THE ANTIMICROBIAL PROPERTIES AND CHEMICAL COMPOSITION OF LEAF ESSENTIAL OILS OF INDIGENOUS *PLECTRANTHUS* (LAMIACEAE) SPECIES

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

Johannesburg, 2003

DECLARATION

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

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10th day of NOVEMBER, 2003

To my beloved Swami.

Thank you for the unconditional guidance, courage and love.

I dedicate this research report to my loving parents and family.

Thank you for all your love, support and understanding.

To the love of my life, Jeeteen.

You are my pillar of strength and love, Thank You.

ABSTRACT

Species of the genus *Plectranthus*, a member of the mint family (Lamiaceae), have been used in alternative medicines by third world countries dating back to the early Chinese empire.

Plectranthus species have been used in the past for coughs and colds (*P. ambiguus*) and as a mouth-wash for loose and bleeding teeth (*P. laxiflorus*). The crushed leaves of *P. madagascariensis* are used by the Xhosa as an ointment for scabies. *P. hadiensis* is used orally as a cough mixture.

Eight species from the genus were chosen to study the essential oil composition and antimicrobial activity. Antimicrobial assays incorporated four bacteria, two yeasts and one fungus. Disc diffusion assays on *Bacillus cereus* showed *P. venteri* to be one of the more active oils. Using gas chromatography-mass spectroscopy the essential oil composition was determined of the hydro-distilled oils. The major compounds identified in *P. venteri* were ρ-cymene (4.32%), linalool (0.77%) and limonene (1.04%). Other species exhibiting antimicrobial activity include *P. ciliatus*, *P. hadiensis* and *P. porphyranthus*. Identified compounds in these oils included germacrene D, bicyclogermacrene, spathulenol, α-fenchone, cubenol, T-cadinol, β-caryophyllene and cubenol.

Plectranthus ciliatus showed good results in the MIC/microplate assays with no growth occurring in any of the wells after 24 hours. Compounds identified in the oil included bicyclogermacrene (16.83%), spathulenol (15.52%) and germacrene D (9.37%). Comparatively the dark blue form *P. zuluensis* showed growth in all wells when tested against *B. cereus* and *P. grandidentatus* showed growth in all the wells when tested against *Staphylococcus epidermidis*.

It is evident that the essential oils that hail from indigenous *Plectranthus* species show antimicrobial activity, providing scientific evidence for their use in traditional herbal preparations.

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1. INTRODUCTION

Historically, plants have been a source of inspiration for novel drug compounds, as plant derived medicines have made a large contribution to human health and well-being. Pharmacognosy research is pivotal in the discovery of lead compounds for the development of new medicinal compounds.

The development of phytomedicines is represented by three generations. The first generation of plant drugs were usually simple botanicals employed in more or less their crude form. Several effective medicines used in their natural state such as *Cinchona*, *Opium*, *Belladonna* and *Aloe* were selected as therapeutic agents based on evidence of their clinical application. Following the industrial revolution, a second generation of plant based drugs emerged based on scientific processing of the plant extracts to isolate their "active constituents". The second generation of phytopharmaceutical agents were pure molecules and some of the compounds were even more pharmacologically active than their synthetic counterparts. Examples include quinine from *Cinchona* and reserpine from *Rauvolfia.* The development of third generation phytotherapeutic agents adopts a top-bottom approach. This consists of first conducting a clinical evaluation of the treatment modalities and therapy as administered by traditional doctors or as used by the community as folk medicine. This evaluation is then followed by acute and chronic toxicity studies in animals. Cytotoxicity studies should also be included. It is only if the substance has an acceptable safety index would it be necessary to conduct detailed pharmacological or biochemical studies.

Examples of plant derived drugs are presented below.

The isoquinolone alkaloid, emetine, obtained from the underground part of *Cephaelis ipecacuanha* has been used for many years as an amoebicidal drug as well as for the treatment of abscesses due to the spread of *Escherichia histolytica* infections. Another important drug of plant origin with a long history of use is quinine. This alkaloid occurs naturally in the bark of *Cinchona* trees. Apart from its continued usefulness in the treatment of malaria, it can also be used to relieve nocturnal leg cramps.

Other classical examples of drugs of plant origin include the analgesic morphine and the well-known antitussive codeine from *Papaver somniferum* (Papaveraceae), atropine from *Atropa belladonna* and other Solanaceae species, and the cardiac glycoside digoxin from *Digitalis* spp (Scrophulariaceae).

Some higher plants have also made important contributions in areas such as cancer therapies. Early examples include the antileukaemic alkaloids, vinblastine and vincristine, which were both obtained from the Madagascar periwinkle, *Catharanthus roseus*. Other anti-cancer agents include taxol, homoharringtonine and several derivatives of camphothein (Hostettmann and Marston 2000).

Almost no mouth wash or other dental product today contains a herbal ingredient (e.g. menthol) extracted as a natural product from members of the Lamiaceae. This family is one of the largest groups of plants included in the Angiosperms. Containing some 252 genera and over 6700 species (Leistner 2000), numerous members of this cosmopolitan family have been used in alternative medicines by third world countries dating back to the early Chinese Empire. Although these fragrant compounds are usually associated with "freshness", the scientific rationale for their inclusion in many toiletries and medicinal products are their known antimicrobial properties.

The diversity or distribution of members of this family can be quite widespread in some cases, while others exist only in restricted areas. Examples are *Glechoma hederacea* (ground ivy) which is widely distributed throughout the United States and Western Europe, while species such as *Hemiandra pungens* (snake bush) is endemic to the Australian continent. South Africa harbours a large number of species belonging to the Lamiaceae group of plants with the genus *Plectranthus* L'Herit being the largest representative in South Africa (Codd 1985). This aromatic genus known for its healing properties is represented by approximately 350 species distributed through Africa to Asia and Australia with over 40 species indigenous to southern Africa (Codd 1985). In South Africa *Plectranthus* occurs in or along forest margins of woodlands, while some species occur in drier regions on rocky outcrops or in open grassland. There is a large concentration of species in KwaZulu-Natal, east of the Drakensberg escarpment, where they are subject to a moist and warm climate (Rabe and van Staden 1997). *Plectranthus* species are annual or perennial herbs or subshrubs with herbaceous, semi-succulent or succulent stems and leaves.

An important use for many of the herbs found in this family is in their essential oils. These oils are used as fragrances in bath oils, soaps, potpourri, perfumes, candles and many other items requiring a fragrance. These oils are extracted by a method known as steam distillation.

Table 1. Medicinal uses of some *Plectranthus* species (Rabe and van Staden 1997).

Plant species	Traditional uses and route of administration
P. ambiguus (H.Bol.) Codd	Infusion of crushed leaves made with hot water, strained and sipped for colds.
P. ciliatus E.Mey.ex Benth.	Used for washing of persons, clothes and animal skins.
P. elegans Britten	Used as a remedy for sore throat.
P. hadiensis (Forssk.)	Infusions are used as sprinkling charms against evil spirits and are also administered as enemas but are not recommended for oral use as a cough mixture.
P. hirtus Benth.	A decoction of the root or an infusion of the leaf is used for cough and chest complaints. In the Grahamstown district the Xhosa rub the crushed leaf into the skin for scabies.
P. laxiflorus Bent.	Teas taken for coughs and colds. Root infusions used as a mouth-wash for loose and bleeding teeth. Zulus inject powdered leaf as an enema for feverishness and abdominal upsets. Powdered aerial parts also administered as an enema for influenzae. The Vhavenda use crushed leaves and stems as mosquito repellents and leaves for eye complaints.
P. madagascariensis (Pers.) Benth. var *madagascariensis*	Zulus take a decoction of the root or an infusion of the leaf for coughs and chest complaints. Xhosa use the crushed leaves rubbed into the skin for scabies.

According to Rabie and van Staden (1997), the Lamiaceae family has been well characterised with respect to their secondary metabolites, largely dominated by the flavonoids and terpenoids. They are a rich source of terpenoids possessing anti-insect, antibacterial and antifungal activity (Cole 1992). Glasby (1991) also reports that *Plectranthus* species that contain volatile oils in their leaves yield a wide range of diterpenoids possessing antibacterial activity (Batista *et al.* 1994, Batista *et al.* 1996, Dellar *et al.* 1996, Teixeira *et al.* 1997).

Rabie and van Staden (1997) also mentions that it is difficult to conclude whether the traditional medicinal uses of the species evaluated are supported by pharmacological effects, or merely based on folklore, as reported ailments are very generalized and their causative agents could cover a wide range of micro-organisms. Therefore, although these *Plectranthus* species do exhibit a certain degree of antibacterial activity, further investigation of their essential oils by gas chromatography (GC) and gas chromatography-mass spectroscopy (GC-MS) as mentioned by the authors is needed to identify the volatile compounds present and establish whether they contribute to the medicinal benefits of the plants. The objective of my study was aimed at recording for the first time the essential oil composition of some indigenous *Plectranthus* species using GC-MS. Once determining the animicrobial activity of the essential oils of these species, we would be able to provide a scientific rationale for the traditional use of these plants.

A recent study by Teixeira *et al.* (1997) shows that the whole plant acetone extract of *P. grandidentatus* yielded a variety of abietane derivatives that showed moderate antibacterial activity. Another study by Batista *et al.* (1996) using a bioautographic agar overlay assay with *S. aureus* as the indicator strain and abietane diterpenoids identified from *P. hereroensis*, showed that these compounds possess antibacterial activity. Fournier *et al.* (1986) describes sabinyl acetate, the major component of the essential oil of *P. fruticosus*, as teratogenic or highly foetotoxic.

Much of the exploration and utilization of natural products as antimicrobials arise from natural sources. It was the discovery of penicillin that led to later discoveries of antibiotics such as streptomycin, aureomycin and chloromycetin . Though most of the clinically used antibiotics are produced by soil micro-organisms or fungi, higher plants have also been a source of antibiotics. Examples of these are the bacteriostatic and antifungicidal properties of lichens or the antibiotic action of allinine in *Allium sativum* (garlic). Plant based antimicrobials represent a vast untapped source for potential medicines. Continued and

further exploration of plant antimicrobials needs to be encouraged. Phytochemicals may be effective in the treatment of infectious diseases while simultaneously mitigating many of side effects that are often associated with synthetic antimicrobials. Phytomedicines usually have multiple effects on the body and their actions often act beyond the symptomatic treatment of disease.

2. STUDY OBJECTIVES

1. To determine the antimicrobial properties of the essential oils distilled from indigenous *Plectranthus* species.

2. To record, for the first time, the essential oil composition of some indigenous *Plectranthus* species.

3. To provide a scientific rationale for the traditional use of *Plectranthus* 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:

Members of the genus *Plectranthus* are mostly abundant in KwaZulu-KwaZulu-KwaZulu-Natal and in areas of the Northern Province. Due to the complex floral morphology, the genus is known to be taxonomically problematic and for this reason collaboration was established with Mr. A Hankey, a taxonomist at the Witwatersand Botanical Garden. Mr. Hankey has an impressive collection of almost all South African *Plectranthus* species and the plant material was harvested from his collection for this study **(Figure 1)**. Plant material was also received from Mrs C. Potgieter from the University of KwaZulu-Natal.

The eight species investigated are given in Table 2. Duplicate samples were collected for *P. ciliatus* and *P. zuluensis*.

Table 2. *Plectranthus* species studied.

Species	Source/Locality
P. ciliatus	Ferncliff (ex C. Potgieter)
P. ciliatus	Witwatersrand Botanical Garden
P. fruticosus	Witwatersrand Botanical Garden
P. grandidentatus	Witwatersrand Botanical Garden
P. hadiensis	Witwatersrand Botanical Garden
P. neochilus	Witwatersrand Botanical Garden
P. porphyranthus	Witwatersrand Botanical Garden
P. venteri	Witwatersrand Botanical Garden
P. zuluensis (dark blue form)	Oribi Gorge (ex C. Potgieter)
P. zuluensis	Witwatersrand Botanical Garden
P. zuluensis (pale blue form)	Oribi Gorge (ex C. Potgieter)

3.2. Distilling of plant material:

The essential oils were extracted using conventional hydro-distillation in a Clevenger-type apparatus **(Figure 2)**. This was a three hour process. Due to the stability problems often encountered with essential oils, the samples were stored in amber vials and in a fridge.

3.3. Chemical analysis of essential oils:

3.3.1. Gas chromatography

The essential oils were analysed by gas chromatography (GC) using the following operating conditions; Shimadzu 17A gas chromatograph Column: JandW-DB1 (30m x 0.25mm id., 0.25μm film thickness), temperatures: injection port 230 °C, column 60 °C for 1 min., 5 °C min to 180 °C for 2 min., (total = 25 min). This method was used to determine any qualitative and quantitative variation with and among populations.

Samples were also analysed by gas chromatography-mass spectroscopy (GC-MS) and this part of the project was completed during my visit to 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 (60 m x 0.25 mm id., 0.25 μm film thickness), temperatures; injection port 250 °C, column 60 °C for 10 min., 4 °C / min. to 220 °C, 220 °C for 10 min., 1 °C / min. to 240 °C (total = 80 min.). Helium as carrier gas. 0.9 μL of hexane with 0.1 μL 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 three separate microbiological assays:

- Disc diffusion assays
- Minimum inhibitory concentration (MIC) / microplate method

3.4.1. 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), *Staphylococcus epidermidis* (ATCC 2223) and *Bacillus cereus* (ATCC 11778). A series of fungal test organisms; *Candida albicans* (ATCC 10231), *Cryptococcus neoformans* (ATCC 90112) and *Aspergillus niger* (clinical isolate). The disc diffusion method was employed whereby the bacterial cultures yielding an inoculum size of $1x10^6$ CFU/mL were incorporated into a suitable agar. Using aseptic manipulation, discs with plant essential oil were introduced onto the seeded agar plate **(Figures 3 and 4)**. Positive (discs impregnated with Neomycin) and negative controls were included for each test organism. The plates were incubated as follows:

- bacteria plates were incubated at 37 °C for 24 hours.
- mould plates were incubated at 25 °C for seven days.
- yeast plates were incubated at 37 °C for 48 hours.

Zones of inhibition were examined after 24 hours to seven days depending on the test organism.

3.4.2. Minimum inhibitory concentration (MIC using the microplate method)

Once antimicrobial activity was identified, a minimum inhibitory concentration was determined using the ρ-Iodonitrotetrazolium violet (INT) microplate method. This involved the dilution of the essential oil in microplate test conditions. 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. ρ-Iodonitrotetrazolium violet solution was then added to the wells to determine a colour change in relation to concentration of microbial growth. The MIC was then determined by observing the lowest concentration having no microbial growth.

Staphylococcus aureus, Psuedomonas aeruginosa, Escherichia coli, Enterococcus faecalis, Candida albicans, Staphylococcus epidermidis (ATCC), *Bacillus cereus* (ATCC) and *Cryptococcus neoformans* were inoculated in a suitable broth (Mueller Hinton/Tryptone Soya broth). They were incubated at 37 °C for 24 hours. Inoculum was transferred into 10 mL sterile broth and incubated at 37 °C for four hours (Mc Farlands standard). Samples were prepared so that a starting concentration of 32 mg/mL was obtained in the first well. Micro titre plates were then aseptically prepared:

- 100 µL sterile water was placed in all wells.
- 100 µL extract sample was placed in rows A1-A12 (**Figure 5**).
- serial dilutions of 100 µL were performed from A1 to B1 and B1 to C1 etc.

Micro titre wells were moved to the microbiology laboratory and 100 µL of culture was added to all wells. The plates were incubated at 37 °C for 24 hours. A 0.2 mg/mL INT solution was prepared. An amount of 40 µL of prepared INT solution was added into all inoculated wells. The plates were examined after 30, 60, 120 min and 24 hours.

3.4.3. Scanning electron microscopy (SEM)

Plant material was collected and small pieces of plant material (leaves and stems) were fixed in 2.5 % glutaraldehyde in 0.075 M phosphate buffer at pH 7.4 – 7.6 for one hour. The material was rinsed in 0.075 M phosphate buffer three times for five minutes each, and then

postfixed in 1 % aqueous osmium tetroxide for two hours after which they were 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. They were mounted on aluminium stubs using doubled-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.

Figure 1: Collecting *P. hadiensis* at Witwatersrand Botanical Garden.

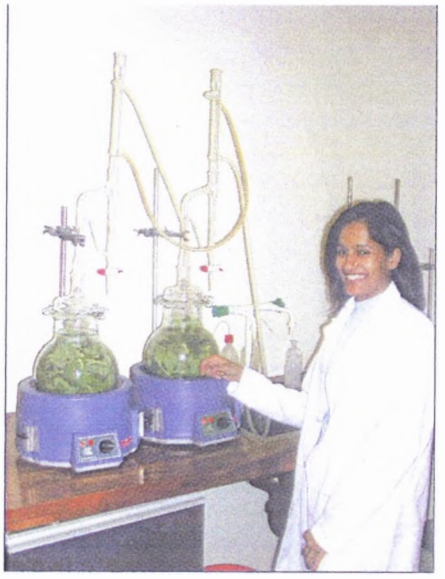


Figure 2: Hydro-distillation of plant material using the Clevenger apparatus.

Figure 3: Discs being saturated with essential oil samples.

Figure 4: Discs being placed onto agar plates.

Figure 5: Aseptic preparation of micro titre wells.

4. SPECIES MONOGRAPHS

4.1. *Plectranthus ciliatus* E. Mey

Botanical description:

This is a soft, branched herb up to 0,6 m tall. The stems are decumbent to ascending. The leaves are petiolate, the blade being thin to thickish and rugose in texture. The leaves are broadly elliptic ovate with the under-surface usually suffused with purple as well as honey-coloured gland-dots. The leaf margin is regular and shallowly crenate-dentate. Flowers form in sessile, 3-flowered cymes. The corolla has a whitish background freely speckled with purple (Codd 1985).

Distribution:

Distribution extends from Uniondale and Knysna in the Cape, along the semi-coastal areas of the eastern Cape and Mpumalanga to KwaZulu-Natal, Swaziland and the mountains of eastern Mpumalanga; common in forests and moist, shady places.

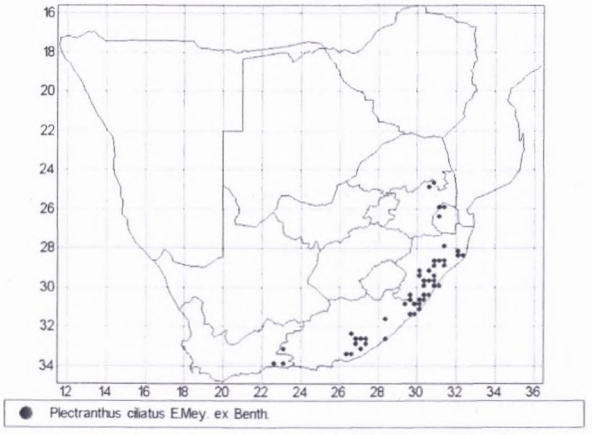
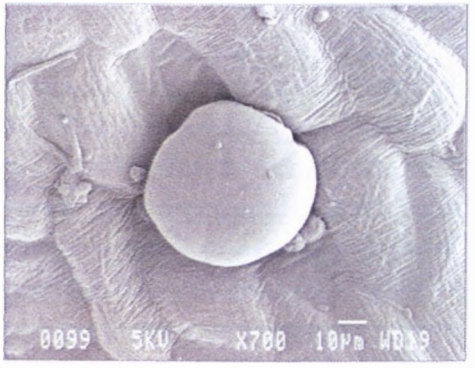
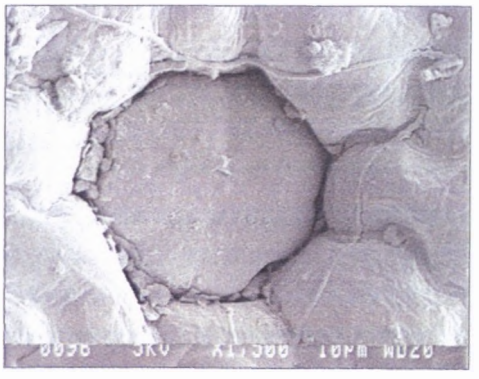


Figure 6: Geographical distribution of *Plectranthus ciliatus*.

Figure 7: *Plectranthus ciliatus* in flower.





Figures 8 and 9: Scanning electron micrograph showing the oil glands on the abaxial leaf surface.

Essential oil composition:

Essential oil was obtained by hydro-distillation (3 hours). The oil yield was:

1. *Plectranthus ciliatus* ex Ferncliff: 0.08%
2. *Plectranthus ciliatus ex hort* Witwatersrand Botanical Garden: 1.0%

GC-MS profile and analysis:

1. *Plectranthus ciliatus* ex Ferncliff

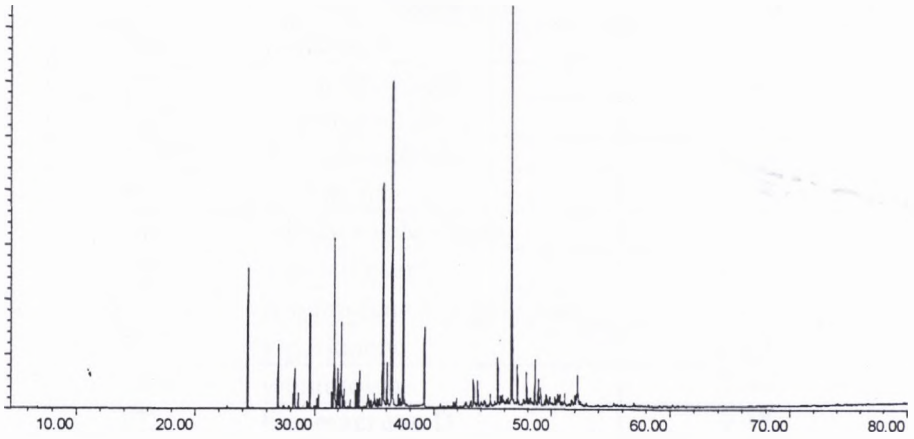


Figure 10: GC-MS profile of the hydro-distilled essential oil obtained from *Plectranthus ciliatus* ex Ferncliff.

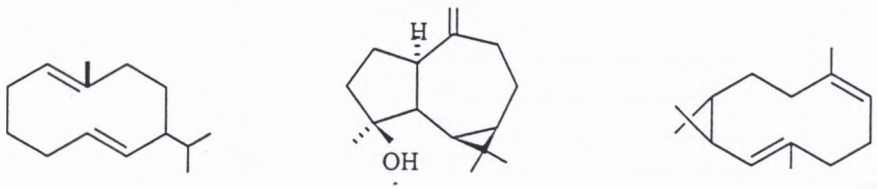
Table 3: Compounds identified in the essential oil of *Plectranthus ciliatus*.

RRI	Compound	Area percentage
1118	β-pinene	0.01
1132	Sabinene	0.03
1203	Limonene	0.04
1213	1,8-cineole	0.01
1280	ρ-cymene	0.02
1345	3-octyl acetate	0.07
1386	1-octanyl acetate	4.25
1393	3-octanol	0.03
1452	1-octen-3-ol	1.83
1466	α-cubebene	0.10
1474	*trans* sabinene hydrate	0.02
1479	δ-elemene	0.09
1495	Bicycloelemene	0.43
1493	α-ylangene	1.23
1497	α-copanene	0.48
1528	α-bourbonene	0.22

RRI	Compound	Area percentage
1535	β-bourbonene	3.08
1544	α-gurjunene	0.08
1545	*cis*-α-bergamotene	0.08
1549	β-cubebene	0.28
1553	Linalool	0.30
1568	1-methyl-4-acetyl-cyclohex-1-ene	0.04
1571	*trans*-ρ-menth-2-en-1-ol	0.01
1572	β-ylangene	0.51
1597	Bornyl acetate	5.48
1594	*trans*-β-bergamotene	0.34
1600	β-elemene	1.16
1612	β-caryophyllene	2.64
1617	6, 9 guaiadiene	0.61
1628	Aromadendrene	0.20
1650	γ-elemene	0.48
1661	Alloaromadendrene	1.12
1668	*Z*-β-farnesene	0.03
1687 +1684	α-humulene + β-guaiaene	0.55
1674	γ-gurjunene	0.20
1704	γ-muurolene	0.40
1726	**Germacrene D**	**9.37**
1741	β-bisabolene	1.32
1755	**Bicyclogermacrene**	**16.83**
1773	δ-cadinene	0.33
1776	γ-cadinene	0.25
1784	*E*-α-bisabolene	5.97
1819	Aromadendra-4, 10(14)-diene	0.24
1854	Germacrene B	2.60
1900	Epi-cubebol	0.10
1941	α-calacorene 1	0.03
1945	15 epoxy-salvial-4 (14)-ene	0.16
1953	Palustrol	0.04
1957	Cubebol	0.27
2001	Iso-caryophyllene oxide	0.14
2008	Caryophyllene oxide	1.13
2033	Epiglobulol	0.80
2037	Salvial 4 (14)-en-1-one	0.12
2057	Ledol	0.11
2063	Germacrene D 1,10 epoxide	0.09
2069	Germacrene D-4-ol	0.46
2096	Elemol	1.52
2104	Viridiflorol	0.27
2144	**Spathulenol**	**15.52**
2209	T-muurolol	0.22
2232	α-bisabalol	1.33

RRI	Compound	Area percentage
2247	*trans*-α-bergamotol	0.86
2255	α-cadinol	0.66
	TOTAL	**87.19**

Sixty-two compounds were identified in the essential oil of *Plectranthus ciliatus*. Bicyclogermacrene, a sesquiterpene, contributes 16.83% of the total composition of the oil. The three sesquiterpenes; spathulenol (15.52%), germacrene D (9.37%) and *E*-α-bisabolene (5.97%) collectively constitute 31%.



germacrene D spathulenol bicyclogermacrene

Figure 11: Chemical structures for the major compounds identified in the essential oil of *Plectranthus cilatus* ex Ferncliff.

2. *Plectranthus ciliatus ex hort* Witwatersrand Botanical Garden

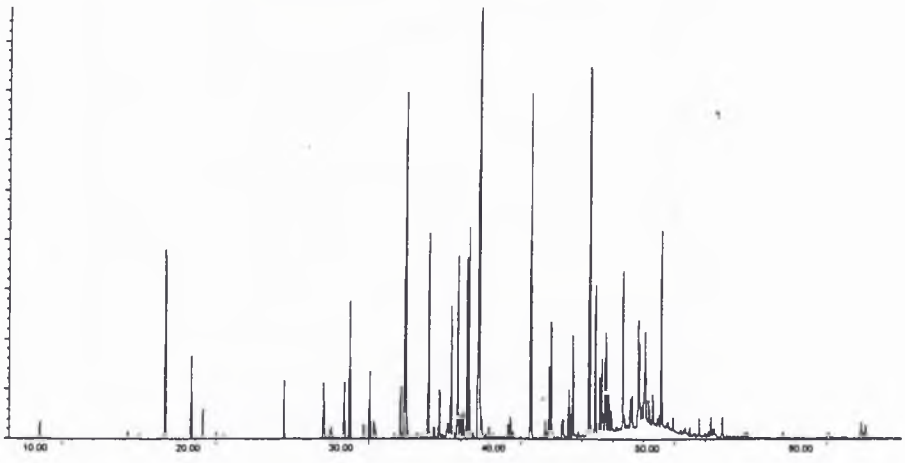


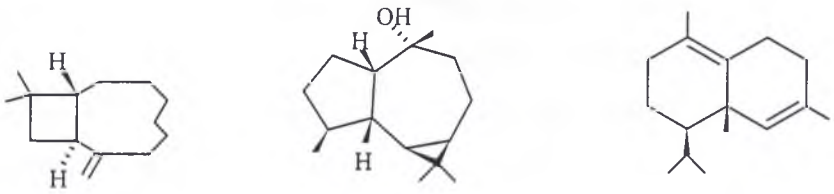
Figure 12: GC-MS profile of the hydro-distilled essential oil obtained from *Plectranthus cilatus ex hort* Witwatersrand Botanical Garden.

Table 4: Compounds identified in the essential oil of *Plectranthus ciliatus*.

RRI	Compound	Area percentage
1032	α-pinene	0.26
1118	β-pinene	0.01
1132	Sabinene	0.01
1159	δ-*Z*-carene	0.12
1174	Myrcene	0.09
1205	Sylvestrene	0.02
1203	Limonene	3.78
1246	*Z*-β-ocimene	1.27
1255	γ-terpinene	0.02
1266	*E*-β-ocimene	0.42
1278	m-cymene	0.01
1280	ρ-cymene	0.12
1290	Terpinolene	0.07
1305	*Z*-1, 5-octadien-3-ol	0.01
1319	*E*-2, 6-diene-1, 3, 7-nonatriene	0.01
1382	*cis*-allocymene	0.02
1386	1-octenyl acetate	0.76
1412+1415	*E*-2-Hexen-1-ol + Rosefuran	0.03
1452	α-ρ-dimestyrene	0.01
1452	1-octen-3-ol	1.11
1466	α-cubebene	0.15
1474	*trans*-sabinene hydrate	0.23
1476	*Z*-β-ocimene epoxide	0.01
1495	Bicycloelemene	0.07
1492	Cyclosativene	0.90
1497	α-copaene	2.25
1528	α-bourbonene	0.01
1544	α-gurjunene	0.99
1549	β-cubebene	0.26
1553	Linalool	0.21
1544	α-gurjunene	0.11
1571	*trans*-ρ-menth-2-en-1-ol	0.03
1572	β-ylangene	0.06
1597	Bornyl acetate	0.10
1600	β-elemene	0.81
1612	**β-caryophyllene**	**9.29**
1617	*Z*-9-Guaiadiene	0.12
1628	Aromadendrene	0.05
1639	*trans*-ρ-menth-2, 8-dien-1-ol	0.06
1650	γ-elemene	0.02
1661	Alloaromadendrene	3.82
1677	Epizonarene	0.16
1687	α-humulene	0.86

RRI	Compound	Area percentage
1704	γ-muurolene	0.26
1706	α-terpineol	0.17
1708	Ledene	2.09
1726	Germacrene D	3.10
1740	α-muurolene	0.38
1747	*trans*-carvyl acetate	2.54
1755	Bicyclogermacrene	3.54
1773	**δ-cadinene**	**11.77**
1782	*cis*-carvyl acetate	0.34
1799	Cadina-1, 4-diene (cubenene)	0.15
1810	3, 7-guaiadiene	0.08
1830	2, 6-dimethyl-3*E*, 5*E*, 7 Octatriene-2-ol	0.05
1845	*trans*-carveol	0.22
1853	*cis*-calamenene	0.39
1864	ρ-cymene-8-ol	0.10
1871	ρ-mentha-1, 8-dien-10-yl acetate	0.01
1882	*cis*-carveol	0.04
1900	epi-cubebol	6.19
1941	α-calacorene	0.26
1953	Palustrol	1.07
1957	Cubebol	1.95
1984	γ-calacorene	0.27
2008	Caryophyllene oxide	1.50
2057	**Ledol**	**7.42**
2069	Germacrene D-4-ol	2.24
2080	Cubenol	0.93
2088	1-epi-cubenol	1.18
2104	Viridiflorol	0.55
2144	Spathulenol	2.23
2187	T-cadinol	3.38
2202	1(10), 6 Germacradien-5α-ol	0.15
2219	δ-cadinol	0.68
2209	T-muurolol	1.26
2247	*trans*-α-bergamotol	0.16
2255	α-cadinol	2.99
2324	Caryophylladienol 2	0.06
2438	Kaur-16-ene	0.07
2607	14-OH-δ-Cadinene	0.02
2676	Epi-13-manool	0.37
	TOTAL	**88.89**

δ-cadinene is the major essential oil compound contributing 11.77% of the total. The sesquiterpenes β-caryophyllene and ledol represent 9.3% and 7.42% respectively.



β-caryophyllene Ledol δ-cadinene

Figure 13: Chemical structures for major compounds identified in the essential oil of *Plectranthus cilatus ex hort* Witwatersrand Botanical Garden.

In essence the two samples of *P. ciliatus* differ in their essential oil composition. Both oils have different major compounds and this can be indicative of the two different areas that they were obtained from i.e. Ferncliff and Witwatersrand Botanical Garden.

4.2. *Plectranthus fruticosus* L'Herit

Botanical description:

This is a soft shrub 0,6-2 m tall, freely branched with fibrous roots. The leaves are petiolate, broadly ovate to ovate-elliptic shaped. They are sparingly pubescent or glandular-hispidulous. The under-surface is covered with honey-coloured gland-dots and usually suffused with purple. The margins are often regularly crenate-dentate. Flowers are formed in sessile 3-flowered cymes. The corolla is bluish mauve, rarely pink or pale blue, 5-13 mm long narrowing slightly towards the throat (Codd 1985).

Distribution:

The distribution extends from the Caledon district in south-western Cape along the semi-coastal southern and eastern Cape to Mpumalanga, eastern KwaZulu-Natal, Swaziland and the mountains of eastern, central and northern Mpumalanga. Common in forest, scrub forest and shady places among rocks.

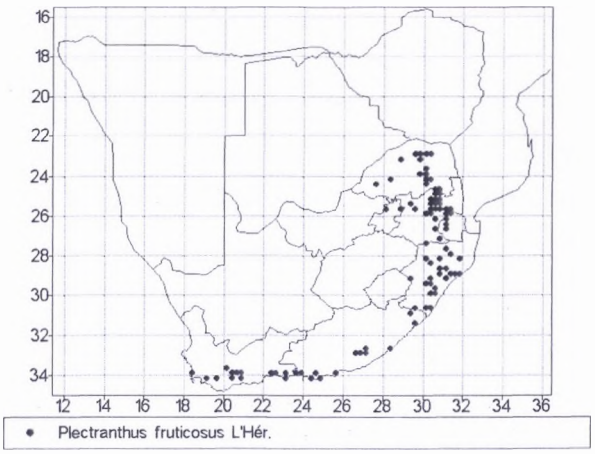
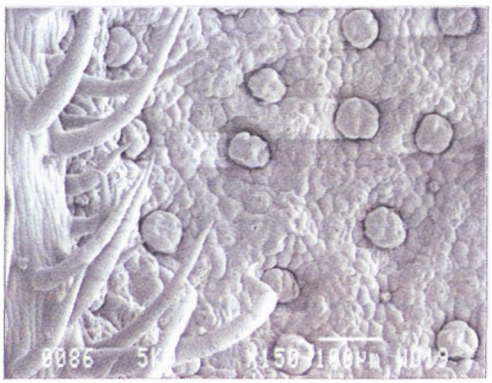


Figure 14: Geographical distribution of *Plectranthus fruticosus*.

Figure 15: Flowers of *Plectranthus fruticosus*.



Figures 16 and 17: Scanning electron micrograph of the abaxial leaf surface showing conspicuous oil glands.

Essential oil composition:

Essential oil was obtained by hydro-distillation (3 hours). The oil yield was 0.02%.

GC-MS profile and analysis:

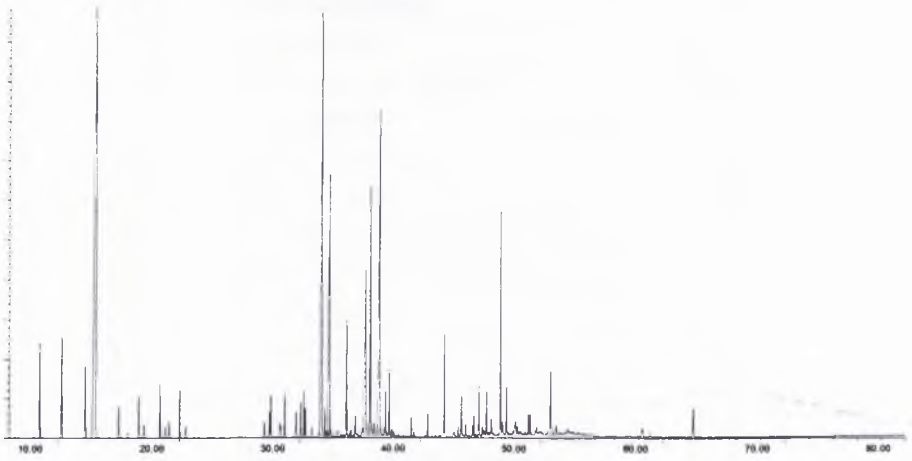


Figure 18: GC-MS profile of the hydro-distilled essential oil obtained from *Plectranthus fruticosus*.

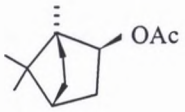
Table 5: Compounds identified in the essential oil of *Plectranthus fruticosus*.

RRI	Compound	Area percentage
1014	Tricyclene	0.05
1032	α-pinene	1.65
1035	α-thujene	0.17
1076	Camphene	2.21
1118	β-pinene	1.68
1132	**Sabinene**	**20.28**
1159	δ-3-carene	0.01
1174	Myrcene	0.53
1183	Pseudolimonene	0.01
1188	α-terpinene	0.08
1203	Limonene	0.73
1213	1,8-cineole	0.06
1218	β-phellandrene	0.23
1246	*Z*-β-ocimene	0.91
1266	*E*-β-ocimene	0.26
1280	ρ-cymene	0.79
1290	Terpineolene	0.19
1382	*cis*-allocymene	0.01
1386	Octanyl acetate	0.01
1393	3-octanol	0.02
1415	Rosefuran	0.02
1451	3-thujone	0.01

RRI	Compound	Area percentage
1452	1-Octen-3-ol	0.35
1466	α-cubenene	0.44
1474	*trans*-sabinene hydrate	0.68
1482	*Z*-3-Hexenyl-2-Mebutyrate	0.01
1495	Bicycloelemene	0.25
1493	α-ylangene	0.19
1498	*E*-β-ocimene epoxide	0.01
1497	α-copanene	0.75
1528	α-bourbonene	0.03
1535	β-bourbonene	0.52
1556	*cis*-sabinene hydrate	0.49
1571	*trans*-ρ-menth-2-en-1-ol	0.18
1545	α-bergamotene	0.03
1572	β-ylangene	0.09
1597	**Bornyl acetate**	**12.81**
1600	β-elemenene	0.46
1783	Bicyclosesquiphellandrene	0.10
1611	Terpinen-4-ol	3.21
1612	**β-caryophyllene**	**4.88**
1617	6, 9-guaiadiene	0.13
1628	Aromadendrene	0.05
1638	*cis*-ρ-menth-2-en-1-ol	0.09
1642	Thuj-3-en-10-ol	0.01
1650	γ-elemene	0.05
1661	Alloaromadendrene	2.08
1668	*Z*-β-farnesene	0.07
1695	*E*-β-farnesene	0.01
1740	α-fuurolene	0.44
1706	α-terpineol	0.09
1708	Ledene	1.67
1719	Borneol	3.04
1726	**Germacrene D**	**5.51**
1741	β-bisabolene	0.31
1755	**Bicyclogermacrene**	**9.04**
1773	δ-cadinene	0.68
1776	γ-cadinene	0.06
1783	β-sesquiphellendrene	1.11
1799	Cadina-1,4-diene	0.03
1807	Perilla aldehyde	0.04
1814	ρ-mentha-1, 5-diene-7-ol	0.03
1815	2, 6-diene-3-*E*, 5-*E*, 7-Octatriene-2-ol	0.03
1854	Germacrene-B	0.35
1864	ρ-cymene-8-ol	0.08
1900	Epicubebol	0.38
1941	α-calacorene	0.04
1953	Palustrol	0.21

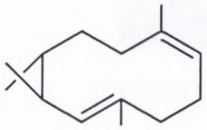
RRI	Compound	Area percentage
1957	Cubebol	1.70
1984	γ-calacorene	0.08
2001	Isocaryophyllene oxide	0.18
2008	Caryophyllene oxide	0.80
2037	Salvial-4 (14)-en-1-one	0.04
2050	*E*-Nerolidol	0.17
2057	Ledol	0.33
2069	Germacrene-D-4-ol	0.90
2088	1-epi-cubenol	0.08
2103	Guaiol	0.76
2104	Viridiflorol	0.05
2144	**Spathulenol**	**4.05**
2187	T-cadinol	0.04
2202	1 (10), 5-Germacradiene-5α-ol	0.34
2209	T-muurolol	0.14
2247	*trans*-α-bergemotol	0.32
2255	α-cadinol	0.35
2676	Epi-13-manool	0.89
	TOTAL	**92.47**

Sabinene is the major compound (20.28%) identified in the essential oil. Bornyl acetate contributes 12.81% and bicyclogermacrene 9.04% to the total essential oil composition.

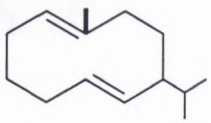


bornyl acetate

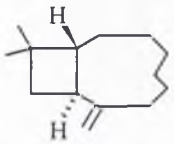
sabinene



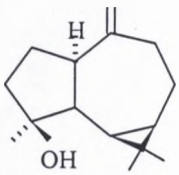
bicyclogermacrene



germacrene D



β-caryophyllene



spathulenol

Figure 19: Chemical structures for major compounds identified in the essential oil of *Plectranthus fruticosus*.

4.3. *Plectranthus grandidentatus* Gürke

Botanical description:

A perennial semi-succulent procumbent herb. Stems grow up to 2 m long, leaves fairly broad ovate in shape, densely pubescent with the under-surface covered with red to brownish gland dots. The flowers occur in 3-6 flowered sessile cymes. The corolla is white, finely pubescent and gland-dotted (Codd 1985).

Distribution:

These plants are distributed from the Soutspansberg to Mpumalanga, Swaziland, KwaZulu-Natal Midlands and Eastern Cape to Queenstown growing in relatively dry, rocky places in open woodland.

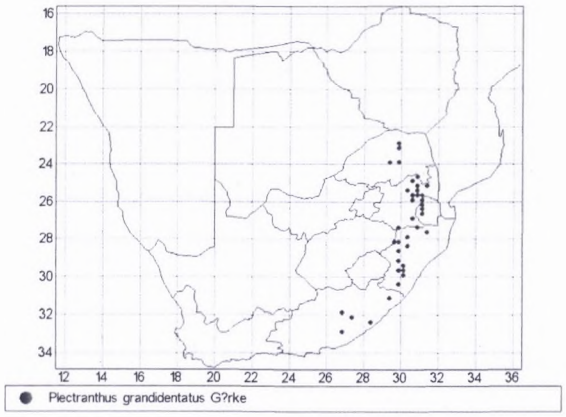
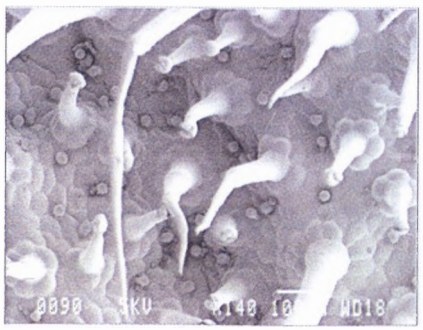
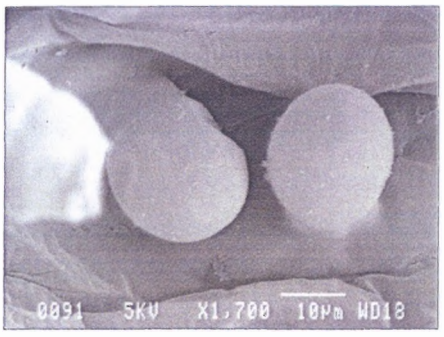


Figure 20: Geographical distribution of *Plectranthus grandidentatus*.

Figure 21: The inflorescence of *Plectranthus grandidentatus*.





Figures 22 and 23: Scanning electron micrograph displaying glandular hairs and oil glands on the abaxial leaf surface of *Plectranthus grandidentatus*.

Essential oil composition:

Essential oil was obtained by hydro-distillation (3 hours). The oil yield was 0.07%.

GC-MS profile and analysis:

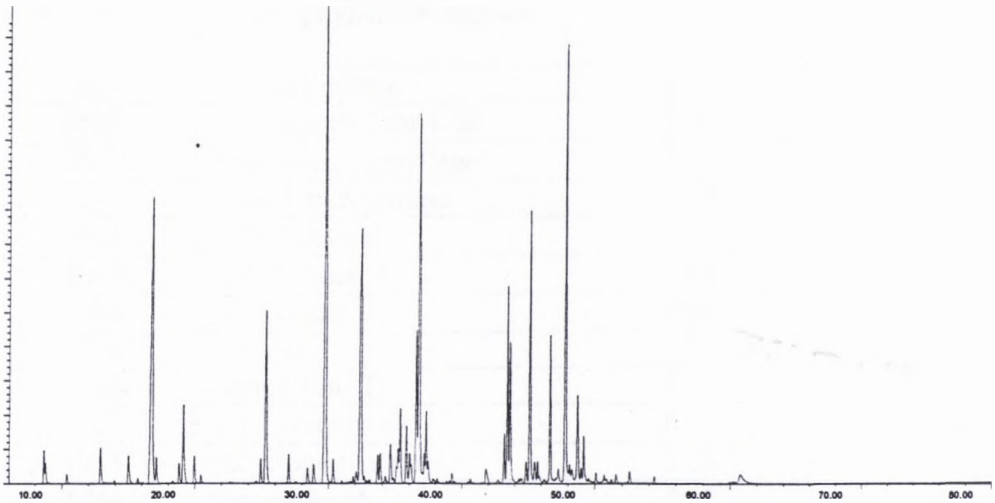


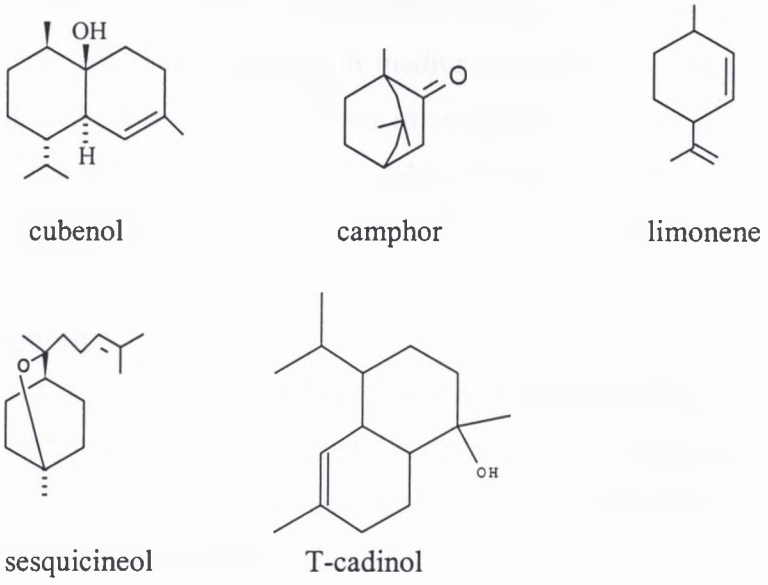
Figure 24: GC-MS profile of the hydro-distilled essential oil obtained from *Plectranthus grandidentatus*.

Table 6: Compounds identified in the essential oil of *Plectranthus grandidentatus*.

RRI	Compound	Area percentage
1032	α-pinene	0.56
1035	α-thujene	0.38
1076	Camphene	0.19
1132	Sabinene	0.84
1176	α-phellandrene	0.74
1188	α-terpinene	0.12
1203	**Limonene**	**9.43**
1218	β-phellandrene	0.54
1246	*Z*-β-ocimene	0.05
1255	γ-terpinene	0.44
1266	*E*-β-ocimene	1.89
1280	ρ-cymene	0.60
1290	Terpinolene	0.18
1391	*Z*-3-hexene-1-ol	0.04
1393	3-octanol	0.45
1406	α-fenchone	4.26
1452	1-octen-3-ol	0.55
1495	Bicycloelemene	0.32

RRI	Compound	Area percentage
1505 + 1497	Dihydroedulane 2 + α-copanene	0.45
1532	**Camphor**	**15.19**
1553	Linalool	0.49
1571	*trans*-ρ-menth-2-en-1-ol	0.04
1594	*trans*-β-bergamotene	0.13
1600	β-elemene	0.27
1612+1611	β-caryophyllene + terpinen-4-ol	7.71
1628	Aromadendrene	0.12
1638	*cis*-ρ-menth-2-en-1-ol	0.06
1655	9-epi-β-caryophyllene	0.62
1661	Alloaromadendrene	0.62
1668	*E*-β-farnesene	0.08
1687	α-humulene	0.95
1706	α-terpineol	0.64
1719	Borneol	1.54
1726	Germacrene D	1.17
1737	*Z-E*-α-farnesene	0.51
1755	Bicyclogermacrene	3.28
1764	**Sesquicineol**	**9.17**
1773	δ-cadinene	0.35
1776	γ-cadinene	1.21
1784	*E*-α-bisabolene	0.45
1810	3,7 guaiadiene	0.08
1845	*trans*-carveol	0.05
1853	*cis*-calamenene	0.19
1864	ρ-cymene-8-ol	0.04
2001	Isocaryophyllene oxide	0.97
2008	Caryophyllene oxide	3.91
2050	*E*-nerolidol	0.04
2051	Gleenol	0.03
2069	Germacrene D-4-ol	0.52
2080	**Cubenol**	**5.56**
2098	Globulol	0.46
2104	Viridiflorol	0.32
2144	Spathulenol	2.66
2170	β-bisabulol	0.29
2187	**T-cadinol**	**10.26**
2209	T-muurolol	0.17
2232	α-bisabolol	1.86
2247	*trans*-α-bergamotol	0.17
2255	α-cadinol	0.70
2324	Caryophyllandienol-2	0.11
2389	Caryophyllenol-1	0.18
2392	Caryophyllenol-2	0.25
	TOTAL	**95.45**

Sixty-one compounds were identified in the hydro-distilled essential oil. The most abundant compound is camphor representing 15.19%. T-cadinol makes up 10.26% while the monoterpene, limonene represents 9.43%.



cubenol camphor limonene

sesquicineol T-cadinol

Figure 25: Chemical structures for major compounds identified in the essential oil of *Plectranthus grandidentatus*.

4.4. *Plectranthus hadiensis* Forssk

Botanical description:

A perennial, semi-succulent herb with erect to decumbent stems that are sparsely to densely tomentose. Petiolate leaves with medium to thick textured blades, ovate to subrotund and gland-dotted. Flowers are formed in sessile 4-15 flowered cymes. The corolla is usually shades of mauve to purple, rarely white. It is 7-13 mm long, finely pubescent and gland-dotted on the lips (Codd 1985).

Distribution:

Abundant in the midlands and semi-coastal KwaZulu-Natal. Also found in the mountainous parts of Mpumalanga, among rocks in dry woodland or on exposed rocky places in grassland where it is subjected to periodic burning. It extends through east tropical Africa to the southern Arabian Peninsula.

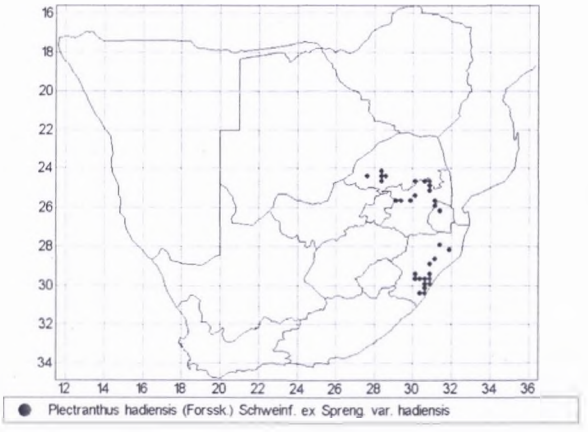


Figure 26: Geographical distribution of *Plectranthus hadiensis*.

Figure 27: *Plectranthus hadiensis*.

Figures 28 and 29: Scanning electron micrograph of the abaxial surface of the leaf showing oil glands.

Essential oil composition:

Essential oil was obtained by hydro-distillation (3 hours). The oil yield was 0.045%.

GC-MS profile and analysis:

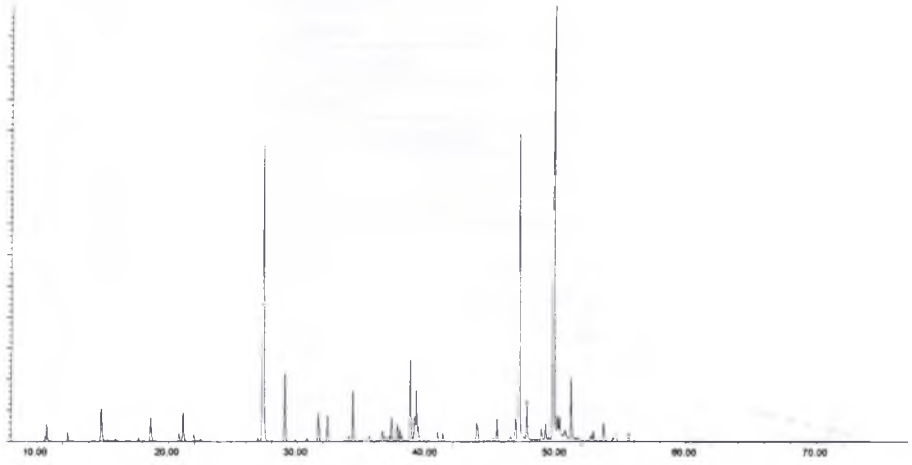
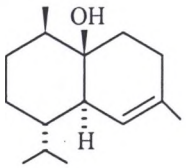


Figure 30: GC-MS profile of hydro-distilled essential oil obtained from *Plectranthus hadiensis*.

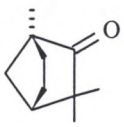
Table 7: Compounds identified in the essential oil of *Plectranthus hadiensis*.

RRI	Compound	Area percentage
1032	α-pinene	0.16
1035	α-thujene	0.65
1076	Camphene	0.39
1132	Sabinene	1.64
1174	Myrcene	0.06
1188	α-terpinene	0.15
1203	Limonene	1.17
1255	γ-terpinene	0.37
1266	*E*-β-ocimene	1.40
1280	ρ-cymene	0.34
1290	Terpinolene	0.10
1393	3-octanol	0.15
1406	**α-fenchone**	**17.46**
1452	1-octen-3-ol	2.87
1497	α-copaene	0.20
1532	Camphor	1.34
1553	Linalool	1.10
1600	β-elemene	0.23
1611+1612	Terpinen-4-ol + β-caryophyllene	2.32
1628	Aromadendrene	0.08
1706	α-terpineol	0.22

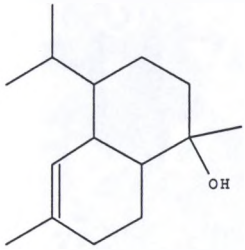
RRI	Compound	Area percentage
1719	Borneol	0.99
1726	Germacrene D	0.74
1737	*Z-E*-α-farnesene	0.45
1773	α-cadinene	1.13
1776	γ-cadinene	2.32
1853	*cis*-calamanene	0.35
1931	Shyabunol	0.71
2008	Caryophyllene oxide	1.03
2051	Gleenol	0.07
2069	Germacrene-D-4-ol	1.28
2080	**Cubenol**	**15.08**
2104	Viridiflorol	1.60
2187	**T-cadinol**	**26.86**
2209	T-muurolol	0.95
2255	α-cadinol	2.45
2320	14-nor-cadin-5-en-4-one-isomer A/B	0.42
2357	14-hydroxy-β-caryophyllene	0.79
2438	Kaur-16-ene	0.38
	TOTAL	**89.82**



cubenol



fenchone



T-cadinol

Figure 31: Chemical structures for major compounds identified in the essential oil of *Plectranthus hadiensis*.

4.5. *Plectranthus neochilus* Schltr

Botanical description:

Perennial or sometimes annual, decumbent to erect, often much branched and bushy, succulent herb growing to about 0.12-0.5 m tall. The stems are sparingly to densely villous and the roots are sometimes tuberous. The leaves are obovate to elliptic-ovate in shape, pubescent and covered with orange gland-dots on the under-surface. Flowers form 3-flowered sessile cymes. The calyx is 6 mm long in fruit while the corolla is mauve to purple and 12-20 mm long (Codd 1985).

Distribution:

This plant is recorded from Zambia, Zimbabwe, northern Namibia, Botswana, central and eastern Mpumalanga, Swaziland, KwaZulu-Natal Midlands and Eastern Cape as far south as the Albany district; usually under trees in open woodland and among rocks (especially dolomite) in grassland.

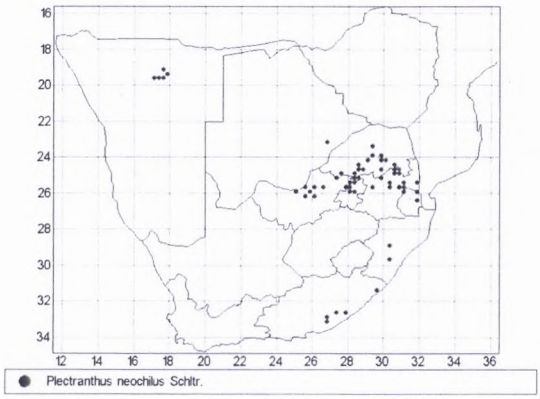
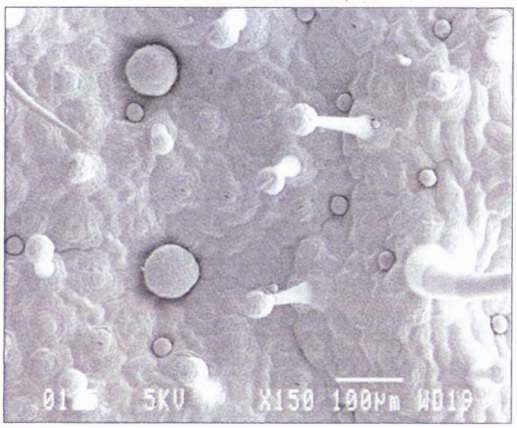
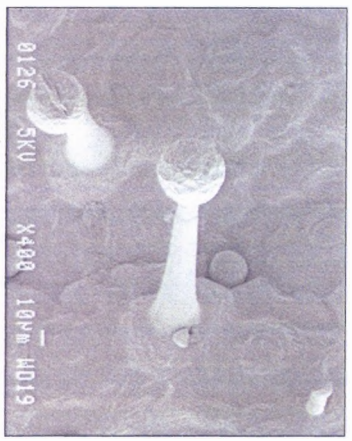


Figure 32: Geographical distribution of *Plectranthus neochilus*.

Figure 33: *Plectranthus neochilus* in flower.





Figures 34 and 35: Scanning electron micrograph of the abaxial surface of the leaf showing the stalked and sessile oil glands.

Essential oil composition:

Essential oil was obtained by hydro-distillation (3 hours). The oil yield was 0.008%.

GC-MS profile and analysis:

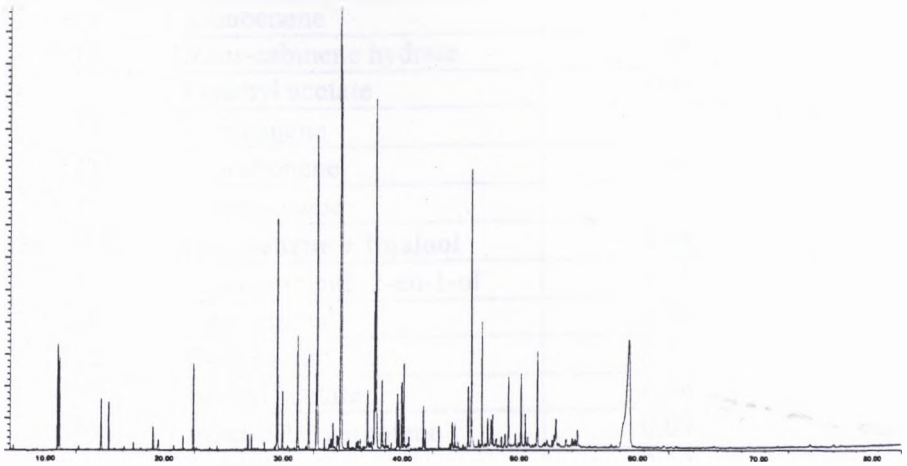


Figure 36: GC-MS profile of hydro-distilled essential oil obtained from *Plectranthus neochilus*.

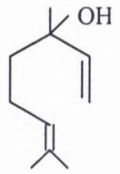
Table 8: Compounds identified in the essential oil of *Plectranthus neochilus*.

RRI	Compound	Area percentage
1032	α-pinene	1.95
1035	α-thujene	1.54
1076	Camphene	0.09
1118	β-pinene	1.08
1132	Sabinene	0.97
1159	δ-z-carene	0.08
1174	Myrcene	0.15
1203	Limonene	0.42
1213+1218	1,8 cineol + β-phellandrene	0.27
1246	*Z*-β-ocimene	0.08
1255	γ-terpinene	0.02
1266	*E*-β-ocimene	0.25
1265	5-methyl-3-heptanone	0.04
1280	ρ-cymene	1.53
1285	Isoamyl isovalerate	0.05
1319	*E*-2, 6-dimethyl-1, 3, 7-nonatriene	0.02
1327	*Z*-3-hexenyl acetate	0.08
1360	1-hexanol	0.03
1384	α-pinene oxide	0.03
1391	*Z*-3-hexen-1-ol	0.27
1393	3-octanol	0.25

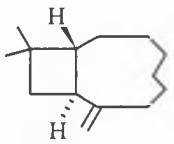
RRI	Compound	Area percentage
1406	α-fenchone	0.02
1415	Rosefuran	0.04
1451	β-thujone	0.05
1452	1-octen-3-ol	4.13
1466	α-cubenene	1.05
1474	*trans*-sabinene hydrate	0.02
	Fenchyl acetate	0.09
1497	α-copanene	2.07
1528	α-borbonene	0.08
1535	β-boubonene	1.71
1549+1553	**β-cubenene + linalool**	**6.98**
1571	*trans*-ρ-menth-2-en-1-ol	0.21
1586	Pinocarvone	0.09
1572	β-ylangene	0.24
1597	Bornyl acetate	0.44
1594	*trans*-β-bergamotene	0.09
1600	β-elemene	0.22
1611+1612	**Terpinen-4-ol** + β-**caryophyllene**	**15.58**
1638	*cis*-ρ-menth-2-en-1-ol	0.14
1643	dehydro sabinaketone	0.04
1648	Myrtenal	0.05
1661	Alloaromadandrene	0.18
1664	*trans*-pinocarveol	0.19
1668	*Z*-β-farnesene	0.04
1681	*Z*-3-hexenyl tiglate	0.06
1687	α-humulene	0.02
1704	γ-muurolene	0.21
1706	α-terpineol	2.10
1709	**α-terpinyl acetate**	**7.35**
1726	Germacrene D	1.34
1740	α-muurolene	0.30
1744	α-selinene	0.05
1740	Geranial	0.07
1758	*cis*-piperitol	0.12
1773	δ-cadinene	0.94
1776	γ-cadinene	0.47
1786	Kessane	1.19
1804	Myrtenol	0.13
1808	Nerol	0.11
1830	2, 6-dimethyl-3-*E*, 5-*E*, 7-octatriene-2-ol	0.03
1838	β-damascenone	0.04
1845	*trans*-carveol	0.05
1853+1857	Calamanene + Geraniol	0.84
1864	ρ-cymene-8-ol	0.34
1900	Epicubebol	0.37

RRI	Compound	Area percentage
1918	α-calacorene 1	0.09
1957	Cubebol	0.41
1941	α-calacorene 2	0.08
2001	Iso caryophyllene oxide	1.15
2008	**Caryophyllene oxide**	**6.05**
2037	Salvial-4 (14)-en-1-one	0.17
2045	Humulene epoxide 1	0.60
2080	Cubenol	0.69
2088	1-epi-cubenol	0.62
2143	Cedrol	1.27
2187	T-cadinol	1.31
2209	T-muurolol	0.65
2219	δ-cadinol	0.13
2250	α-eudesmol	0.11
2255	α-cadinol	1.90
2324	Caryophellenol 2	0.35
	TOTAL	**74.24**

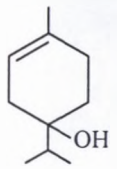
The major compounds terpinen-4-ol and β-caryophyllene represents 15.59% of the total composition. α-terpinyl acetate makes up 7.35% while the combination β-cubenene and linalool represents 6.98%. Caryophyllene oxide occupies 6.05% of the composition.



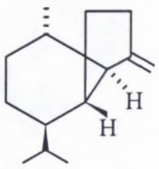
linalool



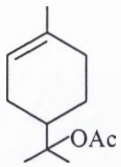
β-caryophyllene



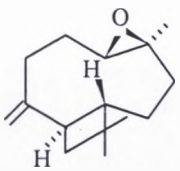
terpinen-4-ol



β-cubenene



α-terpinyl acetate



caryophyllene oxide

Figure 37: Chemical structures for major compounds identified in the essential oil of *Plectranthus neochilus*.

4.6. *Plectranthus porphyranthus* Edwards

Botanical description:

Perennial semi-succulent aromatic herb; stems are procumbent to scandent. The leaves are softly succulent, petiolate and densely hirsute. Leaf margins are markedly revolute in immature leaves becoming less so with age. Inflorescence terminal is 10-40 cm long usually 10-flowered, 1-3 cm apart. The calyx is 3 mm long enlarging to 6 mm in fruit while the corolla is intense violet with pale median markings on the throat and upper lobes. The species has a protracted flowering period starting early in December and continuing to late March (Edwards *et. al.* 2000).

Distribution:

Plectranthus prophyranthus occurs only in xerophytic plant communities on granite lithosols. These communities are often desiccated. Its distribution is along Southern Zimbabwe.

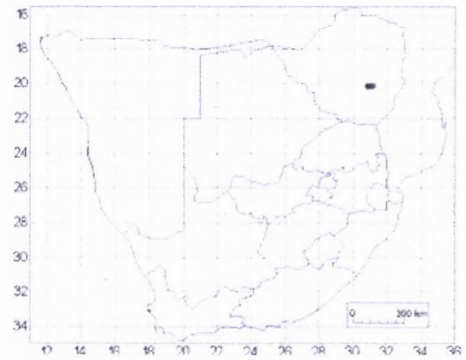


Figure 38: Geographical distribution of *Plectranthus porphyranthus*.

Figure 39: The leaves and inflorescence of *Plectranthus porphyranthus*.

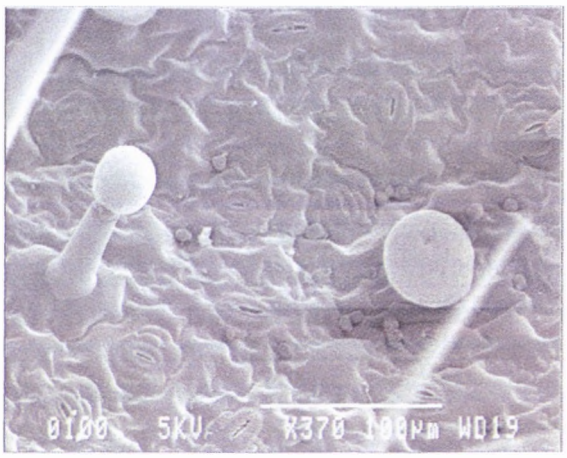


Figure 40: Scanning electron micrograph of the abaxial surface of the leaf showing the oil glands.

Essential oil composition:

Essential oil was obtained by hydro-distillation (3 hours). The oil yield was 0.78%.

GC-MS profile and analysis:

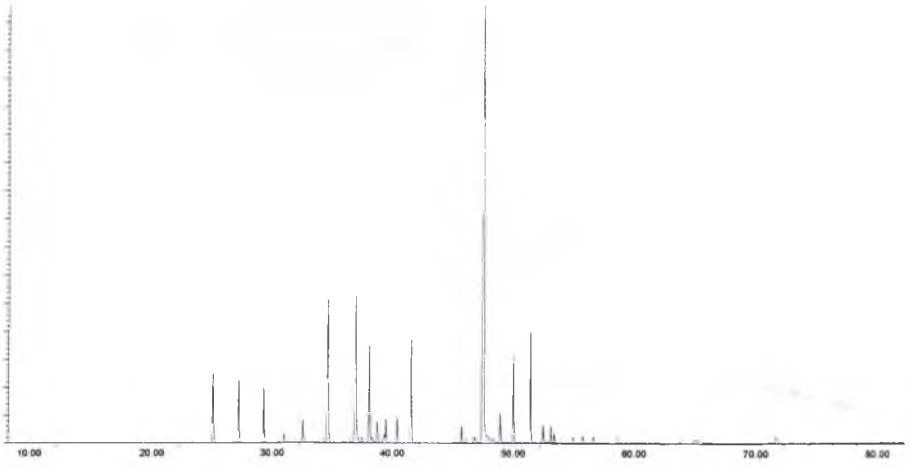
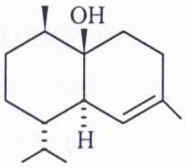


Figure 41: GC-MS profile of hydro-distilled essential oil obtained from *Plectranthus porphyranthus*.

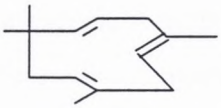
Table 9: Compounds identified in the essential oil of *Plectranthus porphyranthus*.

RRI	Compound	Area percentage
1483	Octanyl acetate	3.93
1393	3-octanol	3.10
1452	1-octen-3-ol	2.67
1497	α-copanene	0.43
1535	β-bourbonene	0.16
1612	**β-caryophyllene**	**9.03**
1687	**α-humulene**	**10.16**
1693	β-acoradiene	0.23
1726	**Germacrene D**	**5.49**
1755	Bicyclogermacrene	1.06
1773	δ-cadinene	0.40
1776	γ-cadinene	1.17
1810	3,7 guaiadene	1.15
1853	***cis*-calamenene**	**5.36**
1957	Cubebol	0.17
2001	Isocaryophyllene	0.14
2008	Caryophyllene oxide	0.82
2051	Gleenol	0.39
2071	Humulene epoxide 2	0.11
2080	**Cubenol**	**36.15**
2144	Spathulenol	1.47

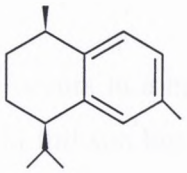
RRI	Compound	Area percentage
2187	T-cadinol	3.95
2232	α-bisbalol	0.07
2255	**α-cadinol**	**5.26**
2320	14-nor-cadin-5-en-4one isomer A or B	0.78
2524	Abietatriene	0.27
	TOTAL	**94.25**



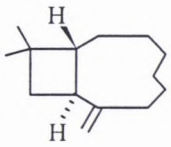
cubenol



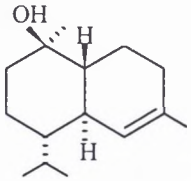
α-humulene



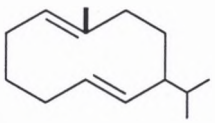
cis-calamenene



β-caryophyllene



α-cadinol



germacrene D

Figure 42: Chemical structures for major compounds identified in the essential oil of *Plectranthus porphyranthus*.

4.7. *Plectranthus venteri* Hankey

Botanical description:

It is an erect, pleasantly aromatic, multi-stemmed herb or subshrub to 700 mm high and to 1m in diameter. The branches are 2-6 mm in diameter, mostly erect and light to dark brown in colour. The leaves are ascending freely, softly succulent in opposite pairs. Flowers are arranged in 6-8 flowered cymes (van Jaarsveld and Hankey, 1997).

Distribution:

Plectranthus venteri is a succulent herb that occurs in a hilly area of Sekukuneland (altitude ca. 1680 m) among norite boulders, usually in full sun but also in the shade of rocks, usually in shallow soil and rock pockets that dry out between rainy spells.

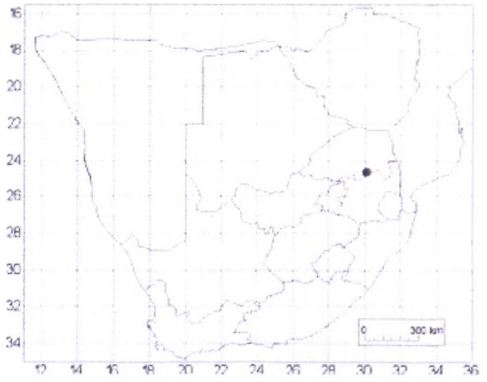
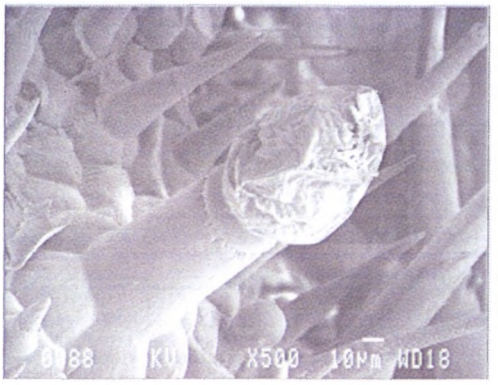


Figure 43: Geographical distribution of *Plectranthus venteri*.

Figure 44: *Plectranthus venteri*.



Figures 45 and 46: Scanning electron micrograph of the abaxial surface of the leaf showing the oil glands and hair follicles.

Essential oil composition:

Essential oil was obtained by hydro-distillation (3 hours). The oil yield was 0.97%.

GC-MS profile and analysis:

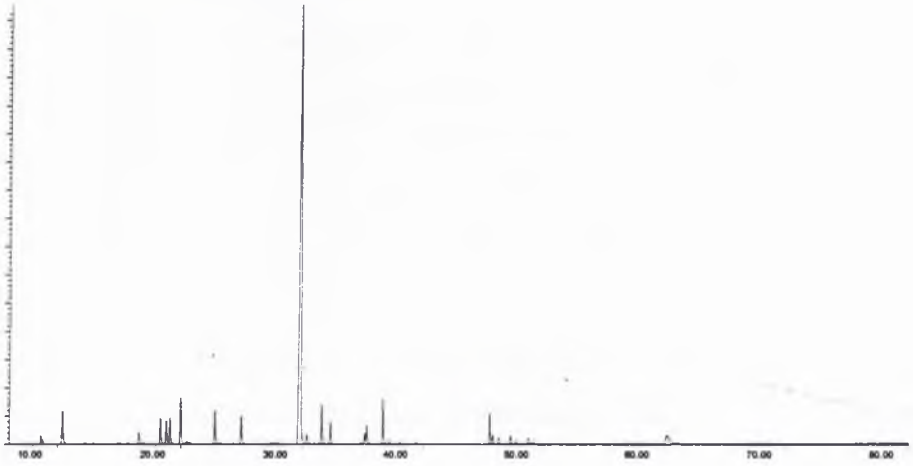


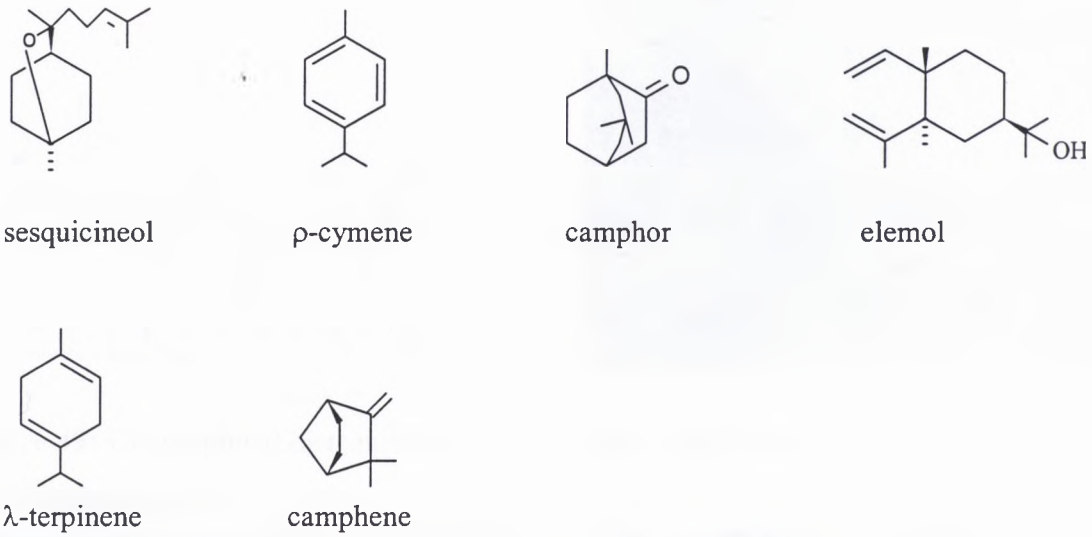
Figure 47: GC-MS profile of hydro-distilled essential oil obtained from *Plectranthus venteri.*

Table 10: Compounds identified in the essential oil of *Plectranthus venteri.*

RRI	Compound	Area percentage
1032	α-pinene	0.59
1035	α-thujene	0.36
1076	**Camphene**	**3.03**
1118	β-pinene	0.09
1174	Myrcene	0.30
1188	α-terpinene	0.15
1203	Limonene	1.04
1246	*Z*-β-ocimene	2.37
1255	γ-terpinene	2.14
1266	*E*-β-ocimene	2.26
1280	**ρ-cymene**	**4.31**
1290	Terpinolene	0.26
1345	3-octyl acetate	2.90
1393	3-octanol	2.11
1474	*trans*-sabinene hydrate	0.12
1478	*cis*-linalooloxide (furanoid)	0.36
1532	**Camphor**	**58.13**
1553	Linalool	0.77
1575	α-santalene	0.17
1597	Bornyl acetate	3.52
1611	Terpinen-4-ol	1.70
1700	ρ-mentha 1,8 dien 4-ol	0.16

RRI	Compound	Area percentage
1706	α-terpineol	0.74
1719	Borneol	1.44
1792	**Sesquicineole**	**3.44**
1864	ρ-cymene-8-ol	0.22
2096	Elemol	2.21
2104	Viridiflorol	0.64
2144	Rosiflorol	0.53
2185	γ-eudesmol	0.32
2232	α-bisabolol	0.51
2250	α-eudesmol	0.22
2257	β-eudesmol	0.30
	TOTAL	**97.41**

The major compound is identified as camphor (58.13%). The monoterpene, ρ-cymene, occupies 4.31% of the total while sesquicineole represents 3.44%.



sesquicineol ρ-cymene camphor elemol

λ-terpinene camphene

Figure 48: Chemical structures for major compounds identified in the essential oil of *Plectranthus venteri.*

4.8. *Plectranthus zuluensis* T. Cooke

Botanical description:

This is an erect soft shrub 1-2 m tall, freely branched with ascending stems. The leaves are petiolate with soft, semi-succulent blades. They are ovate to broadly ovate in shape, short and pubescent on both surfaces. The under-surface of the leaves are covered with colourless gland-dots. The flowers are arranged in sessile 3-flowered cymes. The corolla is 10-11 mm long, pale blue-mauve to almost white (Codd 1985).

Distribution:

The plants are found in the semi-coastal parts of KwaZulu-Natal and are abundant around Port Shepstone. They are also found in the southern regions of Swaziland. The habitat includes forest margins and are often common along streams.

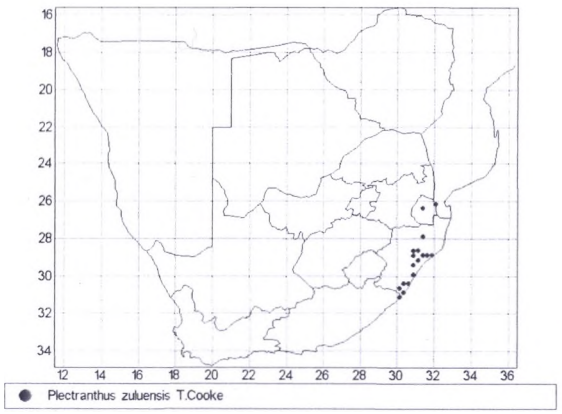
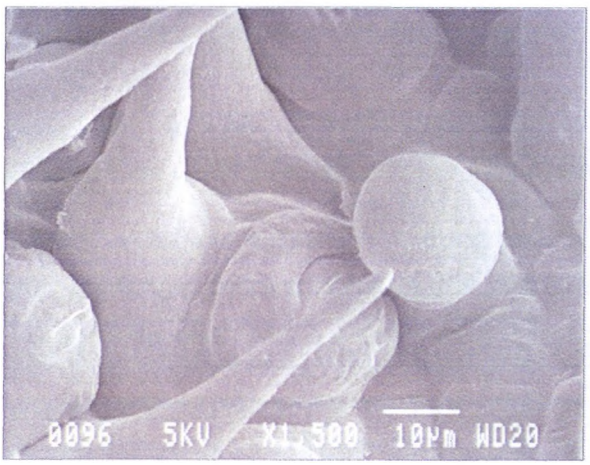
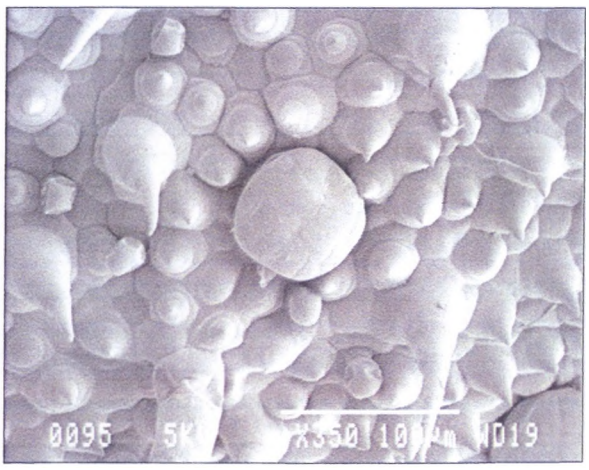


Figure 49: Geographical distribution of *Plectranthus zuluensis*.

Figure 50: *Plectranthus zuluensis* in flower.





Figures 51 and 52: Scanning electron micrograph of the abaxial surface of the leaf showing the oil glands and hair follicles.

Essential oil composition:

Essential oil was obtained by hydro-distillation (3 hours). The oil yields were

1. *Plectranthus zuluensis* (pale blue form) ex. C. Potgieter: 0.12%.
2. *Plectranthus zuluensis* (dark blue form) ex. C. Potgieter: 0.11%.
3. *Plectranthus zuluensis* ex. Witwatersrand Botanical Garden: 0.14%.

The two different forms of *Plectranthus zuluensis* i.e. pale and dark blue refer to two different flower varieties of this species.

GC-MS profile and analysis:

1. *Plectranthus zuluensis* (pale blue form) ex hort C. Potgieter:

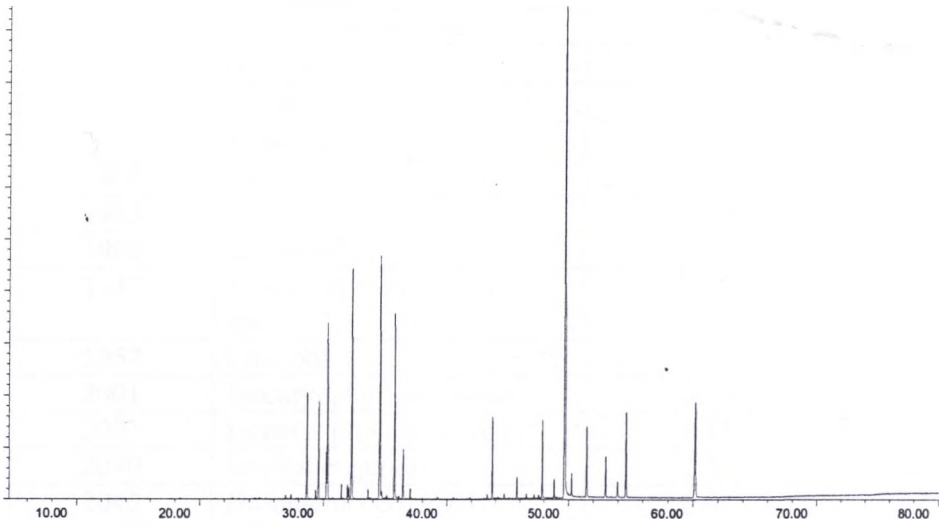


Figure 53: GC-MS profile of the hydro-distilled essential oil obtained from *Plectranthus zuluensis* (pale blue form).

Table 11: Compounds identified in the essential oil of *Plectranthus zuluensis* (pale blue form).

RRI	Compound	Area percentage
1157	m-xylene	0.02
1345	3-octyl acetate	0.04
1360	1-hexanol	0.02
1386	1-octanyl acetate	0.02
1391	*Z*-3-hexen-1-ol	0.03
1393	3-octanol	0.04
1412	*Z*-2-hexen-1-ol	0.02
1452	1-octen-3-ol	0.13
1466	α-cubebene	0.15
1495	Bicycloelemene	0.03

RRI	Compound	Area percentage
1497	α-copaene	3.87
1528	α-bourbonene	0.28
1535	β- bourbonene	3.28
1541	Benzaldehyde	0.02
1549	β-cubebene	0.85
1553	**Linalool**	**5.80**
1565	Linalyl acetate	0.07
1572	β-ylangene	0.50
1600	β-elemene	0.43
1612	**β-caryophyllene**	**9.21**
1668	*Z*- β-farnesene	0.02
1687	**α-humulene**	**10.78**
1706	α-terpineol	0.11
1726	**Germacrene D**	**7.52**
1743	Eremophilene	0.11
1755	Bicyclogermacrene	1.62
1773	δ-cadinene	0.33
1776	γ-cadinene	0.01
1784	*E*-α-bisabolene	0.02
1808	Nerol	0.03
1857	Geraniol	0.09
1900	Epi-cubebol	0.05
1945	1-5-epoxy-salvial-4 (14)-ene	0.02
1957	Cubebol	0.05
2001	Isocaryophyllene oxide	0.03
2001	Isocaryophyllene oxide	0.18
2030	Methyl eugenol	2.58
2045	Humulene epoxide 1	0.02
2050	*E*-nerolidol	0.06
2071	Humulene epoxide 2	0.17
2109	*cis*-methyl isoeugenol	0.62
2144	Spathulenol	0.13
2172	Trimethoxybenzene	0.10
2200	*trans*-methyl-isoeugenol	2.53
2245	Elemicine	0.59
2255	α-cadinol	0.02
2282	***cis*-iso-elemicine**	**30.98**
2361	β-asarone (*Z*-asarone)	2.38
2403	*trans*-iso-elemicine	1.50
2478	α-asarone (*E*-asarone)	3.65
	TOTAL	**91.13**

Fifty-one compounds are identified in the essential oil of *Plectranthus zuluensis*. This makes up 91.13% of the total oil composition. The major compound *cis*-iso-elemicine contributes 30.98% of this total. The sesquiterpene α-humulene comprises 10.78% of the oil while β-

caryophyllene, another sesquiterpene, makes up 9.21%. The acyclic monterpene alcohol, linalool, represents 5.80% of the total oil composition.

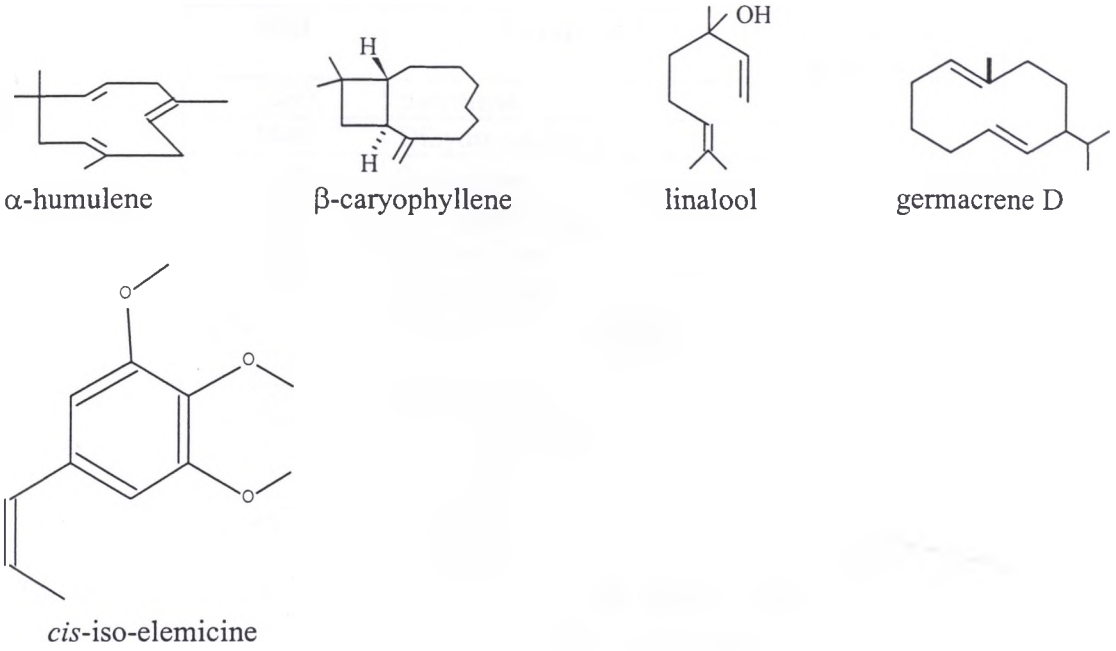


Figure 54: Chemical structures for major compounds identified in the essential oil of *Plectranthus zuluensis* (pale blue form).

2. *Plectranthus zuluensis* (dark blue form) ex hort C. Potgieter:

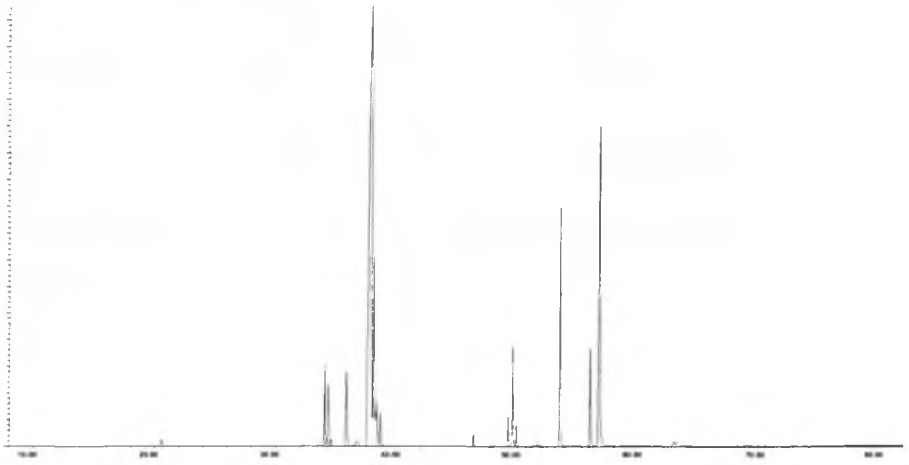


Figure 55: GC-MS profile of hydro-distilled essential oil obtained from of *Plectranthus zuluensis* (dark blue form).

Table 12: Compounds identified in the essential oil of *Plectranthus zuluensis* (dark blue form).

RRI	Compound	Area percentage
1280	ρ-cymene	0.03
1440	Dihydroedulan-1	0.05
1600	β-elemene	3.10
1612	β-caryophyllene	2.60
1687	α-humulene	0.15
1740	**α-muurolene**	**46.65**
1740 +1742	Valencene + β-selinene	5.97
1744	α-selinene	1.31
2050	*E*-nerolidol	0.35
2282	*cis*-isoelemicine	0.15
2361	**β-asarone**	**8.60**
2478	**α-asarone**	**17.62**
	TOTAL	**87.46**

Thirteen compounds have been identified. The major compound, the sesquiterpene, α-muurolene, makes up 46.65% of the total composition which is probably responsible for the aromatic herbaceous character of the oil. The enantiomers α-asarone and β-asarone make up 17.62% and 8.60% of the oil respectively. Valencene and β-selinene makes up 5.97% of the oils' composition. In total, these compounds comprise 78.84% of the total oil composition.

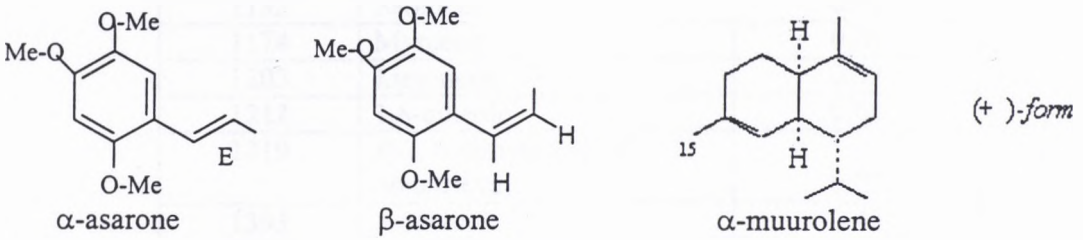


Figure 56: Chemical structures for major compounds identified in the essential oil of *Plectranthus zuluensis* (dark blue form).

3. *Plectranthus zuluensis* ex hort Witwatersrand Botanical Garden:

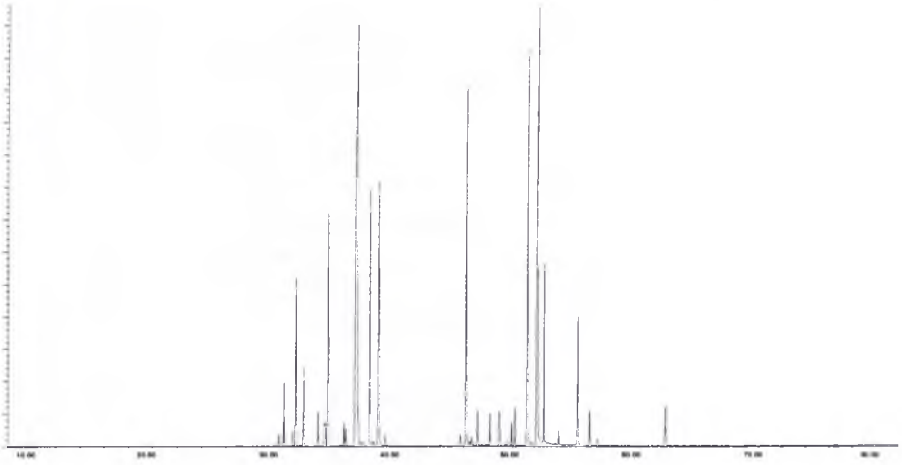


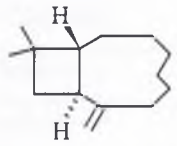
Figure 57: GC-MS profile of the hydro-distilled essential oil obtained from *Plectranthus zuluensis* (WBG).

Table 13: Compounds identified in the essential oil of *Plectranthus zuluensis* (WBG).

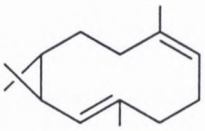
RRI	Compound	Area percentage
1032	α-pinene	0.02
1118	β-pinene	0.01
1132	Sabinene	0.01
1174	Myrcene	0.09
1203	Limonene	0.53
1213	1,8-cineole	0.64
1319	*E*-2, 6-dimethyl-1, 37 nonatrieve	0.27
1393	3-octanol	0.45
1452	1-octen-3-ol	0.71
1466	α-cubenene	0.43
1495	Bicycloelemene	0.21
1497	λ-copanene	0.98
1528	λ-bourbonene	3.85
1535	β-bourbonene	3.85
1544	λ-gujurene	0.25
1548	*E*-2-nonenal	0.16
1549	β-cubenene	0.60
1553	Linalool	1.24
1612	**β-caryophyllene**	**6.19**
1661	Alloaromadendrene	0.40
1668	*Z*-β-farnesene	0.22
1687	**λ-humulene**	**21.40**
1544	λ- gujurene	0.54
1726	**Germacrene D**	**7.11**

RRI	Compound	Area percentage
1743	Eremophilene	0.55
1755	**Bicyclogermacrene**	**7.28**
1773	δ-cadinene	0.80
1776	γ-cadinene	0.06
1817	4, 8, 12-trimethyl-1, 3-*E*, 7-*E*,11-tridecatetraene	0.16
1857	Geraniol	0.08
1900	Epicubebol	0.15
1945	1, 5-epoxy-salvial-4 (14)-ene	0.60
1981	*Z*-methyl cinnamate	0.40
2001	Isocaryophyllene oxide	0.49
2008	Caryophyllene oxide + 1 allyl-2, 4-di-(0.19) methoxybenzene (0.1)	0.29
2030	**Methyl eugenol**	**8.56**
2045	Humulene epoxide 1	0.10
2050	*E*-nerolidol 1	0.16
2071	Humulene epoxide 2	0.71
2081	Humulene epoxide 3	0.51
2096	*E*-methyl cinamate	0.20
2104	Viridiflorol	0.30
2109	*cis*-methyl isoeugenol	0.51
2144	Spathulenol	0.60
	TOTAL	**72.99**

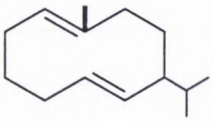
Forty-seven compounds make up the composition of this essential oil. The compound making up the highest percentage of the oil is the sesquiterpene, λ-humulene (21.40%). Methyl eugenol makes up 8.56% of the total composition while bicyclogermacrene makes up 7.28%. Another sesquiterpene, β-caryophyllene, contributes 6.19%. The enantiomers λ-bourbonene and β-bourbonene both make up 3.85% each of the total composition.



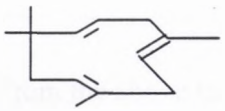
β-caryophyllene



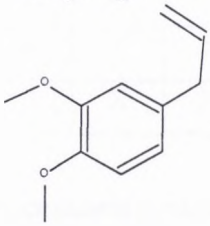
bicyclogermacrene



germacrene D



α-humulene



methyleugenol

Figure 58: Chemical structures for major compounds identified in the essential oil of *Plectranthus zuluensis* (WBG).

Table 14: Common detected compounds in various of the essential oils of the plant species

Oil sample → / Compound ↓	*P. ciliatus*	*P. frutocosus*	*P. grandidentatus*	*P. hadiensis*	*P. porphyranthus*	*P. venteri*	*P. zuluensis* (dark blue)	*P. zuluensis* (pale blue)	TOTALS
α-asarone								X	1
β-asarone								X	1
α-bourbonene							X		1
β-bourbonene							X		1
α-cadinol					X				1
β-caryophyllene		X	X		X		X	X	5
α-fenchone			X	X					2
α-humulene					X			X	2
λ-humulene							X		1
bicyclogermacrene	X	X					X		3
bornyl acetate	X	X				X			3
camphene						X			1
camphor			X			X			2
cis-iso-elemicine								X	1
cubenol			X	X	X				3
germacrene D	X	X			X		X	X	5
limonene			X						1
linlool								X	1
sabinene		X							1
sesquicineol			X			X			2
spathulenol	X	X							2
T-cadinol			X	X					2

From the above table, the most common compounds can be determined. The two compounds that proved to be most common were germacrene D and β-caryophyllene. Both these compounds were identified in five of the eight species above, which represents 62.5%.

5. RESULTS AND DISCUSSION

5.1. Disc diffusion

It must be noted that these are only preliminary results and I suggest verification by allowing one to repeat them.

Bacteria:

Using aseptic manipulation, discs with plant essential oil were introduced onto the seeded agar plates. Zones of inhibition were examined after 24 hours to 7 days depending on the test organism and the results are summarized in Table 15.

Table 15: Zones of inhibition of the essential oils of various *Plectranthus* species on bacteria.

Species	Zone of inhibition (mm) from disc edge					
	S. aureus	***E. coli***	***E. faecalis***	***P. aeruginosa***	***B. cereus***	***S. epidermidis***
P. ciliatus (Ferncliff)	0	0	0	0	4	0
P. ciliatus (WBG)	0	0	0	0	1	0
P. fruticosus	0	0	0	0	<1	0
P. grandidentatus	0	0	<1	0	4	3
P. hadiensis	0	0	0	0	2	0
P. neochilus	0	0	0	0	2	0
P. porphyranthus	0	0	0	0	8	0
P. venteri	<1	0	0	0	8	3
P. zuluensis (pale blue)	0	0	0	0	2	0
P. zuluensis (dark blue)	0	0	0	0	1	0
P. zuluensis (WBG)	0	0	0	0	0	0
Control-Neomycin 30μg	8	6	3	5	11	4

From the microbiological results, it is evident that these species exhibit some antimicrobial activity. Almost all the species tested proved to be active against *B. cereus* with the exception of *P. zuluensis* (WBG). *P. porphyranthus* and *P. venteri* showed remarkable antimicrobial activity against *B. cereus* almost comparable to that of the control. All species showed no activity against *E. coli* and *P. aeruginosa* while *P. venteri* exhibited minimal activity against *S. aureus*. Two of the eleven species tested displayed activity against *S. epidermidis* comparable to that of the Neomycin control.

Impregnated discs with the essential oil samples that were most active against *B. cereus* were re-introduced onto a new seeded agar plate. Refer to Table 16 for results.

Table 16: Zones of inhibition of various *Plectranthus* species against *B. cereus*.

Species	Zones of inhibition (mm) from disc edge
P. ciliatus (Ferncliff)	2
P. grandidentatus	2
P. hadiensis	6
P. porphyranthus	5
P. venteri	8
P. zuluensis (pale blue)	3
Control-Neomycin 30µg	6

Results show that *P. porphyranthus* exhibited decreased activity compared to the first disc diffusion assay. *P. zuluensis* (pale blue) and *P. ciliatus* proved to have little activity. *P. hadiensis* displayed activity equivalent to that of the control while *P. venteri* had a 8 mm zone of inhibition showing high activity, when compared to the control, being Neomycin, having a 6 mm zone of inhibition.

A study conducted by Chao *et al.* (2000) showed limonene as one of the major identified compounds present in the essential oils of Bergamot (11.4%), Angelica root (12.9%), Dill–Indian (50.9%), Lemon (68.7 %), Lime (55.5%) and Neroli (12.9%). All of these essential oils exhibited activity against *B. cereus* with a common zone of inhibition of 4 mm. Limonene is one of the compounds identified in *P. hadiensis* and could be responsible for some of the activity observed by this essential oil against *B. cereus*. In the same study, the essential oils of Savory, Coriander and Cumin had ρ-cymene as one of the major compounds in quantities of 25.9%, 6.1% and 5.8% respectively. The disc diffusion assay showing the antimicrobial activity of these oils against *B. cereus* showed that Savory had a 15 mm zone of inhibition while Coriander's was larger than 33 mm and Cumin's was 7 mm. *P. venteri* also had ρ-cymene (4.31%), linalool (0.77%) and limonene (1.04%) as identified compounds and displayed a zone of inhibition of 8 mm. An interesting point to note is that there are only two major compounds identified in the essential oil of Coriander that are linalool (72%) and ρ-cymene (6.1%). ρ-cymene (0.34 %), linalool (1.10 %) and limonene (1.17 %) are all present in small quantities in *P. hadiensis* as well as *P. venteri* and it is these two essential oils that have been proven to possess antimicrobial activity against *B. cereus*.

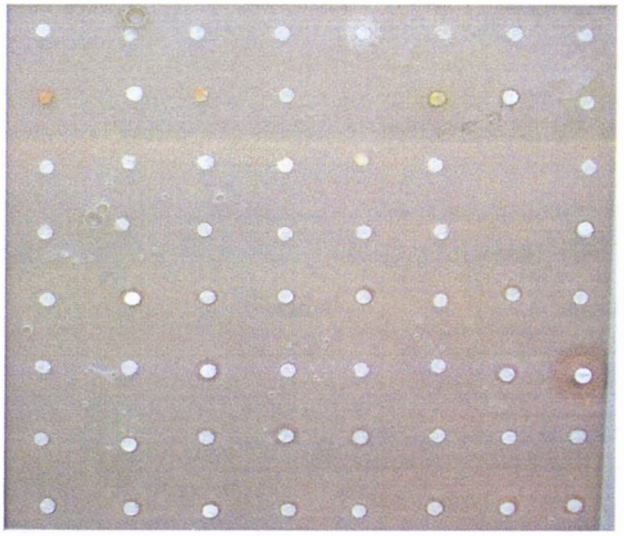


Figure 59: Zones of inhibition as observed with the disc diffusion assay against *Staphylococcus aureus*.

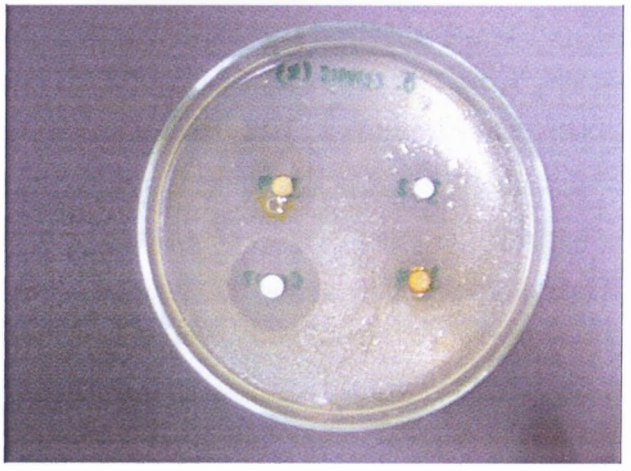


Figure 60: An example of the disc diffusion assay with zones of inhibition against *B. cereus*. The zone of inhibition for the control Neomycin (concentration = 30 ug) is on the bottom left..

Mould and Yeasts:

The disc diffusion technique was also performed on the one mould and two yeasts. The results are summarized in Table 17.

Table 17: Zones of inhibition of the essential oil of various *Plectranthus* species on the mould and yeasts species.

Species	Zone of inhibition (mm) from disc edge		
	Candida albicans	***Aspergillus niger***	***Cryptococcus neoformans***
P. ciliatus (Ferncliff)	<1	0	1
P. ciliatus (WBG)	0	0	5
P. fruticosus	0	0	0
P. grandidentatus	0	0	3
P. hadiensis	0	0	4
P. neochilus	0	0	0
P. porphyranthus	1	0	11
P. venteri	4	1	10
P. zuluensis (pale blue)	3	6	10
P. zuluensis (dark blue)	3	4	0
P. zuluensis (WBG)	3	6	2
Control-Nystatin 100 IU	7	7	5

P. venteri, *P. porphyranthus*, *P. zuluensis* (pale blue) proved to be most active against *Cryptococcus neoformans* with zones of inhibition of 10 mm, 11 mm and 10 mm respectively. *P. zuluensis* (WBG) and *P. zuluensis* (pale blue) showed moderate activity with *Aspergillus niger* (zones of inhibition of 6 mm). Very little activity was exhibited against *Candida albicans* with respect to *P. ciliatus, P. grandidentatus, P. neochilus, P. hadiensis* and *P. fruticoccus*. *P. venteri* had some activity against *Candida albicans, Aspergillus niger* and *Cryptococcus neoformans*.

Oils studied by Chao *et. al.* (2000) inhibited the growth of *Candida albicans* with zones of 30 mm and larger than 33 mm. ρ-cymene was identified in these essential oils as a major compound. *P. venteri* with ρ-cymene (4.31%) as an identified compound proved to be active against *Candida albicans* with a zone of inhibition of 4 mm. The essential oil of Savory (according to Choa *et. al.*, 2000) also inhibited the growth of *Cryptococcus neoformans* with a MIC value of 150 mg/mL. In our assay *P. venteri* inhibited the growth of this organism with a 10 mm inhibition zone. About 8-10 µL of concentrated essential oil of *P. venteri* was used. We could possibly attribute this activity to ρ-cymene.

A tea tree oil study by Carson and Riley (1994) showed that linalool was very effective in inhibiting the growth of *Candida albicans*. One of the most active oils against *Candida albicans*, *P. zuluensis* (pale blue), had a zone of inhibition of 3 mm and contained 5.8% of linalool. The study carried out by Pattnaik *et al.* (1997) also confirms the susceptibility of *Candida albicans* and *Cryptococcus neoformans* to linalool.

Carson and Riley (1994) also showed that tea tree oil (*Malaleuca alternifolia*) is becoming increasingly popular as a naturally antimicrobial agent. The antimicrobial activity of eight components of tea tree oil was evaluated using disc diffusion and broth microdilution methods. The disc diffusion method was used to determine the susceptibility of a range of micro-organisms to 1,8-cineole, 1-terpinen-4-ol, ρ-cymene, linalool, α-terpinene, γ-terpinene, α-terpinene and terpinolene. Terpinen-4-ol was active against all the test organisms including *Bacillus subtilis, Bacteriodes fragilis, Candida albicans, Clostridium perfringens, Enterococcus faecalis, Escherichia coli, Lactobacillus acidophilus, Moraxella catarrhalis, Mycobacterium smegmatis, Psuedomonas aeruginosa, Serratia marcescens and Staphylococcus aureus*. ρ-cymene demonstrated no antimicrobial activity. Linalool and α-terpineol were active against all organisms with the exception of *Psuedomonas aeruginosa*. Minimum inhibitory and minimum cidal concentrations of each component against *Candida albicans*, *Escherichia coli* and *Staphylococcus aureus* were determined using broth microdilution method. Terpinen-4-ol was identified in *P. hadiensis* (zone of inhibition of 4 mm against *Cryptococcus neoformans* and *P. grandidentatus* (zone of inhibition of 3 mm against *Cryptococcus neoformans*).

The compound cubenol is found in both *P. hadiensis* (15.08%) and *P. porphyranthus* (36.15%). Both of these samples of essential oils show activity against *Cryptococcus neoformans* with zones of inhibition of 4 mm and 11 mm respectively.

P. zuluensis (WBG), *P. zuluensis* (pale blue) and *P. zuluensis* (dark blue) all showed activity against *Aspergillus niger* with inhibition zones of 6 mm, 6 mm and 4 mm respectively.

Table 18: Major compounds identified in the three *P. zuluensis* species.

Oil sample	Major compounds identified
P. zuluensis (pale blue)	β-caryophyllene, linalool, germacrene, α-humulene
P. zuluensis (dark blue)	α-muurolene, α-asarone, β-asarone
P. zuluensis (WBG)	β-caryophyllene, germacrene, λ-humulene, linalool

As shown above the two samples from the species that have the same major identified compounds are *P. zuluensis* (WBG) and *P. zuluensis* (pale blue). Both of them also exhibited the same zones of inhibition against *Aspergillus niger* of 6 mm. All three samples of *P. zuluensis* had the inhibition zone of 3 mm against *Candida albicans*. Against *Cryptococcus neoformans P. zuluensis* (pale blue) showed activity with a zone of inhibition of 10 mm. This further substantiates the study by Pattnaik *et al.* (1997) confirming the susceptibility of *Cryptococcus neoformans* to linalool. *P. zuluensis* (dark blue) showed minimal activity and this can also indicate that the compounds α-muurolene, α-asarone, β-asarone may possess no antifungal activity against *Cryptococcus neoformans*.

Table 19: Major compounds identified in *Plectranthus venteri* and *Plectranthus grandidentatus*.

Oil sample	Major compounds identified
P. grandidentatus	camphor, sesquicineole
P. venteri	camphor, sesquicineole

P. grandidentatus and *P. zuluensis* (dark blue) displayed the same minimum inhibitory concentration of greater than 32 mg/mL against the bacteria innoculum *B. cereus*. Also both *P. venteri* and *P. grandidentatus* showed antifungal activity against *Cryptococcus neoformans* with 3 mm and 10 mm zones of inhibition respectively.

The two species below also exhibited similar activity against *B. cereus* with 5 mm and 8 mm zones of inhibition respectively. Their major identified compounds identified by GC-MS can be seen in Table 20.

Table 20: Major compounds identified in *Plectranthus porphyranthus* and *Plectranthus ciliatus*.

Oil sample	Major compounds identified
P. ciliatus	β-caryophyllene, germacrene D, bicyclogermacrene, bornyl acetate
P. porphyranthus	β-caryophyllene, germacrene D, α-humulene

5.2. MIC/microplate

Based on the activity recorded for some species as summarized in Table 14, the microplate method was carried out on species showing promising activity to quantify the antimicrobial activity for some pathogens.

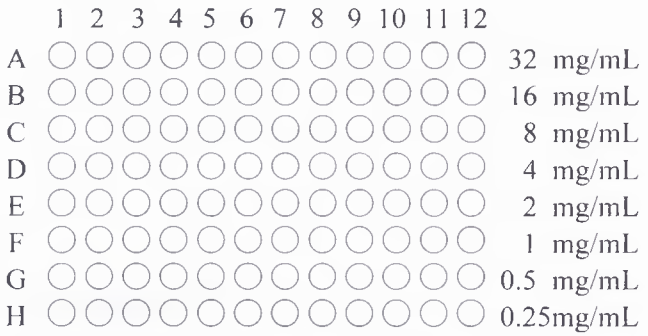


Figure 61: An example of a MIC template.

Key:

The plates were then examined after 30, 60, 120 min and 24 hours. Bacterial growth was ascertained at the different time periods to analyse the intensity of growth of the bacteria. The change in MIC values over the different time periods could be attributed to the fact that the essential oils used are highly volatile and evaporate over time thus growth of bacteria will increase as well.

30 minutes:

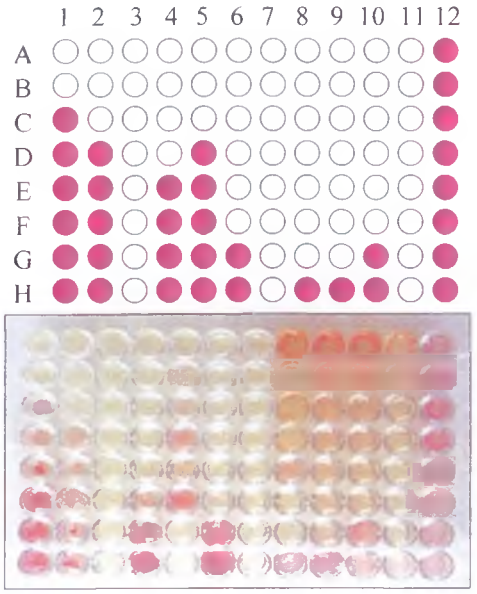


Figure 62: Microplate photographed after 30 minutes.

60 minutes:

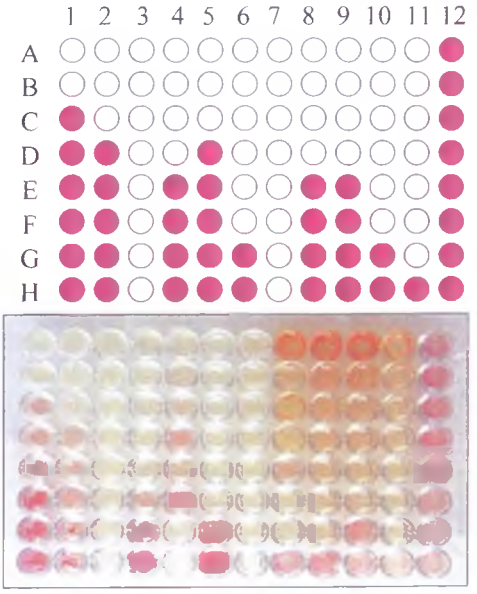


Figure 63: Microplate photographed after 60 minutes.

120 minutes:

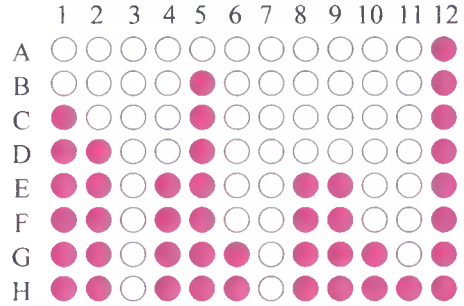
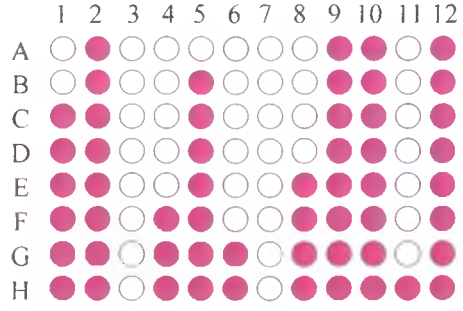


Figure 64: Microplate after 120 min.

24 hours:



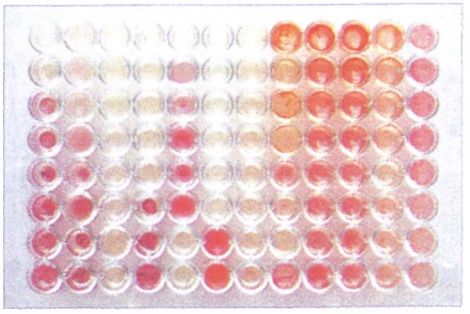


Figure 65: Microplate diagram and photograph after 24 hrs.

Table 21: Results of minimum inhibitory concentration (mg/mL) after 30, 60, 120 minutes and 24 hours.

Column	Oil sample	MIC 30 min	MIC 60 min	MIC 120 min	MIC 24 hours	Bacteria innoculum
1	*P. zuluensis* (pale blue)	16	16	16	16	*B. cereus*
2	*P. zuluensis* (dark blue)	8	8	8	>32	*B. cereus*
3	*P. porphyranthus*	≤0.25	≤0.25	≤0.25	≤0.25	*B. cereus*
4	*P. venteri*	4	4	4	2	*B. cereus*
5	*P. venteri*	8	8	32	32	*S. epidermidis*
6	*P. venteri*	1	1	1	1	*S. aureus*
7	*P. ciliatus*	≤0.25	≤0.25	≤0.25	≤0.25	*B. cereus*
8	*P. grandidentatus*	0.5	4	4	4	*B. cereus*
9	*P. grandidentatus*	0.5	4	4	>32	*S. epidermidis*
10	*P. grandidentatus*	1	1	1	>32	*S. epidermidis*
11	*P. hadiensis*	0.25	0.5	0.5	0.5	*B. cereus*
12	Negative control*	> 32	> 32	>32	>32	*B. cereus*

*negative control indicates that the wells contained the bacteria innoculum without the test substance i.e. the essential oil.

The MIC of *P. zuluensis* (dark blue) is >32 mg/mL indicating minimal activity against *B. cereus*. *P. venteri* had a minimum inhibitory concentration of 32 mg/mL against *S. epidermidis* also indicative of poor antimicrobial properties of the oil sample. *P. grandidentatus* against *S. epidermidis* also displays poor activity that is once again proved in the disc diffusion assay test. Here, we can see that it has greater potency against *B. cereus* than *S. epidermidis*. In the MIC/microplate method, this oil also showed greater activity against *B. cereus* with a minimum inhibitory concentration of 4 mg/mL.

It is interesting to note that with respect to the disc diffusion assay method, *P. hadiensis*, portrayed minimal activity against almost all the bacteria it was tested against except for a 2 mm zone of inhibition against *B. cereus*. However, with regard to the MIC/microplate method, it displayed a value of 0.5 mg/mL. This indicates exceptional activity against this bacteria.

6. CONCLUSIONS

Phytochemical research on the genus *Plectranthus* has hitherto mostly focused on the diterpenoids. This project has recorded the essential oil composition and antimicrobial properties of the lesser studied essential oils. This is the first account on the essential oil chemistry of indigenous *Plectranthus* species. The eight species studied produce complex and different chromatographic profiles.

For some taxa (e.g. *P. ciliatus* and *P. zuluensis*) the essential oil chemistry varies between populations and different taxonomic forms. For *P. ciliatus* for example, the plants collected from Ferncliff mostly accumulate germacrene D, bicyclogermacrene and spathulenol while the hydrodistilled essential oil obtained from plants in the Witwatersrand Botanical Garden mostly yield β-caryophyllene, δ–cadinene, and ledol. Similar chemotypic variation was noted for *P. zuluensis* emphasizing the value of population studies in phytochemical exploration of medicinally used plants.

The essential oil chemistry (albeit variable) together with unique morphological and anatomical characters (trichomes) has allowed us to complete diagnostic monographs for the eight species investigated.

Of the eleven essential oils studied, ten samples showed a degree of antimicrobial activity against the pathogen *B. cereus* in the disc diffusion assay. *P. porphyranthus* and *P. ciliatus* (Ferncliff) showed MIC values of $\leq$ 0.25 mg/ml. *P. hadiensis* and *P. venteri* also showed inhibition of this pathogen in the disc diffusion study. The major compounds identified in the latter two species were p-cymene, linalool and limonene. It is of interest to note that previous studies have shown these essential oil constituents to display antimicrobial activity. Similarly, *P. zuluensis* (pale blue form) had a 3mm zone of inhibition against *B. cereus.* Linalool (present at 5.8%) in *P. zuluensis* was also identified as one of the antimicrobial constituents in the well researched and commercially produced tea tree oil.

Three species; *P. porphyranthus*, *P. venteri* and *P. zuluensis* (pale blue form) showed remarkable activity towards *C. neoformans*. This microbe is an opportunistic pathogen of the respiratory tract. It is interesting to note that the traditional uses of *Plectranthus* species elude to respiratory ailments. The species studied confirms the antimicrobial properties against these respiratory pathogens. Extrapolating from previous studies reporting the antimicrobial

activity for specific essential oil constituents (also detected here in *Plectranthus* species) it is possible that the volatile fraction of these highly aromatic plants may contribute to their therapeutic effects in traditional healing.

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