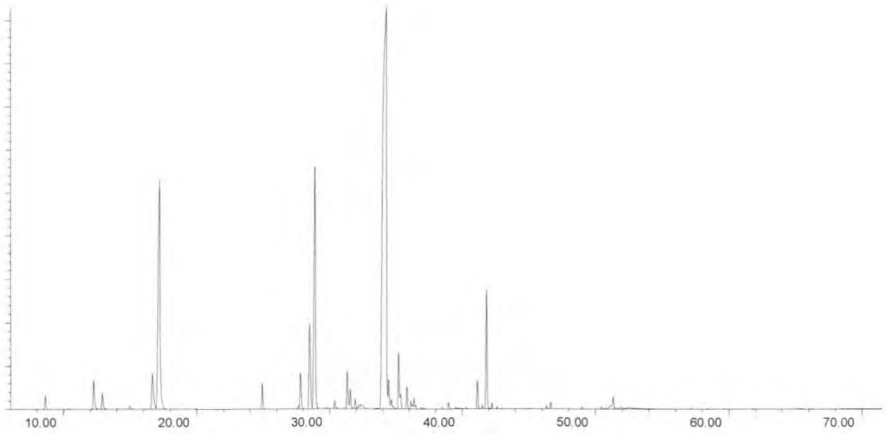
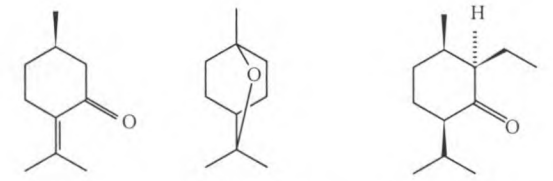


Figure 25: GC-MS profile of the hydro-distilled essential oil obtained from *Mentha longifolia.*

Table 8: Compounds identified in the essential oil of *Mentha longifolia.*

RRI	Compound	Area Percentage
1032	α-pinene	0.49
1118	β-pinene	1.26
1132	Sabinene	0.70
1174	Mycrene	0.16
1203	Limonene	1.97
1213	**1,8-cineole**	**14.72**
1393	3-octanol	0.93
1474	*trans*-sabinene hydrate	0.10
	Menthofuran	4.05
1503	**Isomenthone**	**11.59**
1535	β-bourbonene	0.04
1553	Linalool	0.25
1583	*cis*-isopulegone	0.70
1598	*trans*-isopulegone	0.38
1662	**Pulegone**	**47.57**
1682	δ-terpineol	1.16
	trans-verbenol	0.32
1706	α-terpineol	1.97
1726	Germacrene D	0.80
1748	Piperitone	0.28
1755	Bicyclogemacrene	0.12
1875	*trans*-p-menthane-8-thio-3one	0.23
	Isopiperitenone	0.06
	Myrcen-8-ol	0.04
1475	Menthone	1.49
1904	*cis*-p-menthane-8thio-3one	0.14
1748	Piperitenone	4.13
	cis-jasmone	0.17
	Piperitenone oxide	0.05
	Methofurolactone	0.45
	Total	**96.32**

Thirty compounds were identified in the leaves essential oil of *Mentha longifolia*. The major compound is pulegone, which represents 47.57% of the total composition. There are two other compounds that are also present in quite large quantities, 1,8-cineole (14.72%) and isomenthone (11.60%).



pulegone 1,8-cineole isomenthone

Figure 26: Chemical structures for major compounds detected in the essential oil of *Mentha longifolia.*

4.5 *Ocimum americanum* L. Sp.

Botanical description

A perennial herb or soft shrublet, often woody. This plant grows to about 0.5m tall. The stems are subglabrous or sparingly pubescent to villous, particularly at the nodes. The leaves are petiolate. The calyx grows to about 5-7 mm long, while the corolla grows to about 6mm long. The corolla is lilac to mauve or white.

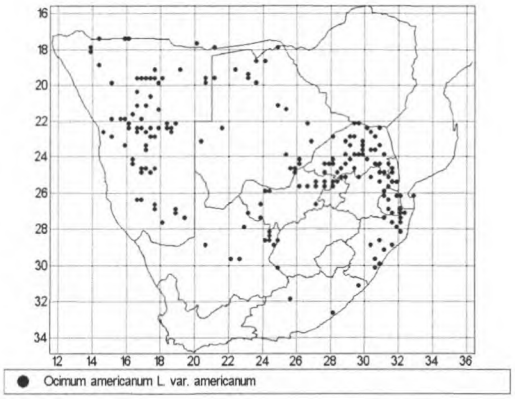


Figure 27: Geographical distribution of *Ocimum americanum*.

Distribution

Found in the warmer parts of Southern Africa. It grows at low and medium altitudes in Swaziland, Natal coast and midlands. The plant may be strongly to slightly aromatic and the description of the scent varies from that of thyme or mint to aniseed, liquorice or *Eucalyptus* oil. The plants tend to spread on overgrazed or disturbed areas.

Common name

Lemon basil.

Uses

Used in snakebite remedies and also used as a diuretic and diaphoretic. (Watt and Breyer-Brandwijk, 1962).

Essential oil composition

The essential oil was obtained by hydro-distillation from plants collected from Swaziland. The essential oil yield was 0.14%.

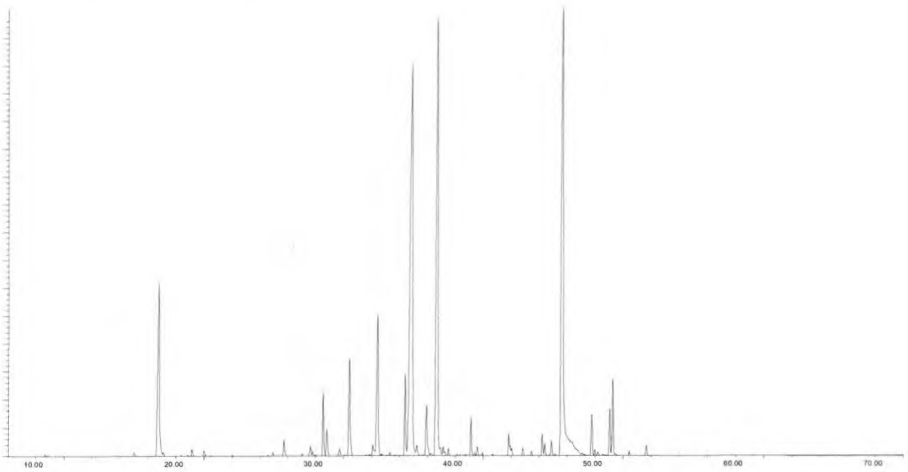


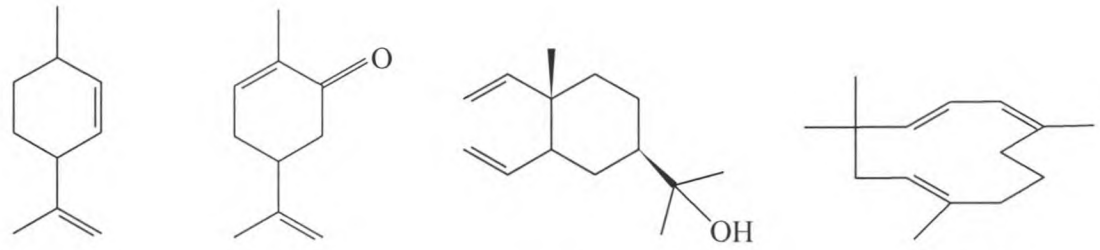
Figure 28: GC-MS profile of the hydro-distilled essential oil obtained from *Ocimum americanum*.

Table 9: Compounds identified in the essential oil of *Ocimum americanum*.

RRI	Compound	Area Percentage
1032	α-pinene	0.05
1174	Myrcene	0.11
1203	**Limonene**	**7.18**
1213 + 1218	1,8-cineole + β phellandrene	0.10
1266	*E*-β ocimene	0.19
1280	ρ-cymene	0.18
1327	*Z*-3-hexen-1-ol	0.03
1393	3-octanol	0.10
1452	1 octane-3-ol	0.05
1474 + 1466	*trans*-sabinene hydrate + α-cubebene	0.32
1476	*Z*-β-ocimene epoxide	0.12

RRI	Compound	Area Percentage
1498	*E*-β-ocimene epoxide	1.78
1497	α-copaene	0.90
1535	β-bourbonene	0.23
1549 + 1553	β-cubebene + linalool	3.07
1600	β-elemene	0.32
1612	β-caryophellene	5.37
	trans-dihydrocarvone	0 05
	cis-isodihydrocarvone	0.08
1668	*Z*-β-farnesene	2.12
1687	**α-humulene**	**24.17**
1706	α-terpineol	0.36
1726	Germacrene D	1.79
1751	**Carvone**	**19.31**
1773	δ-cadinene	0.20
1776	γ-cadinene	0.09
1784	*E*-α-bisabolene	0.17
1845	*trans*-carveol	0.93
	Carvone oxide	0.06
	Isopiperitenone	0.22
1882	*cis*-carveol	0.05
1900	Epicubebol	0.03
1957	Cubebol	0.17
2008	Caryophyllene oxide	0.11
2050	*E*-nerolidol	0.35
2071	Humulene epoxide II	0.42
2082	Cubenol	0.37
2096	**Elemol**	**21.95**
2185	γ-eudesmol	1.20
2237	Carvacrol	0.03
2250	α-eudesmol	1.31
2257	β-eudesmol	2.06
2365	*cis*-methyljasmonate	0.33
	Total	**98. 03**

The major compound present in this oil is α-humulene. There were three other compounds that were also found in large quantities in the oil sample. These were limonene (7.18%), carvone (19.31%) and elemol (21.95%). The major compound α-humulene is a sesquiterpene and makes up 24.17% of the oil.



limonene carvone elemol α-humulene

Figure 29: Chemical structures for major compounds detected in the essential oil of *Ocimum americanum*.

4.6 *Salvia africana-lutea* L

Botanical description

This is a branched shrub growing up to 2m high. The stems are densely leafy, sparse to densely, appressed white-tomentose, dotted with orange-red gland dots. The calyx is broadly campanulate, expanding to 30mm long in fruit. It is purplish and membraneous, with short spreading glandular hairs, dotted with orange-red glands. The corolla is golden brown, reddish brown, khaki or occasionally purplish.

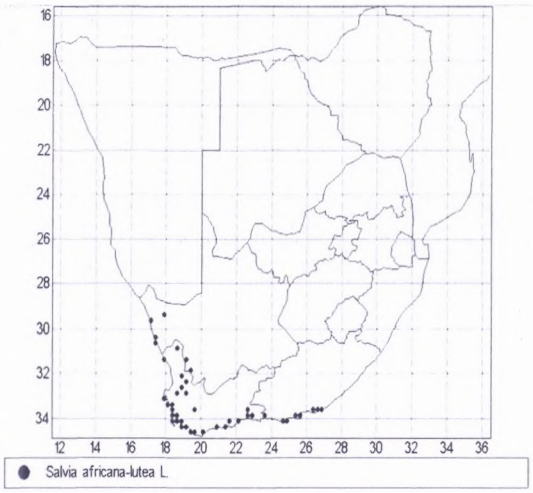


Figure 30: Geographical distribution of *Salvia africana-lutea*.



Figure 31: *Salvia africana-lutea* (Clanwilliam).

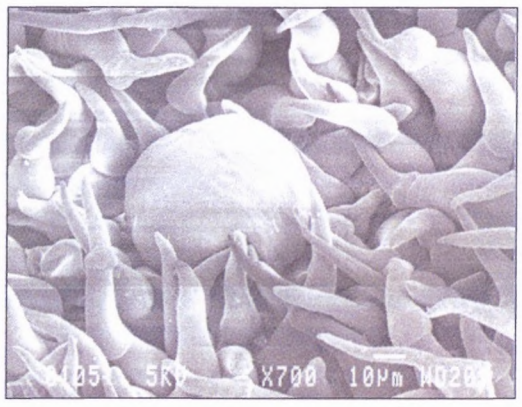




Figure 32 & 33: Scanning electron micrographs of *Salvia africana-lutea* showing the essential oil glands almost obscured by the many epidermal hairs.

Distribution

Distributed from Namaqualand to the Cape Peninsula, on coastal sand dunes and in arid fynbos on rocky slopes to 800m altitudes.

Common name

Beach sage, Bloublomsalie

Uses

Used to treat coughs and colds. (Watt and Breyer-Brandwijk, 1962).

Essential oil composition

The essential oil was obtained by hydro-distillation from plants collected from Clanwilliam. The essential oil yield was 0.13%.

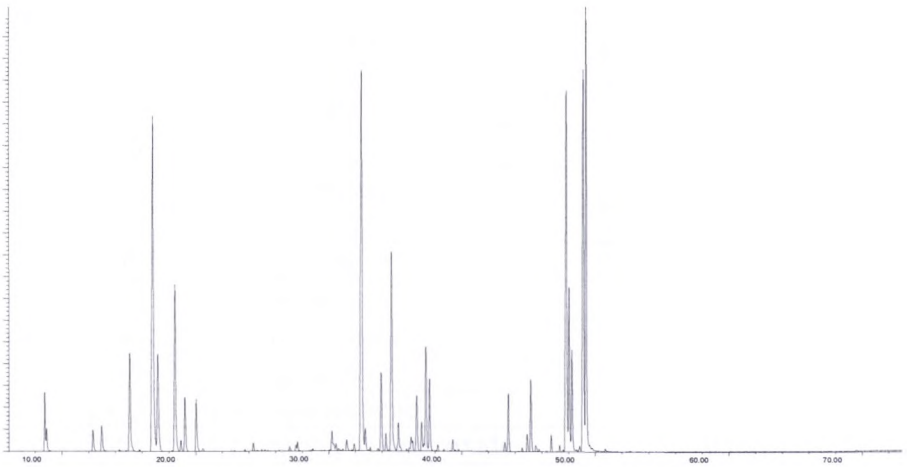
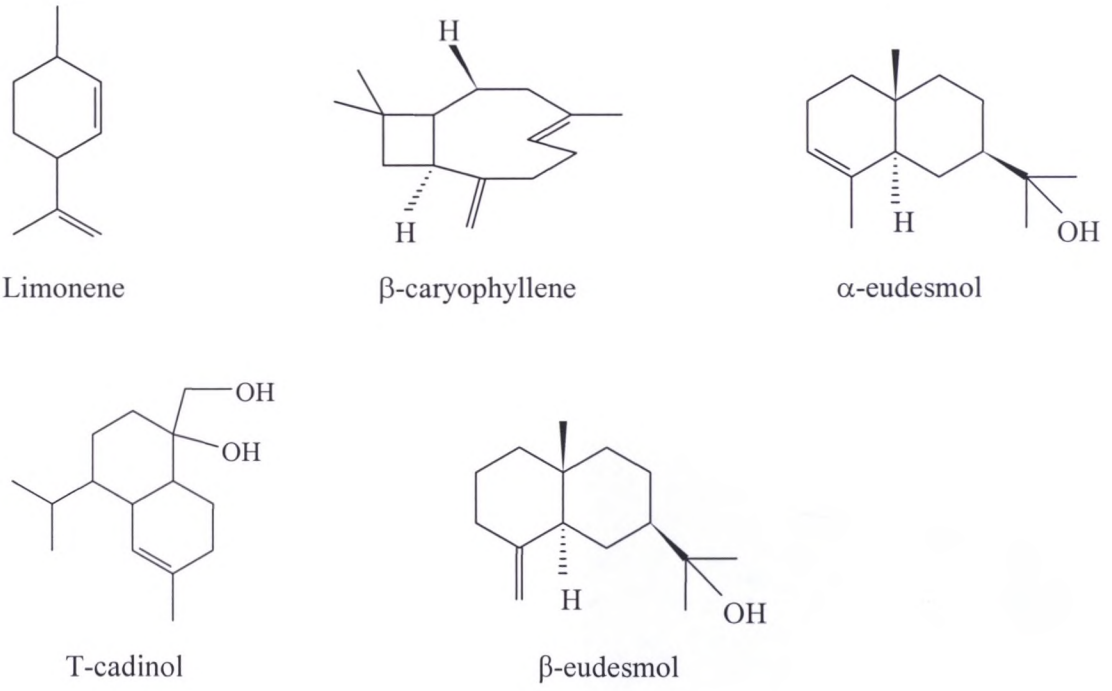


Figure 34: GC-MS profile of the hydro-distilled essential oil obtained from *Salvia africana-lutea*.

Table 10: Compounds identified in the essential oil of *Salvia africana-lutea*.

RRI	Compound	Area percentage
1032	α-pinene	1.15
1035	α-thujene	0.47
1118	β-pinene	0.52
1132	Sabinene	0.65
1174	Myrcene	2.75
1203	**Limonene**	**11.61**
1213 + 1218	1,8-cineole + β-phellandrene	2.89
1246	*Z*-β-ocimene	4.72
1255	γ-terpinene	0.24
1266	*E*-β-ocimene	1.37
1280	ρ-cymene	1.38
1290	Terpinolene	0.06
1382	*cis*-allocimene	0.17
1452	1-octen-3-ol	0.05
1474	*trans*-sabinene hydrate	0.18
1545	*cis*-α-bergamotene	0.48
1556	*cis*-sabinene hydrate	0.15
1594	α-*trans*-β-bergamotene	0.14
1612 + 1611	**β-caryophyllene + terpinen-4-ol**	**13.51**
1628	Aromadendrene	0.53
1638	*cis*-menth-2-en-1-ol	0.05
1661	Alloaromadendrene	1.96
1668	*Z*-β-farnesene	0.37
1687	α-humulene	6.00
1706	α-terpineol	0.72
1742	β-selinene	0.29
1744	α-selinene	0.19
1755	β-curcumene	1.45
1766	I-decanol	0.68
1773	δ-cadinene	0.13
1776	γ-cadinene	2.61
1786	Ar-curcumene	1.67
1810	3,7-guaidiene	0.13
1853	*cis*-calamenene	0.26
2001	Isocaryophyllene oxide	0.09
2008	Caryophyllene oxide	1.35
2071	Humulene epoxide II	0.36
2080	Cubenol	1.57
2096	Elemol	0.17
2144	Spathulenol	0.32
2187	**T-cadinol**	**10.09**
2250	**α-eudesmol**	**10.14**
2257	**β-eudesmol**	**9.79**
	Total	**93.81**

This complex essential oil is made up of many compounds. There are at least five compounds of similar quantities present namely, limonene (11.61%), β-caryophyllene and terpinen-4-ol (13.51%), T-cadinol (10.09%), α-eudesmol (10.14%) and β-eudesmol (9.79%). While terpinen-4-ol and limonene are monoterpenes, T-cadinol, β-eudesmol, α-eudesmol and β-caryophyllene are all sesquiterpenes.



Limonene β-caryophyllene α-eudesmol

T-cadinol β-eudesmol

Figure 35: Chemical structures for major compounds detected in the essential oil of *Salvia africana-lutea*.

4.7 *Salvia disermas* L

Botanical description

This soft shrub or perennial herb usually grows to about 1m tall with one or more stems from a woody rootstock. The leaves are often crowded and larger near the base of the plant. The calyx is glandular-hispid to villous, up to 12mm long. The corolla is whitish, pale blue or mauve, usually around 15-25mm long.

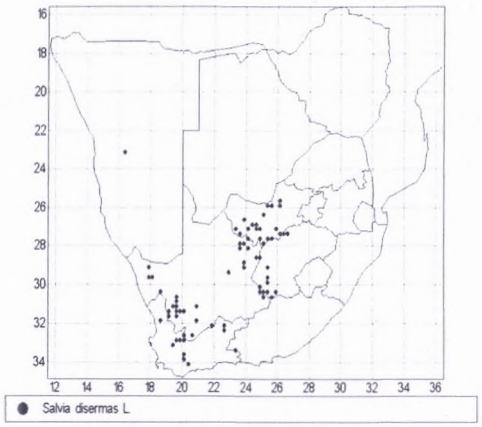


Figure 36: Geographical distribution of *Salvia disermas*.



Figure37: *Salvia disermas* photographed in the Witwatersrand Botanical Garden (Roodepoort).

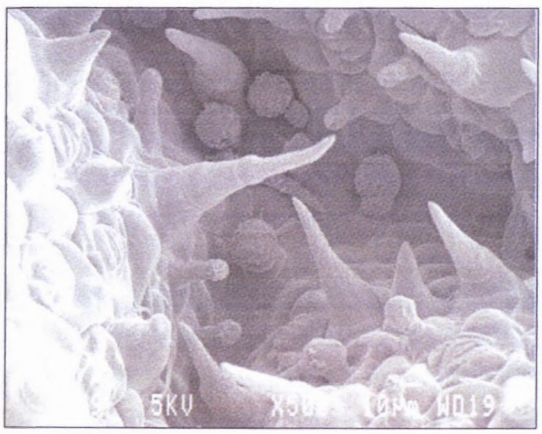




Figure 38 & 39: Scanning electron micrograph showing the numerous oil glands on the abaxial leaf surface of *Salvia disermas*.

Distribution

Salvia disermas is found in the northern to southern districts from south-western Gauteng and northern Cape Province through to Namaqualand and southwards to Uniondale and Swellendam, with a single record from central Namibia. The plants are found on sandy soil in watercourses, on limestone formations and rocky hillsides, tending to spread as a weed along roadsides, on waste places and on overgrazed veld.

Essential oil composition

The essential oil was obtained by hydro-distillation from plants collected from the Kokerboom Woud (Northern Cape). The essential oil yield was 0.34%.

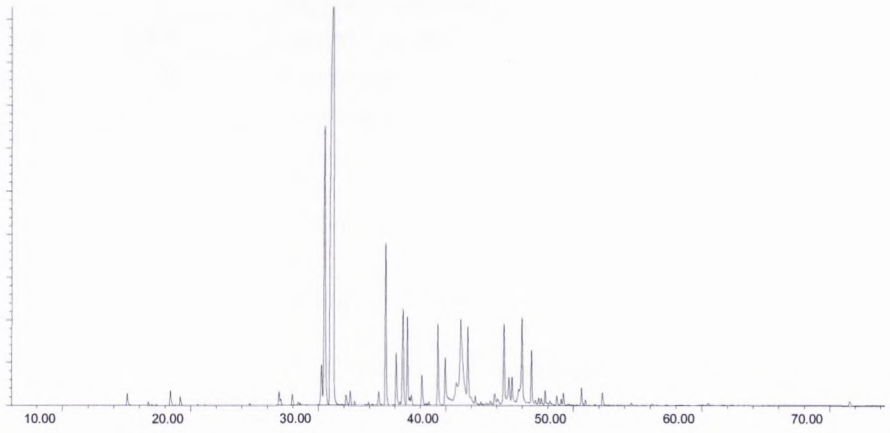


Figure 40: GC-MS profile of the hydro-distilled essential oil obtained from *Salvia disermas*.

Table 11: Compounds identified in the essential oil of *Salvia disermas*.

RRI	Compound	Area percentage
1174	Myrcene	0.44
1203	Limonene	0.12
1220	*cis*-anhydrolinalool oxide	0.03
1246	*Z*-β-ocimene	0.56
1266	*E*-β-ocimene	0.31
1290	Terpinolene	0.03
1391	*Z*-3-hexen-1-ol	0.06

RRI	Compound	Area percentage
1450	*trans*-linalool oxide	0.46
1452	1-octen-3-ol	0.20
1478	*cis*-linalool oxide	0.42
1495	Bicycloelemene	0.11
	cis-3-hexenyl isovalerate	0.06
1544	α-gurjunene	1.71
1553	**Linalool**	**12.71**
1565	**Linalyl acetate**	**37.17**
1600	β-elemene	0.37
1612	β-caryophyllene	0.52
1626	2-methyl-6-methylene-3, 7-octadiene-2-ol	0.12
1628	Aromadendrene	0.09
1668	*Z*-β-farnesene	0.04
1687	α-humulene	0.53
1706	α-Terpineol	5.87
1733	Neryl acetate	1.77
1740	Geranial	0.12
1755	Bicyclogermacrene	3.97
1765	Geranyl acetate	3.02
1776	δ-cadinene	0.27
1773	γ-cadinene	0.37
1808	Nerol	1.10
1815	2,6-dimethyl-3 (E), 5(E), 7-octatriene-2-ol	0.10
1857	Geraniol	2.72
	Diepishyobunone	2.06
	Shyobunone	5.48
1961	3,7-dimethyl-1, 5 octadiene-3, 7-diol	0.27
2008	Caryophyllene oxide	0.15
2069	Germacrene D-4-ol	0.78
2080	Cubenol	0.81
2144	Spathulenol	1.69
2187	T-cadinol	0.47
2209	T-muurolol	0.12
2247	*trans*-α-bergamotol	0.19
2396	8-α-13oxy-14-en-epilabdone	0.49
	Calamenediol	0.06
	Total	**87.94**

The essential oil of *Salvia disermas* constitutes 44 different compounds. These compounds together make up 87.94% of the oil. The major compound is the monoterpene linalyl acetate representing 37.17% of the oil. The other compound that is quite prominent is linalool, accounting for 12.71% of the oil. Linalool is also a monoterpene and together with linalyl acetate make up 49.88% of the total essential oil composition.



linalool linalyl acetate

Figure 41: Chemical structures for major compounds detected in the essential oil of *Salvia disermas*.

4.8 *Salvia dolomitica* Codd

Botanical description

This is a 1.2m tall shrub. The stems are terete, ascending, densely covered with a short whitish tomentum. The leaves are petiolate with a simple blade. The calyx is broadly campanulate, often purple-tinged. The corolla is light pink or lilac with cream or yellow markings on the lower lip.

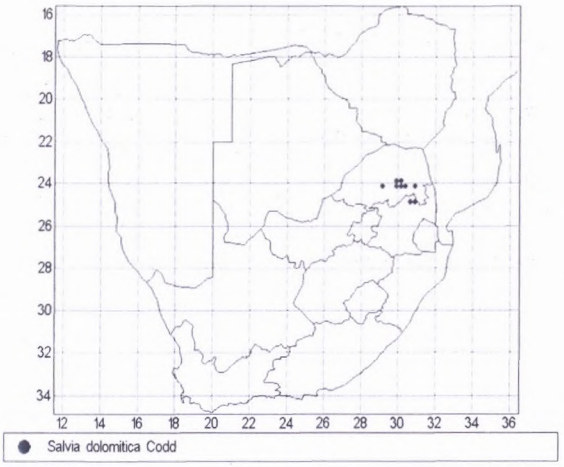


Figure 42: Geographical distribution of *Salvia dolomitica.*

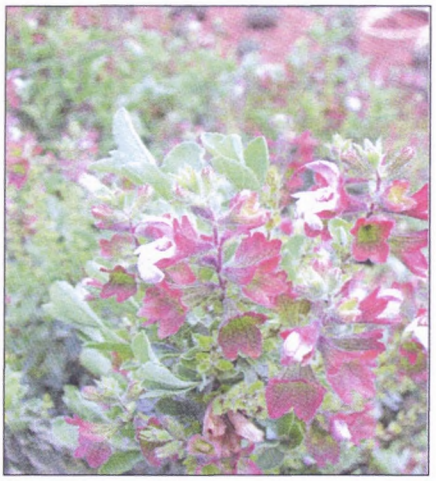


Figure 43: *Salvia dolomitica* photographed in the Witwatersrand Botanical Garden (Roodepoort).

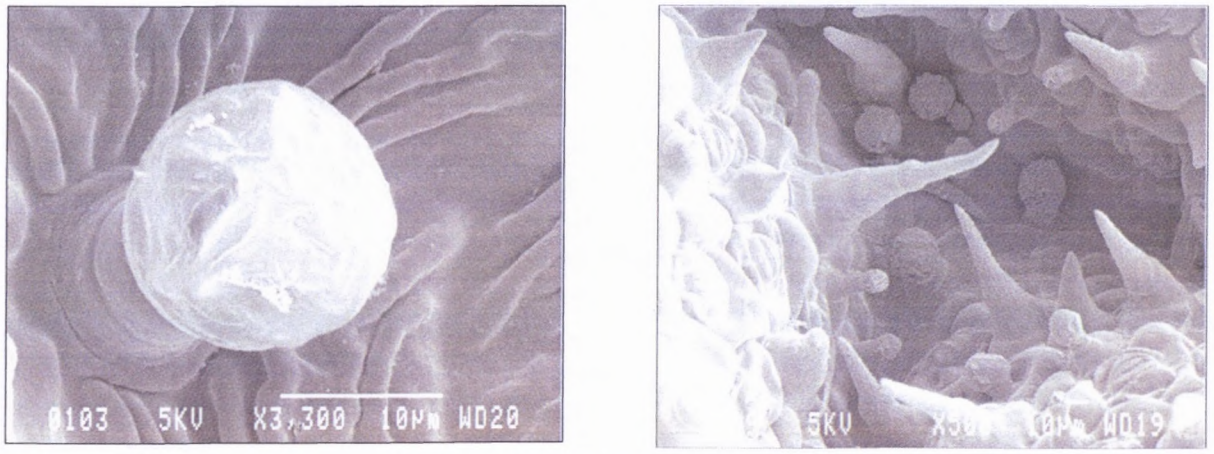


Figure 44 & 45: Scanning electron micrographs of oil glands on the abaxial leaf surface of *Salvia dolomitica.*

Distribution

Restricted to the eastern and north-eastern Gauteng, usually on dolomitic outcrops between 1000-1500m altitudes.

Essential oil composition

The essential oil was obtained by hydro-distillation from plants collected from the Witwatersrand Botanical Garden. The essential oil yield was 0.23%.

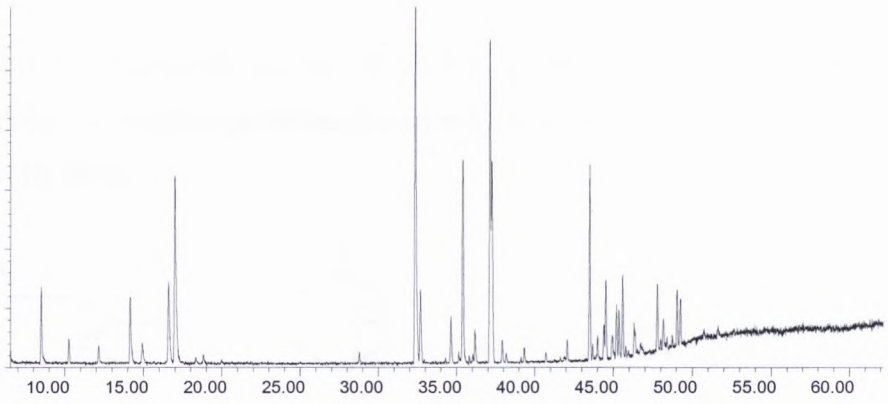


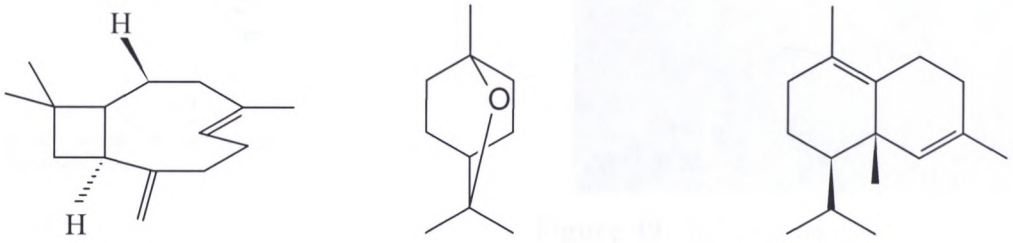
Figure 46: GC-MS profile of the hydro-distilled essential oil obtained from *Salvia dolomitica*.

Table 12: Compounds identified in the essential oil of *Salvia dolomitica*.

RRI	Compound	Area percentage
1032	α-pinene	2.63
1076	Camphene	0.95
1118	β-pinene	0.62
1159	δ-3-carene	2.98
1203	Limonene	4.13
1213	**1,8-cineole**	**10.10**
1612	**β-caryophyllene**	**16.22**
1687	α-humulene	1.81
1719	Borneol	8.50
1740	α-muurolene	1.23
1773	**δ-cadinene**	**11.14**
1776	γ-cadinene	7.45
1799	Cardin-1, 4-diene	0.70

RRI	Compound	Area percentage
1853	*cis*-calamonene	0.54
1957	Cubebol	0.63
2008	Caryophyllene oxide	6.97
2033	Epiglobulol	0.52
2050	*E*-neroledol	0.88
2057	Ledol	2.46
2071	Humulene epoxide II	0.98
2088	1-epi-cubenol	1.38
2098	Globulol	2.64
2144	Rosifordiol	1.58
2187	T-cadinol	2.20
2209	T-muurolol	0.76
2250	α-eudesmol	1.71
2257	β-eudesmol	1.66
	Total	**93.37**

The 27 identified compounds account for 93.37% of the oil composition. There are three major compounds of similar quantities, β-caryophyllene (16.22%), δ-cadinene (11.14%) and 1,8-cineole (10.10%).



β-caryophyllene 1,8-cineole δ-cadinene

Figure 47: Chemical structures for major compounds detected in the essential oil of *Salvia dolomitica*.

4.9 *Salvia namaensis* Schinz

Botanical description

This shrub is usually bushy, has many branches and grows up to 1.2m tall. The branches are often herbaceous above and woody below, yellow-green or grey-green, strongly aromatic and somewhat viscid. The leaves are shortly petiolate, with an irregular blade. The calyx is up to 8mm long and the corolla can grow as long as 12mm. The corolla is white, mauve or blue.

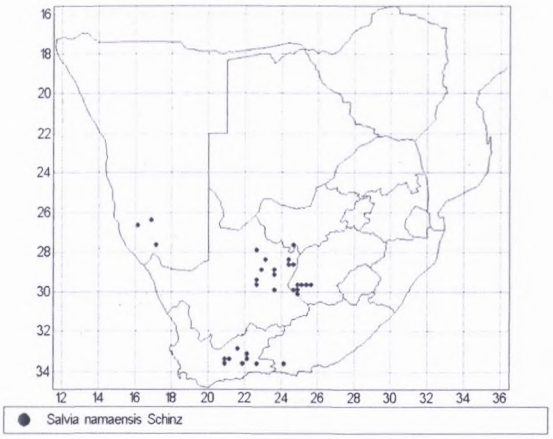


Figure 48: Geographical distribution of *Salvia namaensis*.



Figure 49: *Salvia namaensis* in habitat on the Swartberg Pass.

Distribution

Found in the northern and southern Cape as far south as Oudtshoorn and Willowmore. They are usually found on rocky slopes, in watercourses and on surface limestone.

Uses

At the time of collection, anecdotal evidence was shared with Dr. A. Viljoen that this plant is used by the local people to treat respiratory disorders (reference was made to the strong camphor smell).

Essential oil composition

The essential oil was obtained by hydro-distillation from plants collected from the Swartberg Pass. The essential oil yield was 0.59%.

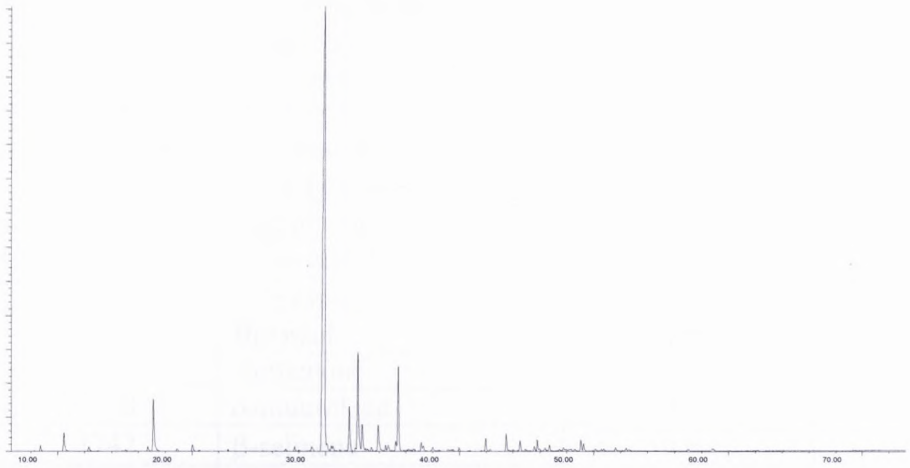


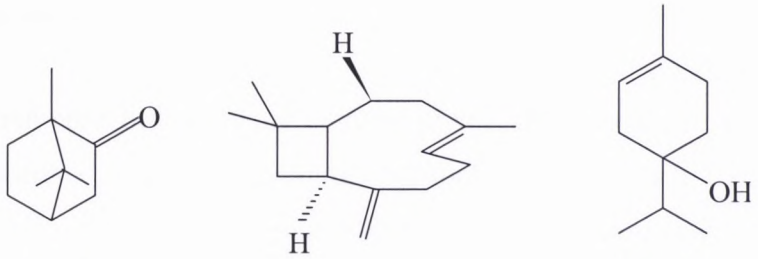
Figure 50: GC-MS profile of the hydro-distilled essential oil obtained from *Salvia namaensis*.

Table 13: Compounds identified in the essential oil of *Salvia namaensis*.

RRI	Compound	Area percentage
1032	α-pinene	0.37
1076	Camphene	1.43
1118	β-pinene	0.34
1132	Sabinene	0.02
1174	Myrcene	0.10
1188	α-terpinene	0.02
1203	Limonene	0.37
1213	**1,8-cineole**	**4.51**
1246	*Z*-β-ocimene	0.01
1255	γ-terpinene	0.17
1280	P-cymene	0.52
1290	Terpinolene	0.08
1430	α-thujene	0.02
1451	β-thujene	0.14
1474	*trans*-sabinene hydrate	0.35
1499	α-campholene aldehyde	0.03
1497	α-copaene	0.40
1532	**Camphor**	**56.89**
1553	Linalool	0.26
1556	*cis*-sabinene hydrate	0.35
1571	*trans*-p-menth-2-en-1-ol	0.01

RRI	Compound	Area percentage
1586	Pinacarvone	0.08
1597	Bornyl acetate	3.24
	Selina-5,11-diene	2.07
1612 + 1611	**β-caryophyllene + terpinen-4-ol**	**7.68**
1628	Aromadendrene	2.07
	Selina-5,11-diene	0.18
1638	*cis*-p-menth-2-en-1-ol	0.15
1804	Myrtenol	0.27
1661 + 1664	Alloaromadendrene + *trans*-pinocarveol	2.14
1682	δ-terpineol	0.36
1687	α-humulene	0.49
1706	α-terpineol	0.65
1719	**Borneol**	**6.08**
1725	Verbenone	0.06
1740	α-muurolene	0.05
1742	β-selinene	0.02
1755	Bicyclogermacrene	0.08
1773	δ-cadinene	0.58
1805	Campholene alcohol	0.01
1845	*trans*-carveol	0.10
1853	*cis*-calamenene	0.01
1864	P-cymen-8-ol	0.12
1882	*cis*-carveol	0.19
1898	1,11-oxidocalamenen	0.03
1941	α-calacorene-I	0.04
1953	Palustrol	0.89
	cis-jasmone	0.12
2008	Caryophyllene oxide	1.17
2029	Perilla alcohol	0.04
1900	Epiglobulol	0.07
2050	*E*-nerolidol	0.02
2057	Ledol	0.68
2071	Humulene epoxide-II	0.07
2098	Globulol	0.31
2104	Viridiflorol	0.72
2144	Spathulenol	0.32
2185	γ-eudesmol	0.19
2221	Isocarvacrol	0.10
2237	Carvacrol	0.11
2250	α-eudesmol	0.72
2257	β-eudesmol	0.48
2324	Caryophylladienol-II	0.11
2389	Caryophyllenol I	0.21
2384	Hexadecanol	0.17
	Total	**96.57**

A total of 96.57% of the oil's compounds were identified, with the major compound being camphor representing 56.89% of the oil composition.



camphor β-caryophyllene terpinen-4-ol

Figure 51: Chemical structures for major compounds detected in the essential oil of *Salvia namaensis*.

4.10 *Salvia runcinata* L. *f.*

Botanical description

This is a perrenial erect herb growing up to 0.7m tall. They have one to several stems from a taproot or, occasionally, from a creeping rootstock. The leaves are shortly etiolate or the upper one sessile. The calyx is about 5-8mm long. The corolla is white or pale blue to mauve or purplish and measures between 7-14mm long.

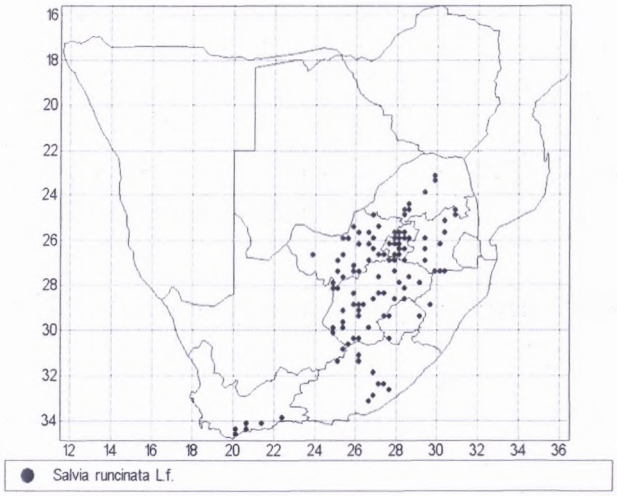
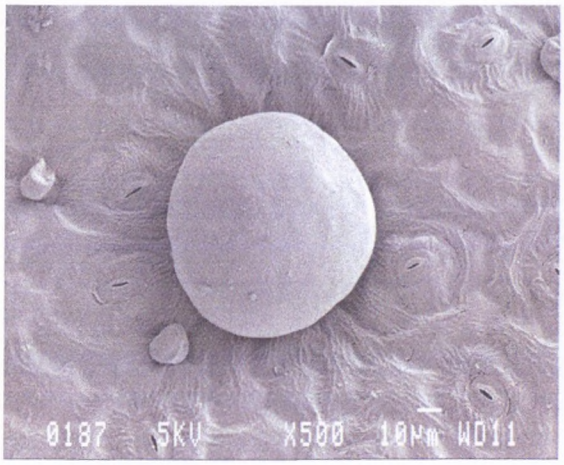


Figure 52: Geographical distribution of *Salvia runcinata*.



Figure 53: *Salvia runcinata* in habitat in Midrand.



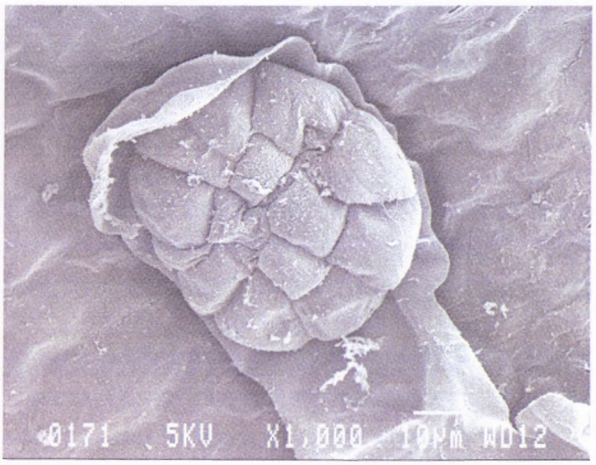


Figure 54 and 55: Peltate trichome on the abaxial leaf surface of *S. runcinata*.

Distribution

This species extends from northern Gauteng and Botswana to northern Cape. The plants are also found in the southern and eastern Cape but are rare in Natal and Lesotho. These plants are found in a variety of habitats.

Common name

"Kruipsalie"

Uses

Used for the relief of urticaria, stomachache. (Watt and Breyer-Brandwijk, 1962).

Essential oil composition

The essential oil was obtained by hydro-distillation from plants collected from Midrand. The essential oil yield was 0.17%.

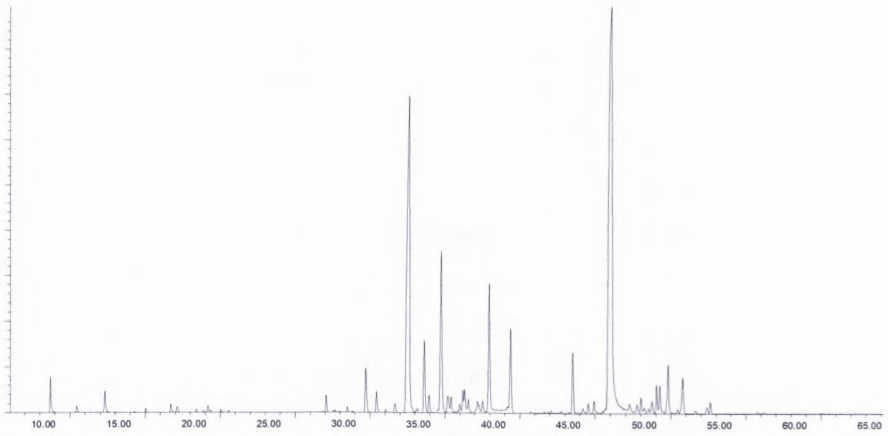
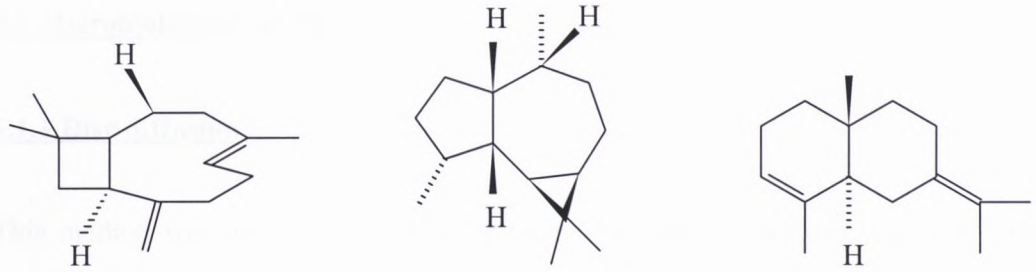


Figure 56: GC-MS profile of the hydro-distilled essential oil obtained from *Salvia runcinata*.

Table 14: Compounds identified in the essential oil of *Salvia runcinata*.

RRI	Compound	Area percentage
1032	α-pinene	1.08
1076	Camphene	0.19
1118	β-pinene	0.79
1174	Myrcene	0.14
1203	Limonene	0.30
1218	β-phellandrene	0.21
1246	*Z*-β-ocimene	0.10
1255	γ-terpinene	0.04
1266	*E*-β-ocimene	0.21
1265	5-methyl-3-heptanone	0.07
1280	ρ-cymene	0.11
1290	terpinolene	0.05
1452	1-octen-3-ol	0.52
1466	α-cubebene	0.05
1474	*trans*-sabinene hydrate	0.06
1495	α-ylangene	0.17
1497	α-copanene	0.06
1532	Camphor	1.69
1553	Linalool	0.69
1589	Isocaryophyllene	0.38
1612	**β-caryophyllene**	**21.73**
1650	γ-elemene	2.70
1661	Alloaromadendrene	0.66
1684	β-guaiene	0.10
1687	α-humulene	6.50
1704	γ-muurolene	0.42
1719	Borneol	0.51
1740	Valencene	0.06
1742	β-selinene	0.64
1744	α-selinene	0.60
1773	δ-cadinene	0.28
1796	**Selina-3,7(11)-diene**	**5.06**
1854	Germacrene B	3.23
1900	Epicubebol	0.03
1941	α-calacorene-I	0.05
2008	Caryophyllene oxide	2.10
2057	Ledol	0.33
2071	Humulene epoxide II	0.47
2104	**Viridiflorol**	**37.63**
2185	γ-eudesmol	0.34
2209	T-muurolol	0.11
2228	Isospathulenol	0.45
2320	Juniper camphor	1.59
2392	Caryophyllenol-II	0.18
	Total	**92.74**

The two major compounds found in *Salvia runcinata* are, viridiflorol (37.63%) and β-caryophyllene (21.73%).



β-caryophyllene viridiflorol Selina-3,7(11)diene

Figures 57: Chemical structures for major compounds detected in the essential oil of *Salvia runcinata*.

5. RESULTS AND DISCUSSION

5.1 Microbiological results

5.1.1 Disc diffusion

This method was used to test the antimicrobial activity of the essential oils. The discs, impregnated with the oils, were introduced onto the seeded agar plates. The zones of inhibition were examined after 24hr – 7 days depending on the test organism. The results were then compared to the results obtained from the MIC tests.

Bacteria

Table 15: Zones of inhibition for the disc diffusion assay measured for six bacteria.

	Zones of inhibition (mm from disc edge)					
	S. aureus	***E. coli***	***E. faecalis***	***P. aeruginosa***	***K. pneumoniae***	***S. typhi***
Hemizygia modesta	0	0	0	2	0	0
Hemizygia pretoriae	0	0	0	0	0	0
Leonotis leonurus	0	0	0	0	0	0
Mentha longifolia	1	0	0	<1	2	1
Ocimum americanum	0	<1	0	0	0	0
Salvia africana-lutea	0	0	2.5	2.5	0	0
Salvia disermas	0	0	0	0	0	0
Salvia dolomitica	0	0	0	0	0	<1
Salvia namaensis	0	0	0	0	2	0
Salvia runcinata	<1	0	0	0	1	<1
Control Neomycin (30 µg)	8	6	3	5	9	9

Table 15 shows that seven of the ten essential oils showed some activity against the bacteria. The control used was neomycin, showing positive antibacterial activity against all the bacteria that were tested. *Salvia africana-lutea* showed relatively good activity against *P. aeruginosa* and *E. faecalis*.

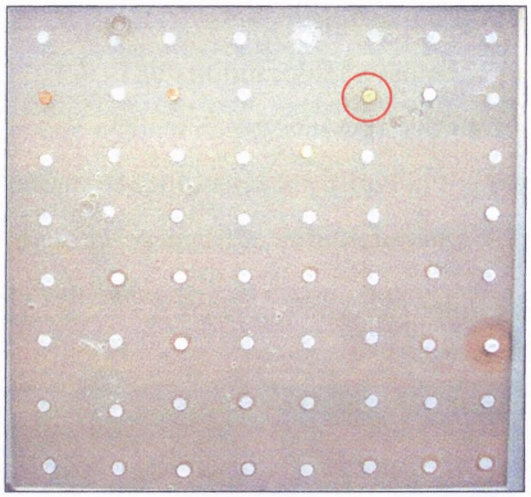


Figure 58: Zone of inhibition (indicated by the red circle) caused by *Mentha longifolia* against *S. aureus*.

Essential oil samples that were most active against *K. pneumoniae* were re-introduced onto a new seeded agar plate to confirm uniformity of results. The results obtained can be seen in **Table 16**.

Table 16: Zones of inhibition of most active oils against *K. pneumoniae*.

Oil sample	**Zone of inhibition (mm from disc)**
Mentha longifolia	2
Salvia namaensis	2
Salvia runcinata	1
Neomycin (control)	9

Major compounds identified in *Mentha longifolia* were pulegone (47.57%), 1,8-cineole (14.72%) and isomenthone (11.59%). Compounds identified in *Salvia namaensis* included Camphor (56.89%), β-caryophyllene and terpinen-4-ol (7.68%), borneol (6.08%) and 1,8-cineole (4.51%). *Mentha longifolia* and *Salvia namaensis* both contained the compound 1,8-cineole. With respect to the chemical composition of both these oils 1,8-cineole was recorded as one of the major compounds i.e. 14.72% in *Mentha longifolia* and 4.51% in

Salvia namaensis. According to a study carried out by Pattnaik *et al.* (1997), *Klebsiella sp.* was found to be susceptible to cineole with a zone of inhibition of 10mm. It is interesting to note that one of the ethnopharmaceutical properties claimed for the essential oil of *Artemisia afra* Jacq. is in the treatment of colds and coughs, conditions related to infection with *K. pneumoniae*. The main components of this volatile oil used in a study carried out by Graven *et al.* (1992), were identified as α- and β-thujone (52%), 1,8-cineole (13%) and camphor (15%). The antimicrobial evaluation in this study revealed the volatile oil to be significantly active against *K. pneumoniae* with a 15.5mm zone of inhibition. Noting that 1,8-cineole is a common compound identified in the essential oils of the above species, we can thus make the assumption that the antimicrobial activity of *Mentha longifolia* and *Salvia namaensis* might be attributed to this compound.

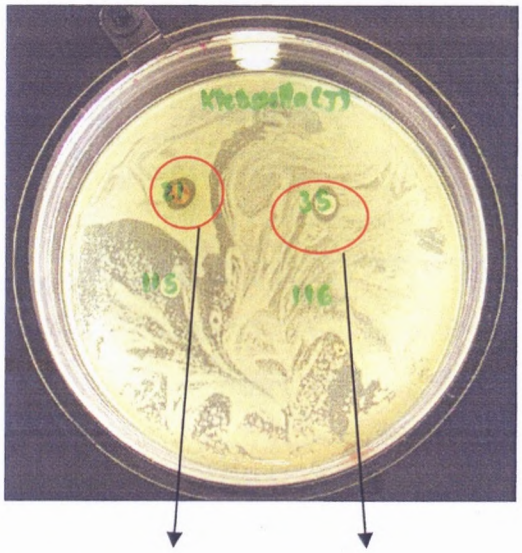


Figure 59: Zones of inhibition caused by *Mentha longifolia* against *K. pneumoniae*.

Two essential oils that showed activity against *P. aeruginosa* were those obtained from *Salvia africana-lutea* and *Hemizygia modesta*. One of the identified compounds present in the essential oil of *Salvia africana-lutea* was β-caryophyllene and terpinen-4-ol (13.51%). According to a study conducted by Carson and Riley (1995), it was found that terpinen-4-ol inhibited the growth of *P. aeruginosa*. Chao *et al.* (2000) demonstrated that tea tree oil showed some activity against *P. aeruginosa*. Some of the compounds that were identified in tea tree oil were terpinen-4-ol (40.1%), γ-terpinene (23%), α-terpinene (10.4%) and 1,8-cineole (5.1%). γ-Terpinene was the major compound (29.36%) identified in the essential oil

of *Hemizygia modesta*. This compound may therefore contribute to the anti-pseudomonal activity found in the disc diffusion and MIC studies.

Mould and yeasts

The essential oils were tested against two yeasts (*C. neoformans* and *C. albicans*) and one mould (*A. niger*). *Cryptococcus neoformans* and *Candida albicans* are responsible for some of the most common opportunistic infections prevalent today.

Table 17: Zones of inhibition caused by the oils against the mould and yeasts.

Oil sample	Zones of inhibition (mm from disc)		
	C. neoformans	***C. albicans***	***A. niger***
Hemizygia modesta	1	0	0
Hemizygia pretoriae	0	0	0
Leonotis leonurus	0	0	0
Mentha longifolia	4	4	0
Ocimum americanum	11	2	<1
Salvia africana-lutea	0	1	1
Salvia disermas	5	3	0
Salvia dolomitica	0	<1	0
Salvia namaensis	0	2	0
Salvia runcinata	1	0	2
Nystatin (100 *i*u)	5	7	7

From **Table 17**, it can be seen that most of the oils showed very good activity against the mould and yeasts, especially against *C. neoformans*. *Ocimum americanum* shows the most activity against *C. neoformans,* while *Mentha longifolia* and *Salvia disermas* showed activity against both *C. neoformans* and *C. albicans*, but no activity against the mould, *A. niger*.

Some of the compounds identified in the essential oil of *Ocimum americanum* were α-humulene (24.17%), elemol (21.95%), carvone (19.31%), limonene (7.18%) and a combination of β-cubebene and linalool (3.07%). According to Chao *et al.* (2000), the essential oils of coriander and rosewood were very active against *C. albicans*. Both of these essential oils contained linalool, 72% and 80% respectively. In the same study, the essential oil of spearmint was also found to show some activity against *C. albicans*. This essential oil comprised of carvone (62.8%) and limonene (11.3%). *Ocimum americanum,* which contains these compounds, does not show the same activity against *C. albicans*. This could suggest that linalool is the major active compound against *C. albicans* and carvone and limonene

have a weaker effect on *C. albicans*. Thus, the activity of *Ocimum americanum* against *C. albicans* can mainly be attributed to the presence of carvone and limonene.

Documented data from previous studies include:

A study carried out by Viollon and Chaumont (1994), on the essential oils of lavender and clary sage exhibit anticryptococcus properties. Both these essential oils contain linalool and carvone, 35.5% and 8% respectively. It was also shown that carvone and linalool both have strong anticryptococcus properties.

Table 18: Disc diffusion susceptibility of organisms to four components of tea tree oil (Carson and Riley, 1995).

Organism	**Zones of inhibition (mm)**			
	Linalool	**Terpinen-4-ol**	***p*-cymene**	**α-terpineol**
C. albicans	40.3	41	15	33.7
P. aeruginosa	0	14.6	0	0
S. aureus	27.7	23	0	20.7

The essential oil of *Salvia disermas* comprises of linalyl acetate (37.17%), linalool (12.71%) and α-terpineol (5.87%). A 3mm zone of inhibition was exhibited by *Salvia disermas* against *C. albicans* using disc diffusion assays. As can be seen from the study by Carson and Riley (1995), growth of *C. albicans* was inhibited drastically by both linalool and α-terpineol. α-terpineol showed significant microbiostatic activity against *C. albicans* (**Table 18**). The inhibitory effect of linalool was found to be weaker, while linalyl acetate was found not have any activity against *C. albicans* (Peana *et al.*, 1999). Results from a study conducted by Chao *et al.,* 2000, showed that rosewood oil displayed the highest level of overall fungal inhibition. The main component of this essential oil is linalool (80%). The activity observed against *C. neoformans* is probably attributed to the presence of linalool, as stated in the study carried out by Viollon and Chaumont (1994).

5.1.2 Microplate (minimum inhibitory concentration)

The minimum inhibitory concentration represents the minimum concentration of the oil needed to inhibit the growth of the test organism. Thus the lower the minimum inhibitory concentration the stronger the antimicrobial properties of that oil sample.

A MIC for *Salmonella typh*i was not done as the oils showed very little activity against this organism as can be seen in **Table 15**. Due to insufficient oil, the MIC for *S. namaensis* against *K. pneumoniae* could not carried out.

The plates were examined after 30 min, 60 min, 120 min and 24 hours

Table 19: Minimum inhibitory concentration results.

Column	Oil sample	Pathogen	Minimum inhibitory concentration (mg/ml)			
			30min	60min	120min	24hrs
1	*Mentha longifolia*	*Staphylococcus aureus*	8	8	8	>32
2	*Mentha longifolia*	*Klebsiella pneumoniae*	16	16	16	>32
3	*Hemizygia modesta*	*Pseudomonas aeruginosa*	32	32	32	32
4	*Salvia runcinata*	*Staphylococcus aureus*	>32	>32	>32	>32
5	*Salvia runcinata*	*Klebsiella pneumoniae*	8	8	8	8
6	*Salvia africana-lutea*	*Enterococcus faecalis*	16	16	32	>32
7	*Salvia africana-lutea*	*Pseudomonas aeruginosa*	16	16	16	16

Table 20: MIC concentrations for selected oils against *K. pneumoniae*.

	Minimum inhibitory concentration (MIC) (mg/ml) against *K. pneumoniae*
Mentha longifolia	16
Salvia runcinata	8

Table 21: MIC concentrations for selected oils against *P. aeruginosa*.

	Minimum inhibitory concentration (MIC) (mg/ml) against *P. aeruginosa*
Hemizygia modesta	32
Salvia africana-lutea	16

As can be seen from **Tables 15, 19** and **20**, *Salvia runcinata* seems more active when comparing the minimum inhibitory concentrations to the disc diffusion assay. With regards to *Mentha longifolia* and *Salvia africana-lutea,* both methods seem to correlate quite well. The results for *Hemizygia modesta* do not correlate as well as the others.

Table 22: Comparison of major compounds found in the essential oils of the different species.

	Hemizyga modesta	*Hemizyga pretoriae*	*Leonotis leomurus*	*Mentha longifolia*	*Ocimum americanum*	*Salvia africana-lutea*	*Salvia disermas*	*Salvia dolomitica*	*Salvia namaensis*	*Salvia runcinata*
δ-cadinene								X		
β-caryophyllene		X	X			X		X		X
α-humulene					X					X
γ-terpinene	X									
1,8-cineole				X				X	X	
Bicyclo-germacrene		X								
Camphor									X	
Carvone					X					
Elemol					X					
Isomenthone				X						
Limonene					X	X				
Linalool					X		X			
Linalyl acetate							X			
P-cymene	X									

	Hemizyga modesta	*Hemizyga pretoriae*	*Leonotis leonurus*	*Mentha longifolia*	*Ocimum americanum*	*Salvia africana-lutea*	*Salvia disermas*	*Salvia dolomitica*	*Salvia namaensis*	*Salvia runcinata*
Pulegone				X						
Spathulenol	X									
T-cadinol						X				
Viridiflorol	X									X
Terpinen-4-ol						X				

From the ten plant species tested, β-caryophyllene was present as one of the major compounds in five of the plants. Of the five *Salvia* species, β-caryophyllene is the most common major compound, present in three of the five *Salvia* species. Another interesting point is that *Hemizygia pretoriae* is not very active against the fungi and the bacteria. *H. pretoriae* contains 69.31% of β-caryophyllene and *L. leonurus* contains 11.3% but neither of these oils showed any antimicrobial activity against the bacteria and fungi tested and therefore this would suggest that β-caryophyllene is not an active compound.

Table 23: Table of identified active compounds according to studies conducted by Nakatsu *et al.*, 2000, Panizzi *et al.*, 1993.

Oil sample	**Major compound**					
	Borneol	Carvacrol	*p*-cymene	Camphene	1,8-cineole	α-pinene
Hemizygia modesta	Yes	No	Yes	No	No	No
Mentha longifolia	No	No	Yes	No	Yes	No
Ocimum americanum	No	Yes	Yes	No	No	Yes
Salvia africana-lutea	No	No	Yes	No	Yes	Yes
Salvia disermas	No	No	No	No	No	No
Salvia namaensis	Yes	Yes	No	Yes	Yes	Yes
Salvia runcinata	Yes	No	Yes	Yes	No	Yes

Studies have been done to identify some of the compounds that are responsible for the antimicrobial activity seen. The essential oil isolated from *Thymus sp.* (thyme) showed activity against both Gram-positive and Gram-negative bacteria, as well as activity against yeasts and filamentous fungi (Nakatsu *et al.*, 2000). Some of the compounds that were isolated from thyme oil that possessed biological activity were thymol, carvacrol, borneol, *p*-cymene, α-pinene and camphene. All these compounds attributed to the activity shown by thyme oil (Nakatsu *et al.*, 2000, Panizzi *et al.*, 1993). 1,8-Cineole, a compound found in *Eucalyptus* oil and rosemary oil (*Rosmarinus officinalis*) (Nakatsu *et al.*, 2000) was found to have antifungal properties but no activity against the Gram-positive bacteria *S. aureus*. Eugenol, commonly found in clove (*Eugenia*) essential oil has been shown to inhibit yeast (Nakatsu *et al.*, 2000).

Table 23 shows which of these compounds were present in the essential oils of the active plants. We can thus speculate that the antimirobial properties of the essential oils are due to the presence of these compounds.

From **Table 18**, we can see that terpinen-4-ol was found to be active against *P. aeruginosa*. Terpinen-4-ol was one of the major compounds found in the essential oil of *Salvia africana-lutea*. This would further indicate that terpinen-4-ol is effective against *P. aeruginosa*. *Ocimum americanum, Salvia disermas* and *Salvia namaensis* all had linalool present and all were active against *C. albicans*. This indicates that linalool could be partially responsible for the activity against *C. albicans*. Another oil that showed activity against *C. albicans* was *M. longifolia* **(Figure 60)**. Both α-terpineol and linalool are present in the essential oil. The oils tested did not show much activity against *S. aureus*, even though they possessed some of the same compounds as those that were found in tea tree oil (Carson and Riley, 1995).

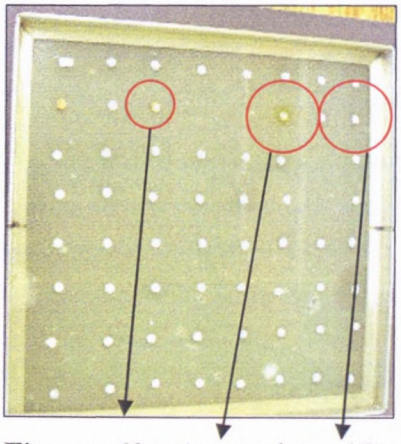


Figure 60: Agar plate (*C. albicans*) showing zones of inhibition by the oils tested.

6. CONCLUSION

Seven of the ten plant species showed some form of antimicrobial activity, either against the bacteria or against the fungi. *Salvia africana-lutea* was active against both *E. faecalis* and *P. aeruginosa*. One of the major compounds identified in the essential oil of *Salvia africana-lutea* was terpinen-4-ol. In studies conducted by Carson and Riley (1995) and Chao *et al.* (2000), terpinen-4-ol was found to show activity against *P. aeroginosa*. Two of the essential oils, *Mentha longifolia* and *Salvia namaensis*, both showed activity against *Klebsiella pneumoniae*. 1,8-cineole was one of the major identified compounds in both these oils. After comparing the results of Pattnaik *et al.* (1997) and Graven *et al.* (1992), to these results, it could be assumed that 1,8-cineole is responsible for the antimicrobial activity of *Mentha longifolia* and *Salvia namaensis*.

From the microbiological tests done in this study, it is clear that the plant species studied show better activity against the fungi. Five of the seven active oils showed activity against *C. neoformans* and against *C. albicans*, while two of the oils also showed activity against *A. niger*. According to a study carried out by Viollon and Chaumont (1994), the essential oils of lavender and clary sage exhibit anticryptococcal properties. Both these essential oils contain linalool and carvone, 35.5% and 8% respectively suggesting that both linalool and carvone could have anticryptococcal properties. Carvone was present in *Ocimum americanum* that showed good activity against *C. neoformans* while linalool was one of the major compounds in *Salvia disermas* that showed activity against *C. albicans*, thus further suggesting the antimicrobial properties of both carvone and linalool.

From the research carried out and the results that followed we have provided some evidence that compounds such as linalool, 1,8-cineol, carvone and terpinen-4-ol possess antimicrobial properties. Potencies of these essential oils may vary according to the percentage of essential oil, or the synergy that might exist amongst compounds.

The results further confirm the possible anti-infective properties of aromatic plants often used in traditional herbal preparations.

7. REFERENCES

Carsen, C.F. and Riley, T.V. 1995. Antimicrobial activity of the major components of the essential oil of *Melaleuca alternifolia*. Journal of Applied Bacteriology 78: 264-269.

Chao, S.C., Young, D.G. and Oberg, C.J. 2000. Screening for inhibitory activity of essential oils on selected bacteria, fungi and viruses. Journal of Essential Oil Research 12: 639-649.

Codd, L.E. 1985. Flora of Southern Africa: Part 4 Lamiaceae. Botanical Research Institute. Pretoria.

Conner, D.E. and Beuchat, L.R. 1984a. Sensitivity of heat-stressed yeasts to essential oils of plants. Applied Environmental Microbiology 47: 229-233.

Conner, D.E. and Beuchat, L.R. 1984b. Effects of essential oils from plants on growth of food spoilage yeasts. Journal of Food Science 49: 429-434.

Davidson, P.M. 1997. Chemical preservatives and natural antimicrobial compounds. Food microbiology fundamentals and frontiers. 520-556.

Denyer, S.P. and Hugo, W.B. 1991. Biocide induced damage to the bacterial cytoplasmic membrane. Mechanisms of action of chemical biocides; their study and exploitation. Oxford: Oxford Blackwell Scientific Publication. The Society for Applied Bacteriology, Technical Series No 27: 171-188.

Graven, E.H., Deans, S.G., Svoboda, K.P., Mavi, S. and Gundidza, M.G. 1992. Antimicrobial and antioxidative properties of the volatile (essential) oil of *Artemisia afra* Jacq. Flavour and Fragrance Journal 7: 121-123.

Griffin, S.G., Wyllie, S.G., Markham, J.L. and Leach, D.N. 1999. The role of structure and molecular properties of terpeniods in determining their antimicrobial activity. Flavour and Fragrance Journal 14: 322-332.

Koga, T., Hirota, N. and Takumi, K. 1999. Bactericidal activities of essential oils of Basil and Sage against a range of bacteria and the effect of these essential oils on *Vibrio parahaemolyticus*. Microbiological Research 3: 276-273.

Nakatsu, T., Lupo, A.T., Chinn, J.W. and Kang, R.K.L. 2000. Biological activity of essential oils and their constituents. Studies in Natural Products Chemistry 21: 571.

Panizzi, L., Flamini, G., Cioni, P.L. and Morelli, I. 1993. Composition and antimicrobial properties of essential oils of four Mediterranean Lamiaceae. Journal of Ethnopharmacology 3: 167-170.

Pattnaik, S., Subramanyam, V.R., Baoaji, M. and Kole, C.R. 1997. Antibacterial and antifungal activity of aromatic constituents of essential oils. Microbios 89: 39-46.

Peana, A.T., Moretti, M.D.L. and Juliano, C. 1999. Chemical composition and antimicrobial action of the essential oils of *Salvia desoleana* and *S. sclarea*. Planta Medica 65: 752-754.

Tassou, C., Koutsoumanis, K. and Nychas, G-J.E. 2000. Inhibition of *Salmonella enteritidis* and *Staphylococcus aureus* in nutrient broth by mint essential oil. Food Research International 33: 273-280.

Van Wyk, B-E., Van Oudtshoorn, B. and Gericke, N. 1997. Medicinal Plants of South Africa. Briza, Pretoriae.

Viollon, C. and Chaumont, J.P. 1994. Antifungal properties of essential oils and their main components upon *Cryptococcus neoformans*. Mycopathologia 128: 151-153.

Watt, J.M. and Breyer-Brandwijk, M.G. 1962. The Medicinal and Poisonous Plants of Southern and Eastern Africa.

Youdim, K.A. and Deans, S.G. 1999. Dietary supplementation of thyme (*Thymus vulgaris* L.) essential oil during the lifetime of the rat: its effects on the antioxidant status in liver, kidney and heart tissues. Mechanisms of Ageing Development 3: 163-175.