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THE PHYTOCHEMISTRY AND ANTIMICROBIAL ACTIVITY OF SELECTED INDIGENOUS *HELICHRYSUM* SPECIES

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

Johannesburg 2007

DECLARATION:

I, Mr Dakshina Reddy, 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 (Pharmaceutical Affairs) at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other university.

_____

_____14th_____ day of _November_____, 2007

DEDICATION:

I would like to thank all those who have assisted me in the completion of this research report.

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ABSTRACT

Helichrysum (Asteraceae) is a large genus consisting of approximately 500 species of which 245 taxa are indigenous to southern Africa. Many *Helichrysum* species are widely used by the indigenous population to treat various ailments, including coughs, colds, fever, infections, headache, menstrual pain and as a treatment for wounds.

Medicinal uses are often not species-specific but often depend on the local availability. Guided by the traditional use and the lack of scientific information, nine species of *Helichrysum* were selected for this study. The essential oils were obtained through hydrodistillation and methanol and acetone extracts of the plant material were prepared.

The essential oil composition was determined using GC-MS. The oil profiles were mostly dominated by the presence of monoterpenes such as α-pinene, 1,8-cineole and *p*-cymene. Monoterpenes were largely absent in the essential oil of *H. felinum*, but this oil was rich in sesquiterpenes with high yields of β-caryophyllene.

The antimicrobial activity of the essential oils and plant extracts were of interest due to their traditional use as an antiseptic. The antimicrobial activity of the essential oils and extracts was determined by disc diffusion assays and, following this, the most active species were further investigated using the minimum inhibition concentration (MIC) assay.

Helichrysum dasyanthum displayed the best activity against *B. cereus* (MIC = 16 mg/ml) and was the only extract that exhibited activity against all three fungal strains tested (*C. neoformans*, 1 mm; *C. albicans*, 3 mm; and *A. alternata*, 2 mm). The essential oil of *H. petiolare* and *H. felinum* exhibited the most pronounced activity against the fungal strains in the disc diffusion assay (*C. albicans*, 2mm).

CHAPTER 1: INTRODUCTION

Humans have used plants to treat various illnesses for hundreds of years. To this extent South Africans have been no different from the rest of the world. The indigenous diverse flora has provided the inhabitants of southern Africa with a source of food and medicine. This unique botanical diversity has been incorporated into the social and spiritual lives of the local inhabitants. A large proportion of the South African population practise traditional medicine to a large extent to meet their physical, mental and psychological health needs (Rabe *et al*., 1997).

Plants are widely used for their medicinal purposes in South Africa and it is estimated that approximately 27 million South Africans use traditional herbal medicines from more than 1020 indigenous plant species. The amount of plant material traded in two of the busiest markets in South Africa, viz. KwaZulu-Natal and the Witwatersrand, is estimated at approximately 4 500 tonnes per year (Fennell *et al.*, 2004).

Principles of traditional African medicine:

In order to understand the widespread use of traditional medicine it is important to understand the principles of this form of practice. In most African communities, commonly used plant remedies are available for the treatment of minor ailments such as headaches, fever, aches and pains. If the illness persists and becomes life-threatening, a specialist is consulted, usually the traditional healer, and divination is used in an attempt to uncover the hidden misdemeanours and to obtain more effective remedies. Plant remedies are used with other rituals that enhance the belief in the efficacy of the treatment, rather than as pharmacological entities. In traditional African medicine, plants are used to treat both the physical and spiritual states of the afflicted individual (Okpako, 1999).

The use of traditional medicine:

The belief in using plants for their healing properties is an ancient pratice and has been used by people from all continents. There is evidence that Neanderthals living 60 000 years ago in present-day Iraq used plants such as the Hollyhock for its healing properties, and these plants are still widely used today in ethno-medicine around the world (Cowan, 1999).

Most of the population in urban as well as smaller rural communities in South Africa are dependent on plant remedies for their medical requirements. Herbal remedies, apart from their traditional and cultural significance, are generally more accessible and affordable. Therefore the trend to integrate traditional Western medicine, especially in the primary health care setting, is becoming increasingly important.

There were approximately 300 000 traditional healers compared with just under 32 000 medical doctors registered with the Health Professions Council of South Africa in 2003 (Meissner, 2004). The realization of this vast resource in providing total health care cover through acceptable and economically feasible means could be utilized. The Traditional Health Practitioners Act of 2004 aims to regulate the practice of traditional medicine in a safe and competent manner. Traditional healers can be integrated as part of the primary health care team and can play an important role in treating many preventable diseases.

The use of traditional medicine in South Africa is currently not regulated, resulting in the possibility of dispensing toxic plant remedies. This raises the need for a scientific evaluation of the methods used by the healers. It is necessary to establish systems to allow for the quality, efficiency and safety of traditional herbal medicines.

Rationalisation of the use of indigenous plants:

The need to provide a scientific rationale for the safe and effective use of indigenous plant remedies in a health care setting has become increasingly important. In addition, this rationalisation could aid in the discovery of novel compounds that could be of pharmaceutical value. The traditional use of indigenous plants could help researchers target plants that may be of medical significance, instead of relying on trial and error. It is estimated that 122 drugs derived from plant species via ethnobotanical leads have been discovered (Fennell *et al*., 2004).

The use of plants as antimicrobials:

With the modernisation of the pharmaceutical industry, the ability to manufacture large volumes of drugs has contributed to the increase in drug-resistance (Cowan, 1999). There has been resurgence in discovering new sources of anti-infectives and it is envisaged that plant-derived phytochemicals may provide the answer.

In the United States, 25-50% of pharmaceuticals dispensed are of higher plant origin, with very few of these intended for use as antimicrobials. Since the advent of antibiotics in the 1950's, the use of plant derivatives as antimicrobials has been virtually non-existent. Clinical microbiologists have two reasons to be interested in the topic of antimicrobial plant extracts.

Firstly, it is very likely that these phytochemicals will find their way into the arsenal of antimicrobial drugs prescribed by physicians; several are already being tested in humans. It is reported that, on average, two to three antibiotics derived from micro-organisms are launched each year. After a downturn in the pace in recent decades, the pace is quickening again as scientists realise that the effective life span of any antibiotic is limited. Worldwide spending on finding new anti-infective agents (including vaccines) is expected to increase to 60 % from the spending levels in 1993. New sources, especially plant sources, are also being investigated (Cowan, 1999).

Secondly, the public is becoming increasingly aware of the problems of over-prescription and misuse of traditional antibiotics. In addition, many people are interested in having more autonomy over their medical care. A multitude of plant compounds (often of unreliable quality) is readily available over the counter from herbal suppliers and natural food stores, and self-medication with these substances is commonplace. The use of plant extracts, as well as other alternative forms of medical treatments enjoyed popularity in the late 1990's. Earlier in the 1990's approximately one third of people surveyed in the United States used at least one "unconventional" therapy during the previous year. It was reported in 1996 that sales of botanical medicines increased 37 % over 1995. It is speculated that the American public may be reacting to over-prescription of drugs, just as their predecessors of the 19th century reacted to the overuse of bleeding, purging and calomel (Cowan, 1999).

It is well known that plants exert defence mechanisms against micro-organisms, insects and herbivores. This defence strategy is partly attributed to the ability of plants to synthesise aromatic substances such as phenols or their oxygen-substituted derivatives.

Various investigators have tried to elucidate a mechanism for the microbiological activity of the above chemical groups. Phenols have been thought to exhibit their antimicrobial activity through the number and site of hydroxyl groups on the phenol group. An increased hydroxylation often results in increased antimicrobial activity. The antimicrobial activity

could include enzyme inhibition by the oxidized compounds through reaction with sulfhydryl groups or through non-specific interactions with proteins. Flavones exhibit their antimicrobial activity through their ability to form complexes with extracellular and soluble proteins and bacterial cell walls. However, with flavones there is no correlation between the degrees of hydroxylation to the level of antimicrobial activity. The mechanism of antimicrobial activity of terpenes is not fully understood but it is thought to involve membrane disruption by lipophilic compounds (Cowan, 1999).

The genus: Helichrysum

A plant group extensively used for its antibacterial properties throughout South Africa in traditional medicine is *Helichrysum*, more commonly known as “imphepo”.

Helichrysum (Asteraceae) is a large genus consisting of approximately 500 species of which 245 are indigenous to southern Africa. The South African *Helichrysum* species are classified into 30 groups based on their morphological diversity (Hilliard, 1983). The Asteraceae is the largest family of the fynbos biome (Cowling *et al*., 1992). Most traditional medicines used by indigenous communities in South Africa have been derived from plants belonging to this family (Leng *et al*., 1996).

Species are mostly distributed in or along forest margins of woodlands, in drier regions or rocky outcrops or even in open grassland. About 83 species predominate in the Free State, east of the Drakensberg escarpment in the former Basotho QwaQwa homeland, where they are subjected to high altitude, moist and warm climate, and conditions conducive for the proliferation of most microorganisms. Members of the genus are characterised as being aromatic perennial herbs with densely hairy or woolly leaves and persistent flower heads. The shape and size of the leaves and flower heads are characteristic, which differentiate the species (Hilliard, 1983).

Use of Helichrysum: Worldwide

Helichrysum species have been used in traditional medicine throughout the world and were already known in the Greek-Roman period (Ruberto *et al*., 2002). *Helichrysum* species have been used in folk medicine for thousands of years in areas as far apart as Europe, Egypt, North America, China and Australia (Jakupovic *et al*., 1989, Lwande *et al*., 1993).

In the Mediterranean regions, *H. italicum* is used widely and commercially for its essential oils (Angioni *et al.*, 2003, Nostro *et al.*, 2001, Pietta *et al*., 1991). These are mostly used for:

1. The healing of scar tissue by the prevention of bleeding and the formation of scar tissue.
2. The anti-inflammatory action and the ability to increase lymphatic flow and circulation, enhance wound healing and the prevention of scarring.
3. The oil is used in aromatherapy to help in detoxifying the body from the excesses of alcohol, nicotine and narcotic drugs. Other aromatherapy uses include improving circulation problems by decreasing inflammation of swollen vessels.

Use of Helichrysum: South Africa

Very little information is available on our indigenous medicinal plants. It has therefore become increasingly important to elucidate a scientific rationale for the use of many of the plant species that are being used traditionally by the people of southern Africa. The antibacterial properties of *Helichrysum* have been reported in literature (Bougatsos *et al.*, 2003; Başer *et al.*, 2002; Leng *et al.*, 1996; Meyer & Afolayan, 1995; Meyer & Dilika, 1996; Meyer *et al.*, 1997; Meyer & Lall, 1999; Jakupovic *et al*., 1986; Roussis *et al.*, 1999; Roussis *et al.*, 2000; and Van Puyvelde *et al.*, 1989).

In southern Africa the indigenous population have traditionally used *Helichrysum* species for their antimicrobial properties (Hutchings and Van Staden, 1994; Watt and Breyer-Brandwijk, 1962). They have been used in the treatment of wounds, infections and respiratory conditions (Hutchings, 1996; Van Wyk *et al.*, 1997; Watt and Breyer-Brandwijk, 1962).

Traditional uses of indigenous Helichrysum species:

All parts of the plant have been used in some form for their healing properties.

Table 1.1 provides a summary of some indigenous *Helichrysum* species and their traditional uses.

Table 1.1: List of traditional uses of indigenous *Helichrysum* species.

Species	Common use	Used by
H. aureonitens	Leaves and stems burned as incense to invoke the goodwill of the ancestors.	Zulu[1,2]
	Decoction of root used to treat enuresis.	Sotho[2]
H. caespititium	Inhalation of smoke from the burnt plant is used to treat head aches and chest colds.	South Sotho[1,2]
H. callicomum	Used in the preparation of an enema to treat colic.	South Sotho[1,2]

Species	Common use	Used by
H. cooperi	Dried leaves made into an ointment and used as an aphrodisiac.	Zulu[1,2]
H. crispum	Used to treat heart conditions and back ache.	Western Cape region[1,2]
H. cymosum subsp. *cymosum*	A tea is made with the leaves to treat coughs and colds. Leaves are burnt and the smoke inhaled to treat pain. Leaves are used to treat wounds as dressings. Used to prevent infection.	Xhosa and Zulu[3,4]
H. dasyanthum	Leaves and stems burned as incense to invoke the goodwill of the ancestors.	Zulu[1,2]
H. decorum	Dried plant is burnt to induce a trance during ritual practices.	Zulu[1,2]
H. foetidum	Leaves are used as wound dressings. Used to treat menstrual pains.	Zulu[1] Xhosa[2]
H. imbricatum	An infusion is used as a demulcent in coughs and also used to treat whooping cough.	Western Cape region[1,2]
H. nudifolium	A tea is prepared from the leaves to treat colds. Decoction of root is used as an infusion to treat catarrh, phthisis and chest conditions. Steam bath prepared from an infusion of the plant is used as an antipyretic.	Xhosa and Zulu[2]
H. odoratissimum	Leaves and stems used as incense to invoke goodwill of ancestors.	Xhosa and Zulu[1]
	Inhaled as protective cleansers and used in medicines for coughs and colds. Plant burnt in fat and used as an ointment to treat pimples. Plant is burnt to fumigate sickrooms.	Xhosa[1]
H. pedunculatum	Leaves are used as dressings during circumcision. Dressings made from leaves used to treat sores. Roots are used for coughs and colds.	Zulu and Xhosa[2]
H. psilolepis	Plant is used to treat menstruation complaints.	South Sotho[2]

References:
[1]Hutchings *et al.* 1996.
[2]Watt & Breyer-Brandwijk. 1962.
[3]Joffe . 2001.
[4]Van Wyk *et al.* 1997

Study objectives:

The elucidation of the chemical composition and the antimicrobial profiles of South African *Helichrysum* species have been studied for a limited number of species. Species that have been widely reported in literature include: *H. aureonitens, H. odoratissimum* and *H. pedunculutum* (Eloff, 1999, Lwande *et al.*, 1993, Meyer *et al.*, 1995; Meyer *et al.*, 1997, Zwaving *et al*, 1993). It is increasingly important to provide a scientific evaluation of many species of *Helichrysum*, as this plant group comprises a large portion of our indigenous flora that is extensively used as a traditional medicine.

This study investigates the composition of the essential oil of nine species of *Helichrysum.* The antimicrobial activities of the essential oil, as well as the solvent extracts (acetone and methanol) are investigated using the disc diffusion and MIC technique.

The specific objectives of this study include:

1. To determine the antimicrobial activity of the solvent extracts and the essential oils distilled from the nine indigenous *Helichrysum* species.
2. To determine the composition of the essential oils from selected species of *Helichrysum* by means of GC-MS and to correlate the antimicrobial activity to the essential oil chemistry.
3. To provide a scientific rationale for the traditional use of the *Helichrysum* species.

CHAPTER 2: MATERIALS AND METHODS

2.1 Collection of plant material:

A total of ten samples (nine species) of *Helichrysum* were collected. With the exception of *H. petiolare* which was collected from the Pretoria National Botanical Garden, all species were collected from natural populations in South Africa. The South African National Biodiversity Institute (SANBI) confirmed the identity of all collections. These species and their specific origin are given in Table 2.1.

Table 2.1: Collection data of the species studied.

Voucher	Species	Locality
JV 2831	*H. asperum* (Thunb.) Hilliard & B.L. Burtt var. *albidulum* (D.C.) Hilliard	Northern base of Robinson's Pass near Moeres River, Oudtshoorn
DBH 6565	*H. callicomum* Harv.	Robinson's Pass, Oudtshoorn
JV 2826	*H. cymosum* subsp. *cymosum* (L.) D.Don	Outeniqua Mountains, near the top of Robinson's Pass, Oudtshoorn
JV 2829	*H. dasyanthum* (Wild.) Sweet	Near the top of Robinson's Pass, Oudtshoorn
JV 2830	*H. excisum* (Thunb.) Less.	Northern base of Robinson's Pass near Paardepoort, Oudtshoorn
JV 2828	*H. felinum* Less.	Outeniqua Mountains about 1km west of Robinson's Pass, Oudtshoorn
JEV 2437	*H. odoratissimum* (L.) Sweet	Amatolas
JEV 2440	*H. odoratissimum* (L.) Sweet	Amatolas
DBH 6567	*H. petiolare* Hilliard & B.L. Burtt	Pretoria National Botanical Garden
JEV 2436	*H. rosum* (P.J. Bergius) Less. var. *rosum*	Amatolas

2.2 Phytochemistry:

2.2.1 Isolation of essential oils:

Hydrodistillation of the fresh aerial parts was carried out using a modified Clevenger-type apparatus (500 mL of water) for a period of 2 ½ hours to isolate the essential oils. The temperature of the apparatus was kept constant at 104 °C. The pure essential oils were refrigerated in amber glass vials.

2.2.2 Preparation of the non-volatile extracts:

Plant material was air dried at 30 °C. The dried plant material was subsequently crushed to a fine powder to enable maximum surface area coverage for the extraction process. At room temperature of 25 °C approximately 5 g of the dried plant sample from each species was submerged separately in approximately 125 ml of acetone and 125 ml of methanol for approximately 2 ½ hours. The extraction process was repeated. The solvent was evaporated on a Rotavap (Buchi).

2.2.3 Thin-layer chromatography (TLC):

To assess patterns of variation the essential oils and solvent extracts were initially investigated by thin-layer chromatography (TLC). The essential oils were diluted with hexane (Rochelle Chemicals) in the ratio 1:7. The solvent extracts required dilution with either methanol or acetone. Using a calibrated micro-capillary tube approximately 2 µl was spotted onto silica plates (silica gel 60 with fluorescent indicator UV_{254}. layer: 0,2 mm. Macherey-Nagel, Germany). For the essential oils, the mobile phase in which the plates were developed comprised toluene:ethyl acetate in the ratio (93:7). The mobile phase for the solvent extracts comprised toluene:dioxane:acetic acid in the ratio (90:25:3). The spotted silica gel plate was placed in a glass developing chamber for between 10-20 minutes to allow for separation. The plates were viewed under UV light (366 nm and 254 nm). The plates used for the essential oils were sprayed with vanillin-sulphuric acid (1 % ethanolic vanillin + 10 % ethanolic sulphuric acid) and the plates containing the solvent extracts were sprayed with natural products reagent. The plates were dried in the oven at 110 °C for 10 minutes.

2.2.4 Gas chromatography/mass spectroscopy (GC-MS):

The GC-MS of the essential oils was conducted during my visit to Anadolu University, Turkey at the Medicinal and Aromatic Plant and Drug Research Centre (TBAM). These oils were analysed using a Hewlett-Packard GCD system. A Hewlett-Packard Innowax column (60 m x 0.25 mm Ø. with 0.25 µm film thickness) with helium as a carrier gas was used. The GC injection port temperature was 250 °C and the column temperature was kept at 60 °C for 10 min and programmed to 240 °C at a rate of 4 °C/min for a total of 80 minutes. The split ratio was 50:1 with electron energy of 70 eV and a mass range of 35-425 *m/z*. Relative percentage amounts of the separated compounds were calculated automatically from peak areas of the total ion chromatogram, and library search for identification of the compounds was carried out using commercial libraries and the Başer Library of Essential Oil Constituents.

2.3 Antimicrobial assays:

The essential oils, acetone and methanol extracts were tested for activity against bacterial and fungal strains. Oils or extracts were initially screened using a disc diffusion assay to determine the level of activity. Samples yielding promising activity in the disc diffusion assay were further evaluated by the minimum inhibitory concentration (MIC) technique.

2.4 Disc diffusion assay:

2.4.1 Reference test strains:

Antimicrobial disc diffusion assays were performed using the hydrodistilled essential oils and extracts. All antimicrobial tests were carried out under aseptic conditions. All assays were conducted in duplicate.

Table 2.2: Test organisms included in the study

Gram-negative test organisms	*Escherichia coli* *Yersinia enterocolytica* *Klebsiella pneunomiae*	ATCC 11775 ATCC 23715 ATCC 13883
Gram-positive test organisms	*Staphylococcus aureus* *Bacillus cereus*	ATCC 12600 ATCC 11778
Yeast test organisms	*Cryptococcus neoformans* *Candida albicans*	ATCC 901125 ATCC 10231
Mould test organism	*Alternaria alternata*	Clinical strain

2.4.2 Preparation of cultures:

The growth medium used for the antimicrobial disc diffusion assays was Tryptone Soya agar (Oxoid), which was inoculated with the pathogens. This medium was prepared by dissolving 30 g of Tryptone Soya powder in 750 ml sterile water. The solution was sterilized by autoclaving at 121 ℃ for 15 minutes. A total of 200 ml of the Tryptone Soya agar was used for every tray. The agar plate was prepared by pouring the molten Tryptone Soya agar onto a sterile glass bottom tray and ensuring that the solution reached all corners of the tray to form a level surface. This formed the base layer that was allowed to cool. One milliliter of standard bacterial solution or fungal solution of inoculum (Mc Farlands standard) was incorporated into 100 ml of the agar solution and poured over the cool base layer to form the top layer and allowed to cool.

2.4.3 Method of disc diffusion:

Sterile paper discs (6 mm in diameter) were impregnated with 0.015 ml of essential oil or 128 mg/ml solution of the solvent extract. These discs were placed onto the set agar with a sterile

needle. The trays were turned upside down and the extract was allowed to pre-diffuse into the agar in a refrigerator (4 °C) for 1 hour. The trays containing the bacterial cultures were incubated for 24 hours and were kept at 37 °C. The mould cultures were incubated at 25 °C for 7 days and the yeast strains at 37 °C for 48 hours.

After the incubation period the zones of inhibition were recorded in millimetres from the edge of the paper disc to the edge of the inhibition zone.

Neomycin (30 µg/disc,Oxoid) and Nystatin (100 IU/disc, Oxoid) were used as positive controls for antibacterial and antifungal activity, respectively.

2.5 Minimum inhibitory concentration (MIC):

The MIC is the lowest concentration of sample that inhibits growth by visual inspection. A microtitre plate containing 96 wells (Figure 2.1) was aseptically prepared by incorporating 100 µl of sterile water and 100 µl of stock solution into row one of the microtitre plate, this was then diluted down the microtitre plate. Stock solution was prepared by dissolving the plant extracts in acetone or methanol. Serial dilutions were made (starting from the first well) to obtain concentrations ranging from 16 to 0.125 mg/ml (Fig. 2.1). A fixed bacterial culture (100 µl) of approximate innoculum size of 1 x 10^6 CFU/ml was added to all wells and incubated at 37 °C for 24 hours. After incubation a 0.02 mg/ml of *p*-iodo-nitrotetrazolium violet (INT) solution (Sigma) (40 µl) was added to each well (Eloff, 1998). Plates were examined after six hours to observe the colour change for the different concentrations. The concentration that inhibited bacterial growth completely, i.e. the first clear well, was taken as the MIC value.

	1	2	3	4	5	6	7	8	9	10	11	12	mg/ml
A	○	○	○	○	○	○	○	○	○	○	○	○	**16**
B	○	○	○	○	○	○	○	○	○	○	○	○	**8**
C	○	○	○	○	○	○	○	○	○	○	○	○	**4**
D	○	○	○	○	○	○	○	○	○	○	○	○	**2**
E	○	○	○	○	○	○	○	○	○	○	○	○	**1**
F	○	○	○	○	○	○	○	○	○	○	○	○	**0.5**
G	○	○	○	○	○	○	○	○	○	○	○	○	**0.25**
H	○	○	○	○	○	○	○	○	○	○	○	○	**0.125**

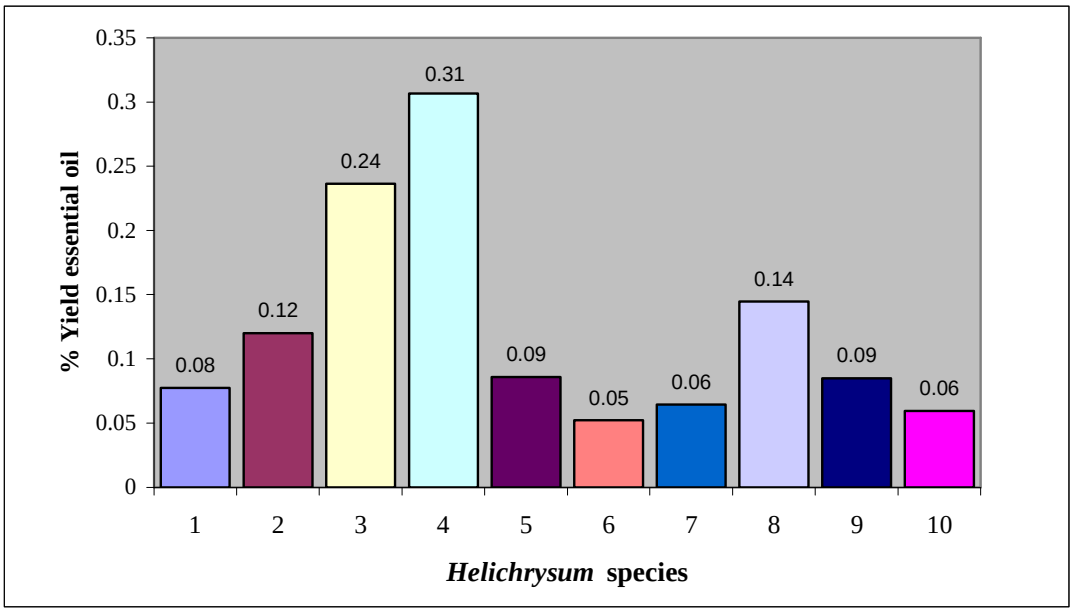
Figure 2.1: An example of an MIC template demonstrating the concentration of the dilutions used.

The INT solution (Eloff, 1998) is used to determine colour change in relation to concentration of microbial growth. The INT solution is used as an indicator of biological activity as the colourless compound acts as an electron acceptor and is reduced to a coloured product by biologically active organisms (Eloff, 1998). The contents of the well turned to red or purple as an indicator of the presence of biological growth. Each MIC value was determined in duplicate. A starting concentration of 0.01 mg/ml Ciprofloxacin (Merck) was used as a positive bacterial control.

CHAPTER 3: RESULTS AND DISCUSSION

3.1 Analytical chemistry: Essential oils

The essential oil yield is represented in Figure 3.1. Most species yielded very little essential oil. *H. dasyanthum* produced the highest amount of essential oil. Factors that may affect the yield of the essential oils obtained, include the location from which the samples were collected



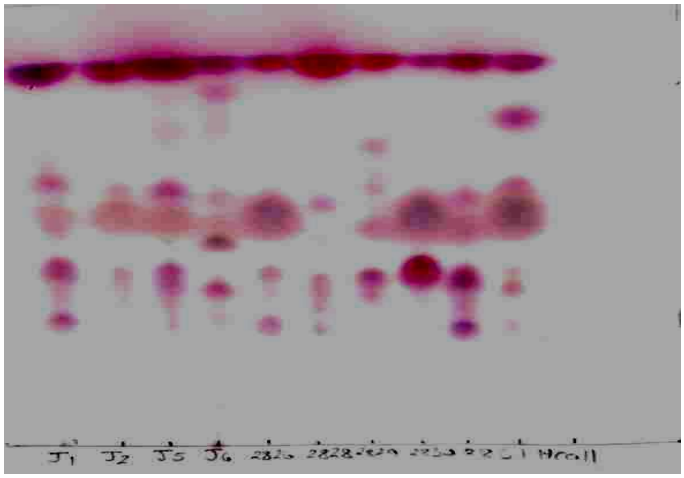
KEY:

1	*H .asperum*
2	*H .callicomum*
3	*H. cymosum subsp. cymosum*
4	*H. dasyanthum*
5	*H. exisum*
6	*H. felinum*
7	*H. odoratissimum* JEV 2437
8	*H. odoratissimum* JEV 2440
9	*H. petiolare*
10	*H. rosum*

Figure 3.1: Graphical representation of the yield of the essential oil (%) obtained through distillation.

The essential oil composition of the eight indigenous *Helichrysum* species was initially asessed by thin layer chromatography (TLC), Figure 3.2. This technique was able to illustrate compositional similarities and differences between the taxa.

Chromatography is an analytical method widely used for the separation and identification of compounds in complex mixtures. Thin layer chromatography uses a specific solvent system as a mobile phase that allows for the separation of different components present in the mixture based, on their polarity. This method is useful for preliminary screening, but not as accurate as gas chromatography which allows for an expeditious quantitative and qualitatve assessment of the sample. Gas chromatography coupled with mass spectrometry (GC-MS) was used to accurately quantify the composition of the essential oils. The results are presented in Table 3.1.



1 2 3 4 5 6 7 8 9 10

KEY:

1	*H. rosum*
2	*H. odoratissimum* (JEV 2437)
3	*H. odoratissimum* (JEV 2440)
4	*H. petiolare*
5	*H. cymosum* subsp. *cymosum*
6	*H. felinum*
7	*H. dasyanthum*
8	*H. excisum*
9	*H. asperum*
10	*H. callicomum*

Figure 3.2: TLC plate of essential oils of *Helichrysum* species studied.

Table 3.1: Chemical composition (%) of the essential oils determined by GC-MS

RRI	Retention time	Compound	Ha	Hc	Hcs	Hd	He	Hf	Ho1	Ho2	Hp	Hr
1014	8.1	Tricyclene	tr									tr
1032	8.5	α-Pinene	3.5	3.6	12.4	16.6	2.8		4.8	8.3	6.8	4.7
1072	9.9	α-Fenchene	tr		0.1	0.1					0.7	
1076	10.3	Camphene	0.2	0.2	0.4	0.3	Tr			0.3	0.4	0.2
1080	11.0	Hexanal	tr									
1118	12.2	β-Pinene	3.5	6.2	3.7	6.2	1.1		1.0	1.9	0.4	16.1
1132	12.8	Sabinene			0.2	tr	0.2					0.3
1122	12.9	Thuja-2,4-(10)-diene	0.1									
1174	14.9	Myrcene	0.2	0.2	1.2	1.0	0.7		0.4	1.0	0.5	tr
1188	15.7	α-Terpinene			1.2	3.5					0.1	
1203	16.8	Limonene	6.2	1.2	7.2	6.1	2.2		11.6	19.6	3.1	5.2
1213	17.3	1,8-Cineole	10.8	34.6	20.4	20.6	34		11.2	17.1	22.4	10.9
1244	18.1	2-Pentylfuran		tr								
1246	18.2	(*Z*)-β-Ocimene			9.5	0.9			0.5	0.2	0.1	
1239	18.5	Methyl isoheptanoate	0.4									
1255	18.7	γ-Terpinene			1.4	3.5			0.3		1.0	
1266	19.0	(*E*)-β-Ocimene			3.6	0.2					tr	
1280	19.9	*p*-Cymene	2.7	6.2	2.4	5.5	6.5		1.0	3.7	9.8	2.6
1290	20.4	Terpinolene			0.4	0.4					0.3	
1282	20.5	Heptenylacetate								0.1		
1282	20.5	2-Octanone	0.2									0.1
1286	20.9	2-Methyl butyl-3-methyl butyrate					0.7					
1320	21.8	2-Heptanol			0.1							
1327	21.8	(*Z*)-3-Hexenyl acetate	tr									
1348	22.7	6-Methyl-5-hepten-2-one		tr		tr	0.1			tr	tr	0.1
1386	23.2	1-Hexanol	tr	tr	tr						tr	
1360	23.7	2-Octylacetate	tr									
1385	24.2	Heptylacetate				0.1						
1382	24.2	*cis*-Alloocimene			0.4							
1384	24.3	α-Pinene oxide	tr	0.1							tr	
1386	24.4	1-Octenyl acetate				tr			1.1	1.7		
1391	24.4	(*Z*)-3-Hexen-1-ol		tr	0.1					0.1		
1393	24.8	3-Octanol								tr		0.6
1398	24.8	2-Nonanone	0.6		0.4							1.8
1400	25.1	Nonanol	0.4									
1406	25.1	α-Fenchone									0.1	
1413	25.2	Rosefuran								tr		
1412	25.8	2-Octanol	0.4		0.5							0.2
1429	25.9	Perillen								tr		0.1
1423	26.2	Hexyl-2-methyl butyrate				tr						
1435	26.2	γ-Campholene aldehyde	0.1									
1452	26.7	*p*-Cymenene = α,*p*-Dimethylstyrene		tr		0.1				tr	0.1	
1450	26.7	*trans*-Linalool oxide (furanoid)	0.1									0.1
1452	26.9	1-Octen-3-ol			0.3	0.1		0.1	0.1	0.4	0.1	

RRI	Retention time	Compound	Ha	Hc	Hcs	Hd	He	Hf	Ho1	Ho2	Hp	Hr
1443	27.0	Methylnonanone	1.4									
1449	27.1	1-Heptanol									tr	
1468	27.4	*trans*-1,2-Limonene epoxide	0.5							0.1		
1474	27.5	*trans*-Sabinene hydrate										0.6
1479	27.6	Furfural		tr								
1482	27.7	Fenchylacetate				0.5				0.1		
1478	27.8	*cis*-Linalool oxide (furanoid)										0.1
1492	28.3	Cyclosativene	0.1				0.1					
1493	28.4	α-Ylangene		0.3	0.1			0.4		0.4		
1495	28.5	α-Amorphene					1.3	1.0				
1497	28.8	α-Copaene	4.0	0.8	1.2	0.1	2.5	4.0	3.2	4.6	1.3	0.7
1506	29.3	Hexyl valerate									tr	
1509	29.3	2-Decanol	0.3									
1510	29.3	2-Nonanol			0.1							0.1
1532	29.5	Camphor	0.3			tr					0.1	
1535	29.6	β-Bourbonene			0.6			tr				0.4
1541	29.7	Benzaldehyde	0.1		0.1							
1525	29.8	Decanone		1.0								
1544	30.0	α-Gurjunene			0.1		0.1	1.0		0.1	0.1	
1553	30.3	Linalool	0.5		0.1			0.2				0.3
1556	30.4	*cis*-Sabinene hydrate										0.6
1557	30.4	Italicene							1.5	0.1	1.0	
1565	30.7	Linalyl acetate	0.2									
1568	30.8	1-Methyl-4-acetyl-cyclohex-1-ene		6.6						tr		
1566	30.8	α-Santalene = β-gurjunene					0.8					
1571	30.9	*trans-p*-Menth-2-en-1-ol	0.2									
1573	31.0	Isoitalicene									0.1	
1586	31.3	Pinocarvone	0.6	0.1	0.1		0.1					0.9
1591	31.5	Fenchyl alcohol	0.4	0.7	0.7	0.5	0.3				1.5	
1597	31.6	Bornyl acetate								0.2		
1582	31.7	Nopinone	1.5									0.9
1594	31.7	*trans*-β-Bergamotene		0.2			0.1	0.9	0.8	0.6		
1604	32.0	Thymol methyl ether									0.6	
1617	32.2	Terpinen-4-ol	0.3									0.3
1612	32.3	β-Caryophyllene		8.4	10.8	13.3	5.7	27.6	25.2	9.3	14	
1628	32.6	Aromadendrene	3.0		1.5			2.2	0.8	0.4	0.3	2.5
1632	32.9	Selina-5,11-diene			0.3			0.7	0.8	0.9		
1638	33.0	*cis-p*-Menth-2-en-1-ol					0.1					
1642	33.3	Thuj-3-en-10-ol										2.1
1648	33.3	Myrtenal	2.2	0.1			0.1			tr		0.1
1661	33.8	Alloaromadendrene					0.7	7.3	1.7	1.9		
1664	33.9	*trans*-Pinocarveol	3.8	0.4	0.6	0.2					0.4	3.1
1674	34.0	γ-Gurjunene						0.1			0.1	
1678	34.3	*cis-p*-Mentha-2,8-diene-1-ol								0.1		
1682	34.3	δ-Terpineol	0.2	0.2	0.9	0.2					tr	0.1
1687	34.5	α-Humulene		1.9	1.3	0.6	0.2	9.4	7.2	4	2	
1683	34.5	*trans*-Verbenol					0.7					0.7
1694	34.8	Limonen-4-ol			0.2							

RRI	Retention time	Compound	Ha	Hc	Hcs	Hd	He	Hf	Ho1	Ho2	Hp	Hr
1698	34.9	Myrtenyl acetate						0.8				
1695	34.9	(*E*)-β-Farnesene						tr				
1704	35.0	γ-Curcumene							5.4			
1704	35.0	γ-Muurolene						1.8		0.6	2.2	
1706	35.1	α-Terpineol	1.3		2.6	0.5	0.8				5.1	1.1
1719	35.2	Borneol	1.0		1.8	0.3	1.2				3.3	0.6
1707	35.5	β-Chamigrene								0.1		
1725	35.7	Verbenone	0.6									0.4
1596	35.7	α-Guaiene				0.1						
1729	35.7	*cis*-1,2-Epoxy-terpin-4-ol		0.1			0.1				0.4	
1741	35.8	δ-Guaiene			1.1				0.4			
1740	35.8	Valencene		0.3				3.0				
1741	35.9	β-Bisabolene				1.5				0.4		
1733	35.9	Nerylacetate	0.1									
1726	36.0	*cis*-α-Bisabolene							1.3			
1741	36.1	β-Bisabolene		0.9								
1740	36.1	α-Muurolene	tr	0.8	0.3							
1742	36.1	α-Selinene								1.7		1.3
1751	36.5	Carvone	0.8	0.1						0.4		0.5
1758	36.5	*cis*-Piperitol	0.1				0.1			0.1		
1765	36.8	Geranyl acetate									0.1	
1771	36.9	γ-Bisabolene							0.6	0.2		
1773	37.0	δ-Cadinene		0.2	0.6	0.2	0.3	2.5	2.0	0.3	0.7	
1776	37.1	γ-Cadinene	0.1	1.4			0.1	2.0		0.2		0.1
1782	37.3	*cis*-Carvylacetate							0.1	0.2		
1786	37.4	ar-Curcumene							3.9		0.6	
1797	37.6	*p*-Methylacetophenone	0.5				tr					0.1
1798	37.6	α-Dihydroagarofuran				3.4						
1796	37.8	Selina-3,7-(11)-diene							1.1	1.7	0.6	
1804	37.9	Myrtenol	3.4	0.2	0.6		0.1					2.0
1810	38.1	3,7-Guaiadiene		0.2				0.2			0.1	
1819	38.6	2-Phenyl ethyl acetate			tr	0.1				0.1		
1845	38.9	*trans*-Carveol	0.9	tr	0.2	0.1				0.2	0.1	0.4
1849	39.2	Calamenene		0.5	0.6	tr	0.1	0.8	0.2	0.5	0.3	
1864	39.3	*p*-Cymen-8-ol	0.7	0.2	0.1	0.1	0.2			0.2	0.3	0.4
1868	39.6	(*E*)-Geranyl acetate	0.3				0.3	0.1		tr	0.1	0.3
1882	39.8	*cis*-Carveol	0.2							0.1		0.2
1867	39.9	Benzylacetone			0.3							
1889	40.1	Ascaridol				0.1						
1885	40.4	Isoshyobunone	0.1									
1896	40.4	*cis-p*-Mentha-1(7), 8-dien-2-ol			tr							
1918	41.1	β-Calacorene					0.2	tr				
1933	41.3	Nerylvalerate									0.3	
1941	41.5	α-Calacorene	0.2	0.1	0.1	0.5	0.1	0.5	0.1	0.1	0.1	
1953	41.8	Palustrol				0.2					0.3	
1984	42.7	γ-Calacorene		0.1				tr				
2001	43.1	Isocaryophyllene oxide	1.1	1.6	0.1	0.2	0.4	0.2	0.1	1.3	0.6	1.4
2008	43.4	Caryophyllene oxide	7.8	7.7	1.6	1.3	2.1	6.9	4.9	5.9	2.5	10.3

RRI	Retention time	Compound	Ha	Hc	Hcs	Hd	He	Hf	Ho1	Ho2	Hp	Hr
2029	43.8	Perilla alcohol						0.2				
2033	43.9	Epiglobulol	0.3		0.3			0.6				0.5
2045	44.1	Humulene epoxide-I		0.3				0.3	0.5	0.2	0.1	0.1
2050	44.3	(*E*)-Nerolidol				0.2	0.8				0.1	
2057	44.4	Ledol				0.3		0.6			0.2	
2088	44.8	6-Epi-cubenol	2.6				1.2					
2071	44.8	Humulene epoxide-II		2.3	0.2			1.7	1.3	1.7	0.7	8.5
2073	44.9	β-Caryophyllene alcohol			0.4	0.1		1.4			1.9	
2081	45.1	Humulene epoxide-III								0.1		
2088	45.2	1-Epi-cubenol							0.1			0.4
2080	45.2	Cubenol	0.1	0.6			0.1	1.0				
2088	45.5	Ascaridol glycol	2.2			0.5						
2098	45.4	Globulol		0.2	0.6			1.4			0.2	1.2
2104	45.6	Viridiflorol	0.8	0.1	0.8		18.2	0.7	1.0	0.8	0.9	0.5
2113	45.8	Cumin alcohol		1.0								
2144	46.2	Rosifoliol			0.2			0.4				
2131	46.5	Hexahydrofamesyl acetone		0.2								
2145	46.6	Valeranone				tr						
2144	46.6	Spathulenol	3.5					0.6		0.1	0.2	1.1
2170	47.1	β-Bisabolol							0.3		0.8	
2184	47.4	*cis*-*p*-Menth-3-en-1,2-diol			0.1	0.3				0.1		
2187	47.6	*T*-Cadinol		0.5			0.1	0.6	0.2	0.1	0.2	
2193	47.7	β-Betulenol				0.5						
2203	47.8	Copaborneol		0.1			3.4	2.2				
2209	48	*T*-muurolol		0.1								
2200	48.2	Valerianol						0.9				
2238	48.2	Clovenol	0.2		0.1	0.1			0.1		0.1	
2204	48.2	Torreyol		0.3								
2239	48.4	Carvacrol		0.1			0.2		0.1	tr	0.1	
2240	48.6	Pogostol				0.2						
2255	49.1	α-Cadinol		0.5	0.1			1.0			0.2	
2256	49.1	Cadalene				0.1				0.1		
2257	49.1	β-Eudesmol	0.4				0.1					
2264	49.5	4,7-Dimethyl-1-tetralone		tr								
2310	49.5	14-Acetoxy-β-caryophyllene				0.2						
2269	49.6	Porosadienol							0.2	0.3		
2289	50.0	*oxo*-α-ylangene	0.7									0.1
2316	50.5	Caryophylladienol-I	tr	0.1	tr			0.2				
2308	50.5	9-Geranyl-*p*-cymene							0.7	1.1		
2324	50.6	Caryophylladienol-II	0.3	0.6	0.4	0.5	0.4	1.5			0.4	0.1
2346	51.2	Kaur-15-ene									0.3	
2389	51.5	Caryophyllenol-I			0.2		0.2	0.8	0.4		0.2	
2390	52.2	10-Hydroxy calamenene		0.4			0.2	0.1		0.2		
2392	52.4	Caryophyllenol-II	0.8	0.3	0.1	0.2		1			0.3	0.8
2404	52.9	4-Isopropyl-6-methyl-1-tetralone		0.1								
2438	53.5	Kaur-16-ene				0.7					1.3	
2498	55.1	1-Heptadecanol						0.2				
2518	55.7	*cis*-Lanceol					0.4					

Abbreviations:

RRI = Relative retention indices
tr = trace value < 0.01 %
Ha = *H. asperum*
Hc = *H. callicomum*
Hcs = *H. cymosum* subsp. *cymosum*
Hd = *H. dasyanthum*
He = *H. excisum*
Hf = *H. felinum*
Ho1 = *H. odoratissimum* (JEV 2437)
Ho2 = *H. odoratissimum* (JEV 2440)
Hp = *H. petiolare*
Hr = *H. rosum*

Table 3.2: Major compounds identified by GC-MS:

Monoterpene hydrocarbons	**Sesquiterpene hydrocarbons**	**Oxygenated sesquiterpenes**
α-Pinene	β-Caryophyllene	Caryophyllene oxide
Camphene	Aromadendrene	Humulene epoxide II
β-Pinene	α-Humulene	Viridiflorol
Myrcene	δ-Cadinene	Spathulenol
Limonene	*trans*-Carveol	T-Cadinol
1.8-Cineole	*cis*-Calamenene	Caryophylladienol II
p-Cymene	*p*-Cymen-8-ol	Caryophyllenol II
α-Copaene	(*E*)-Geranyl acetate	
	α-Calacorene	
	iso Caryophyllene oxide	

The major compounds identified through GC-MS are presented in Table 3.2. The most abundant compounds identified in the essential oils were 1,8 cineole, α-pinene, β-pinene, β-caryophyllene and caryophyllene oxide.

The high concentrations of 1,8-cineole (average = 20.2 %, found in all species except *H. felinum*) and β-caryophyllene (average = 14.2 %, found in all species except *H. asperum* and *H. rosum*) were similar to that of the *Helichrysum* species studied in Madagascar (*H. bracteiferum, H. cordifolium and H. rusillonii*). In the Madagascan species the major compounds identified were 1,8-cineole, α-humulene and β-caryophyllene (Başer *et al.*, 2002).

β-caryophyllene has been reported as a major component in *H. kraussii, H. heldreichii, H. stoechas* subsp. *barrelieri* and *H. odoratissimum* (Bougatsos *et al.*, 2003).

Among the minor compounds identified, the most abundant monoterpenes were α-pinene, β -pinene, camphene, myrcene, limonene, and *p*-cymene. The most abundant sesquiterpenes were α-copaene, aromadendrene, α-humulene, δ-cadinene, *trans*-carveol, *cis*-calamenene, *p*-cymen-8-ol, (*E*)-geranyl acetate, α-calacorene, and iso-caryophyllene oxide. These findings correlate with those reported for *H. italicum* (Manitto and Monti, 1972).

Terpinen-4-ol has been identified in *H. rosum* (0.3%) and *H. asperum* (0.3%). This compound has also been found in *H. gymnocephalum* and *H. bracteiferum* (Ruberto *et al.*, 2002).

The hydrocarbons α-pinene and β-pinene predominated in the *Helichrysum* species studied. Their predominance has also been reported in *H. italicum* (Manitto and Monti, 1972). β-Caryophyllene, α-pinene and α-humulene has been reported in *H.* var. *scadens* and *H.* var. *spathulatum* (Roussis *et al*., 2000).

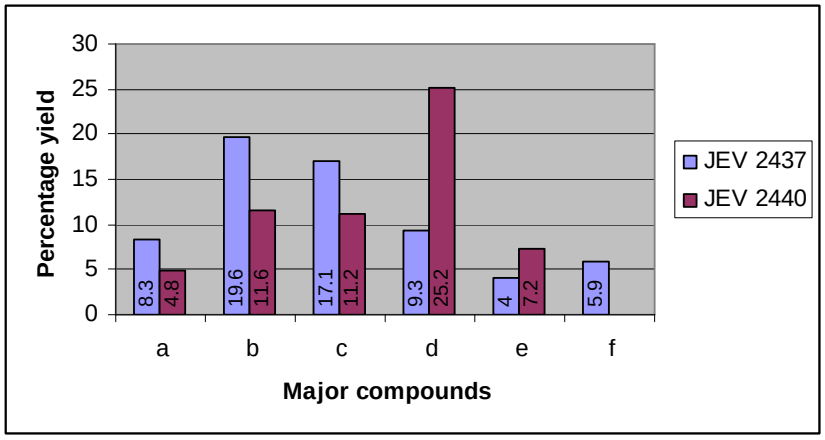
A characteristic compound, neryl acetate, found in *H. italicum* has also been found in *H. asperum.* Linalool and γ-curcumene have been identified in *H. cymosum, H. felinum, H. rosum* and *H. asperum.* The presence of γ-curcumene (5.4 %) that has been detected in *H. odoratissimum* (JEV 2437) has been associated with the turmeric-like odour that is characteristic of this plant (Ruberto *et al.*, 2002).

The commercially important essential oil of *H. italicum* has been widely studied and documented in literature. However, the essential oil of *H. italicum* does differ from the indigenous species studied in that it is composed mainly of sesquiterpene hydrocarbons rather than oxygenated monoterpenes (Bianchini *et al.*, 2003).

Another difference in the chemical composition of the essential oils from this investigation is that these essential oil profiles are characterised by mono- and

sesquiterpene hydrocarbons and oxygenated sesquiterpenes. The Australian *Helichrysums* are characterised by diterpenes (Jakupovic *et al.*, 1989). This correlates with results previously obtained from other South African species (Lwande *et al.*, 1993; Ramanoelina *et al.* 1991).

Two samples of *H. odoratissimum* were investigated in this study. This was done to investigate if there were any chemotypic variations in the chemical composition of these samples growing in different locations within the same geographical area. A study on *H. odoratissimum* reported α-pinene, (*E*)-farnesol and α-humulene as the major constituents (Lwande *et al.*, 1993). Another study on *H. odoratissimum* reported α-pinene, α-humulene, β-caryophyllene, elemol, β-himachalene and 1,8-cineole as the major components of the oils (Zwaving, 1993). It has been reported that there are approximately equal levels of monoterpenes and sesquiterpenes in *H. odoratissimum*, with α-pinene, α-curcumene and β-caryophyllene being in the highest concentration respectively (Ruberto, *et al.*, 2002 and Kuiate *et al.*, 1999). These findings were consistent with the chemical composition of the *H. odoratissimum* reported by Zwaving (1993) and Lwande *et al.*, (1993).



KEY

a	α-Humulene
b	Limonene
c	1,8 Cineole
d	β-Caryophyllene
e	α-Humulene
f	Caryophyllene oxide

Figure 3.3: The major compounds in *H. odoratissimum* JEV 2437 & *H.odoratissimum* JEV 2440 essential oils.

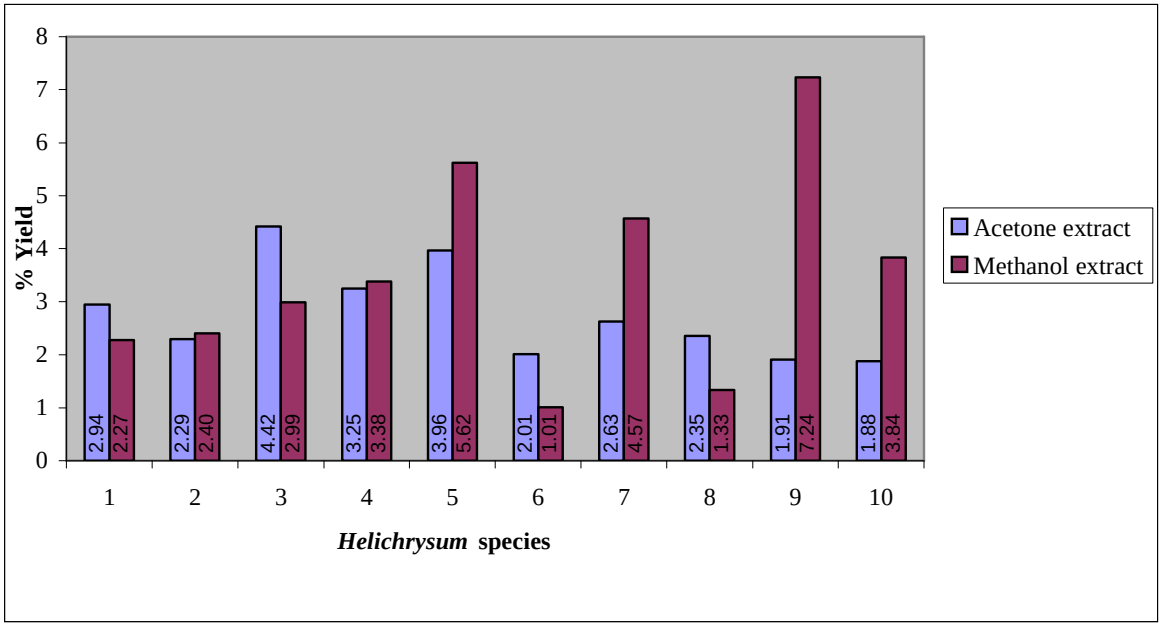
The results (Figure 3.3) show that in general the composition is similar to the characteristic predominance of equal amounts of mono- and sesquiterpene hydrocarbons as described above. The difference was mostly quantitative.

It is difficult to attribute a specific cause to the variation of essential oil profiles of plants belonging to the same species but from different localities. Hypotheses on ecosystem differentiation and its effect on inter-population variation have been proposed. However due to the ability of a species to adapt to its environment makes these hypotheses difficult to prove (Martin & Puech, 2001).

3.2: Analytical chemistry: Phenolic extracts

There was no correlation between the yields produced for the acetone and methanol extracts. *H. asperum* showed a distinct difference where the yield of the methanol extract was approximately double that of the acetone extract.

The yield of the acetone extract produced by the two samples of *H. odoratissimum* was similar to that observed with the essential oil extracts. Sample JEV 2440 produced nearly double the yield of sample JEV 2437. However the methanol yield of these two samples was similar. Both these samples were collected from different localities within the same geographical area.

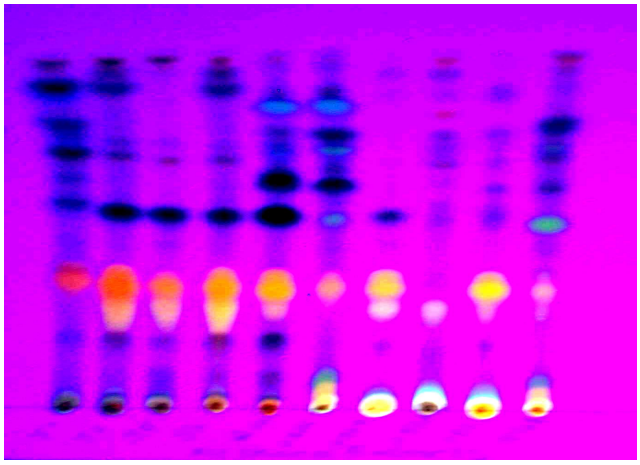


KEY

1	*H. rosum*
2	*H. odoratissimum* JEV 2437
3	*H. odoratissimum* JEV 2440
4	*H. petiolare*
5	*H. felinum*
6	*H. cymosum subsp. cymosum*
7	*H. dasyanthum*
8	*H. exisum*
9	*H. asperum*
10	*H. callicomum*

Figure 3.4: Percentage yield of extracts using methanol and acetone.

A TLC plate was developed (Figure 3.5) for the acetone extracts. Chemical variation was noted between the species. The chromatogram of both the essential oil and acetone extract of *H. felinum* was distinctly different to that of the other species.



KEY:

1	*H. rosum*
2	*H. odoratissimum* (JEV 2437)
3	*H. odoratissimum* (JEV 2440)
4	*H. petiolare*
5	*H. cymosum* subsp. *Cymosum*
6	*H. felinum*
7	*H. dasyanthum*
8	*H. excisum*
9	*H. asperum*
10	*H. callicomum*

Figure 3.5: TLC plate of essential oils of *Helichrysum* species studied.

3.3 Antimicrobial activity

Antimicrobial activity was determined by testing the essential oil and the phenolic extracts against Gram-positive, Gram-negative and fungal strains. These samples were initially screened for their antimicrobial activity using the disc-diffusion assay. The samples were screened against six bacterial and three fungal strains. The minimum inhibitory conecentration (MIC) was determined for the species displaying promising activity in the disc diffusion assay. The disc diffusion assay demonstrated that the extracts exhibited the best activity against *S. aureus* and *B. cereus* (Table 3.3). These pathogens were used in the MIC procedure. The results indicate that high activity in the disc diffusion assay does not always correlate to good activity in the MIC assay.

Table 3.3: Disc diffusion results for the essential oils and extracts tested against bacteria. Measurements are in millimetres from the edge of the disc to the edge of the inhibition zone.

Bacterial strain	**Extract / Essential oil**	**Neomycin control**	**Zones of inhibition (mm)**									
			H. asperum	***H. callicomum***	***H. cymosum* subsp. *cymosum***	***H. dasyanthum***	***H. excisum***	***H. felinum***	***H. odoratissimum* JEV 2437**	***H. odoratissimum* JEV 2440**	***H. petiolare***	***H. rosum***
B. cereus ATCC 11778	Acetone	6	5	5.5	8.5	14	9	7	12	11	4	9
	Essential oil	6	10	<1	0	2	15	0	0	0	0	<1
	Methanol	6	3	2.5	4	10	8	7	9	4	9	4
B. subtilis ATCC 6051	Acetone	6	0	0	0	0	0	0	0	0	0	0
	Essential oil	6	0	0	<1	0	0	0	0	0	0	0
	Methanol	6	<1	0	0	0	0	0	0	0	0	0
E. coli ATCC 1175	Acetone	6	0	0	0	0	0	0	0	0	0	0
	Essential oil	6	0	0	0	0	0	0	0	0	0	0
	Methanol	6	0	0	0	0	0	0	0	0	0	0

Bacterial strain	**Extract / Essential oil**	**Neomycin control**	**Zones of inhibition (mm)**									
			H. asperum	***H. callicomum***	***H. cymosum* subsp. *cymosum***	***H. dasyanthum***	***H. excisum***	***H. felinum***	***H. odoratissimum* JEV 2437**	***H. odoratissimum* JEV 2440**	***H. petiolare***	***H. rosum***
K. pneumoniae ATCC 13883	Acetone	6	0	0	0	0	0	0	0	0	0	0
	Essential oil	6	0	0	0	0	0	0	0	0	0	0
	Methanol	6	0	0	0	0	0	0	0	0	0	0
S. aureus ATCC 12600	Acetone	6	4	3	7	9	8	6	8	9	2.5	3
	Essential oil	6	0	0	0	0	0	0	0	0	<	<1
	Methanol	6	<1	2.5	5	5	4	5	5	3	7	<1
Y. enterocolitica ATCC 23715	Acetone	6	0	0	0	0	0	0	0	0	0	0
	Essential oil	6	0	0	0	0	0	0	0	0	0	0
	Methanol	6	0	0	0	0	0	0	0	0	0	0

0 indicates no apparent activity

.

Table 3.4: Disc diffusion results for the essential oils and extracts tested against fungi and yeasts. Measurements are in millimetres from the edge of the disc to the edge of the inhibition zone.

Strain	Extract / Essential oil	*Nystatin* Control	Zones of inhibition (mm)									
			H. asperum	***H. callicomum***	***H. cymosum* subsp. *cymosum***	***H. dasyanthum***	***H. excisum***	***H. felinum***	***H. odoratissimum* JEV 2437**	***H. odoratissimum* JEV 2440**	***H. petiolare***	***H. rosum***
A. alternata Clinical strain	Acetone	16	0	0	0	2	0	0	0	0	0	0
	Essential oil	16	0	0	0	0	0	0	0	0	0	0
C. albicans ATCC 10231	Acetone	16	2	0	0	3	0	1	0	3	0	0
	Essential oil	16	1	1	0	0	0	2	0	0	2	0
C. neoformans ATCC 901125	Acetone	16	0	0	0	1	0	2	0	2	0	0
	Essential oil	16	0	0	0	0	0	0	0	0	0	0

0 indicates no apparent activity

No antifungal activity was observed in the methanol extract.

Table 3.5: MIC results for essential oils and extracts tested against *S. aureus* and *B. cereus.*

Pathogen	Species	Extract / Essential oil	MIC (mg/ml)
S. aureus ATCC 12600	*H. asperum*	Acetone	<0.25
	H. callicomum	Acetone	<0.25
		Methanol	<0.25
	H. cymosum subsp. *cymosum*	Acetone	<0.25
		Essential oil	16
		Methanol	<0.25
	H. dasyanthum	Acetone	<0.25
		Essential oil	16
		Methanol	2
	H. excisum	Acetone	<0.25
	H. fellinum	Acetone	<0.25
	H.odoratissimum (JEV 2437)	Acetone	<0.25
		Methanol	<0.25
	H.odoratissimum (JEV 2440)	Acetone	<0.25
	H. petiolare	Acetone	<0.25
		Essential oil	8
		Methanol	<0.25
	H. rosum	Acetone	1
	Control	Ciprofloxacin	0.31×10^{-3}
B. cereus ATCC 11778	*H. calilcomum*	Acetone	0.5
		Essential oil	1
		Methanol	<0.25
	H. cymosum subsp. *cymosum*	Acetone	<0.25
		Essential oil	16
		Methanol	<0.25
	H. excisum	Acetone	<0.25
		Essential oil	8
		Methanol	<0.25
	H. odoratissimum (JEV 2437)	Acetone	<0.25
		Methanol	<0.25
	Control	Ciprofloxacin	0.3×10^{-3}

Good activity was exhibited only against *S. aureus* and *B. cereus.* No significant activity was exhibited against the other bacterial strains. These results were consistent with that reported by Meyer and Afloyan. (1997), where it was observed that the acetone extract of *H. aureonitens* was more active against Gram-positive bacteria than Gram-negative bacteria. This result also correlated with their earlier work where similar results were obtained with methanol and dichloromethane extracts (Meyer and Afolayan, 1995; Rios *et al*., 1991; Meyer *et al*., 2000; Roussis *et al*, 1999).

In general the antibacterial activity was the greatest for the acetone extract, whilst the essential oil produced the least activity in the disc diffusion assays. The most active species identified was *H. dasyanthum* (acetone and methanol) against *B. cereus* with an inhibition zone of 14 mm and 10 mm respectively.

The mechanism of action for the antimicrobial activity of the phenolic extracts of *Helichrysum* species has been postulated widely in literature. Van Puyvelde *et al*., (1999) associated the antimicrobial activity of *H. odoratissimum* to the presence of the flavonoids. The following flavonoids were identified to be present in this species: 3,5-dihydroxy-6,7,8-trimethoxyflavone and 3-*O*-methylquercetin, one chalcone and helichrysetin which was isolated from the methanol extract.

Nostro, *et al*. (2001) attributes the antimicrobial activity of the diethyl ether extract of *H. italicum* to flavonoids and terpenes. He demonstrated the basis of this activity in a previous study (Nostro, *et al*. 2000). The mechanism for the antimicrobial activity has been attributed to the lipophilic nature of flavonoids, which have an affinity for the cytoplasmic membrane of microorganisms and affect the structural and functional properties of the membrane (Nostro, *et al*. 2000).

Tomás-Barberán *et al*. (1990) associated the antimicrobial activity of three Spanish *Helichrysum* species (*H. decumbens*, *H. stoechas* and *H. italicum*) to phloroglucinol and acetophenone derivatives. He also proposed the mechanism of antibacterial activity to the lipophilicity of these compounds. Cosar and Cubukcu (1990) determined the antimicrobial activity of *H. italicum* to be due to the presence of flavonoids (apigenin, luteolin, kaempferol, astragalin and carringenin).

Gram-negative bacteria have an outer phospholipidic membrane due to structural lipopolysaccharide components. This makes the cell wall impermeable to lipophilic solutes, while porins constitute a selective barrier to hydrophilic solutes with an exclusion limit of about 600 Da. Gram-positive bacteria are more susceptible, having an outer peptidoglycan layer which is not an effective permeability barrier (Nostro, *et al*, 2000). This difference in permeability can account for the greater antibacterial activity against Gram-positive bacteria than Gram-negative bacteria.

The lipophilic nature of the phenolic extracts enables the compounds described above to act on the cytoplasmic membrane. The accumulation of lipophilic compounds in the cytoplasmic membrane of micro-organisms has considerable effects on the structural and functional properties of these membranes, which become increasingly permeable to protons and ions and lose their integrity (Nostro *et al*. 2001).

Phloroglucinols that have been identified in *Helichrysum* species have demonstrated antimicrobial activity against Gram-positive bacteria as well as some fungal species. Phloroglucinols have demonstrated no activity against Gram-negative bacteria (Meyer *et al*. 2000). The antimicrobial activity of the species studied correlate with the findings mentioned above.

It has been proposed by Van Vuuren *et al.* (2006) that two of the major compounds identified (Table 3.1), 1,8-cineole and α-pinene may be responsible for the anitmicrobial activity present in the essential oils.

H. dasyanthum produced the highest antimicrobial activity of the species studied (Table 3.4). The chemical composition of the essential oils of this species revealed the presence of flavonoids, kaurenic acid derivatives, cadinenes and guaianolides that have also been reported in literature (Jakupovic *et al*., 1989). These compounds contain phenolic hydroxyl groups that have been associated with antibacterial activity (Tomas-Barberan *et al*., 1990).

However, although very similar phenolic hydroxyl containing compounds were previously isolated from *H. felinum* and *H. petiolare* (Jakupovic *et al.*, 1989), the extracts from these plants were less active.

In a previous study on *H. kraussii* (Bremner and Meyer, 2000), the antimicrobial activity was attributed to prenyl-butyrylphloroglucinol and kaurenoic acids found in this species. Kaurenoic acid exhibited much higher activity than the phloroglucinol (which also contains phenolic hydroxyl groups) in the antimicrobial assay. A further

investigation may be needed to ascertain the association of kaurenoic acid derivatives with the antimicrobial activity.

Contrary to the results previously reported for other species of *Helichrysum* (Roussis *et al*., 2000), the essential oils of the species investigated showed little activity in the antimicrobial assays. The antibacterial activity of pure β-caryophyllene, α-pinene, and limonene was investigated in a study by Roussis *et al*. (2002). The antibacterial activity of these compounds was tested. It was found that limonene exerted no activity against the organisms tested, α-pinene exerted antibacterial activity that was similar to that of the species investigated whilst β-caryophyllene produced an MIC of >20 mg/ml. The above findings could partially explain the good antibacterial activity that was observed for the essential oils of *H. dasyanthum, H. asperum* and *H. excisum* (Table 3.3). *H. dasyanthum* contained the highest levels of α-pinene and β-caryophyllene.

Furthermore, the concentration of α-pinene, caryophyllene oxide and viridiflorol has been known to exhibit antibacterial activity (Bougatsos, *et al*., 2003). Although these compounds are common to most of the species in this study, high concentrations of viridiflorol was found only in *H. excisum.* Thus this compound may be associated with the antibacterial property of this species (Tables 3.3 and 3.4).

The activity of the acetone extract against the fungal strains was the most significant, with the methanol extract producing no activity. *H. dasyanthum* produced the greatest antifungal activity in the acetone extract by producing inhibition zones of 1 mm, 3 mm and 2 mm against against *C. neoformans, C. albicans, and A. alternata* respectively (Table. 4.5)

The compounds α- and β-bisabolene are known to have fungicidal activity against phytopathogenic fungi (Bougatsos, *et al.*, 2003). These compounds have been found in the following species that were investigated: *H. callicomum, H. odoratissimum, H. dasyanthum.* Of these species only *H. callicomum* exhibited antifungal activity against *C. albicans.* MIC was not conducted for the fungal strains due to the low activity that was exhibited in the disc diffusion assay.

The mechanism of the antifungal action due to lipophilicity is known to be as a result of the acidic hydroxyl groups that may act by uncoupling oxidative phosphorylation (Thomas-Barberan *et al*, 1990). The antifungal activity of flavones decreases when the methyl group at position five is removed. The presence of these compounds may explain the low antifungal activity that was observed (Thomas-Barberan *et al*., 1988).

CHAPTER 4: SPECIES MONOGRAPHS

4.1 *Helichrysum asperum* (Thunb.) Hilliard & B.L. Burtt var. *albidulum* (D.C.) Hilliard

4.1.1 Botanical description:

Tangled, twiggy, thin grey-woolly shrublet with woolly leaves that are often tufted on short shoots with brownish or white florets (Manning *et al*., 2001).

4.1.2 Distribution:

On stony slopes and flats. Distributed from Namibia to the Northern Cape coast and the Western Cape.

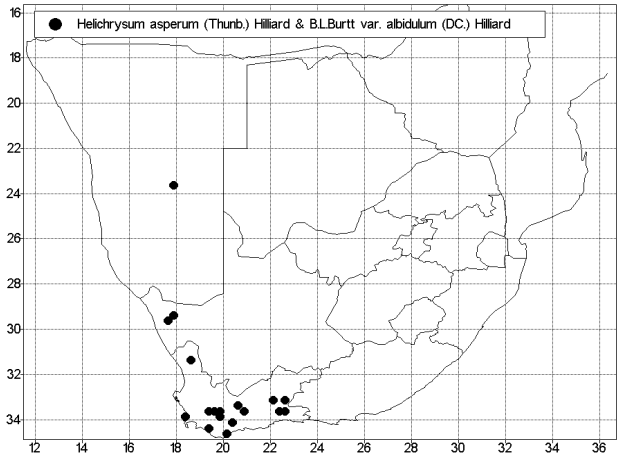


Figure 4.1: Geographical distribution of *H. asperum* var. *albidulum.*
(All distribution maps have been purchased and included with permission from the South African National Biodiversity Institute)

4.1.3 Essential oil composition:

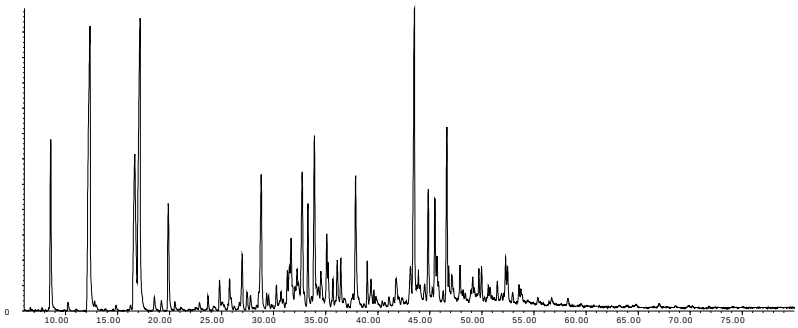


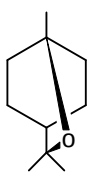
Figure 4.2: GC-MS chromatogram of *H. asperum* var. *albidulum* essential oil**.**

Table 4.1: GC-MS results: Essentail oil composition of *H. asperum* var. *albidulum.*

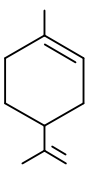
RRI	Retention time (minutes)	Compound	Area Percentage
1014	8.1	Tricyclene	tr
1032	8.5	α-Pinene	3.5
1072	9.9	α-Fenchene	tr
1076	10.3	Camphene	0.2
1080	11.0	Hexanol	tr
1118	12.2	β-Pinene	3.5
1122	12.9	Thuja-2,4-(10)-diene	0.1
1174	14.9	Myrcene	0.2
1203	16.8	Limonene	6.2
1213	17.3	1,8-Cineole	10.8
1239	18.5	Methyl isoheptanoate	0.4
1280	19.9	*p*-Cymene	2.7
1282	20.5	2-Octanone	0.2
1327	21.8	(*Z*)-3-Hexenyl acetate	tr
1384	24.3	α-Pinene oxide	tr
1398	24.8	2-Nonanone	0.6
1400	25.1	Nonanol	0.4
1412	25.8	2-Octanol	0.4
1435	26.2	γ-Campholene aldehyde	0.1
1450	26.7	*trans*-Linalool oxide = Furanoid	0.1
1443	27.0	Methylnonanone	1.4
1468	27.4	*trans*-1,2-Limonene-epoxide	0.5
1492	28.3	Cyclosativene	0.1
1497	28.8	α-Copaene	4.0
1509	29.3	2-Decanol	0.3
1532	29.5	Camphor	0.3
1541	29.7	Benzaldehyde	0.1
1553	30.3	Linalool	0.5
1565	30.7	Linalyl acetate	0.2
1571	30.9	*Trans-p*-Menth-2-en-1-ol	0.2
1586	31.3	Pinocarvone	0.6
1591	31.5	Fenchylalcohol	0.4
1582	31.7	Nopinone	1.5

RRI	Retention time (minutes)	Compound	Area Percentage
1617	32.2	Terpinen-4-ol	0.3
1628	32.6	Aromadendrene	3.0
1648	33.3	Myrtenal	2.2
1664	33.9	*trans*-Pinocarveol	3.8
1682	34.3	δ-Terpineol	0.2
1706	35.1	α-Terpineol	1.3
1719	35.2	Borneol	1.0
1725	35.7	Verbenone	0.6
1733	35.9	Nerylacetate	0.1
1751	36.5	Carvone	0.8
1758	36.5	*cis*-Piperitol	0.1
1776	37.1	γ-Cadinene	0.1
1797	37.6	*p*-Methylacetophenone	0.5
1804	37.9	Myrtenol	3.4
1845	38.9	*trans*-Carveol	0.9
1864	39.3	*p*-Cymen-8-ol	0.7
1868	39.6	(*E*)-Geranyl acetate	0.3
1882	39.8	*cis*-Carveol	0.2
1885	40.4	Isoshyobunone	0.1
1941	41.5	α-Calacorene	0.2
2001	43.1	*Iso* caryophyllene oxide	1.1
2008	43.4	Caryophyllene oxide	7.8
2033	43.9	Epiglobulol	0.3
2088	44.8	6-Epi-cubenol	2.6
2080	45.2	Cubenol	0.1
2088	45.5	Ascaridol glycol	2.2
2104	45.6	Viridiflorol	0.8
2144	46.6	Spathulenol	3.5
2238	48.2	Clovenol	0.2
2257	49.1	β-Eudesmol	0.4
2289	50.0	*oxo*-α-Ylangene	0.7
2316	50.5	Caryophylladienol I	tr
2324	50.6	Caryophylladienol II	0.3
2392	52.4	Caryophyllenol II	0.8
Total area percentage			**80.2**

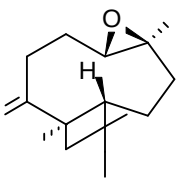
The major compounds in the essential oil of *H. asperum* var. *albidulum* are 1,8-cineole (10.8%), limonene (6.2 %) and caryophyllene oxide (7.8 %)



1,8-cineole



limonene



caryophyllene oxide

4.2 *Helichrysum callicomum* Harv.

4.2.1 Distribution:

Distributed widely over the interior parts of South Africa spreading from the Eastern Cape, along the Drakensberg Mountain region into Mpumalanga.

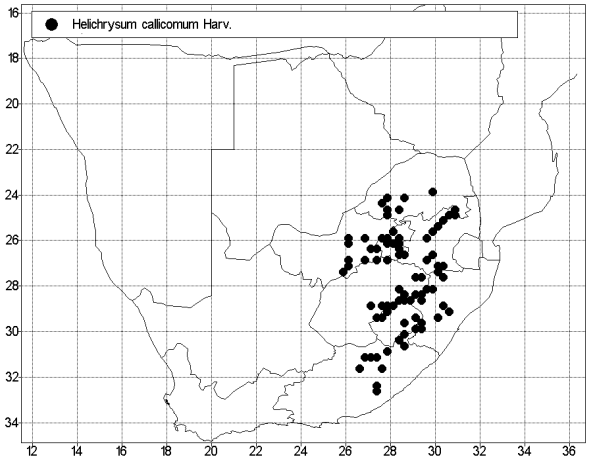


Figure 4.3: Geographical distribution of *H. callicomum.*
(All distribution maps have been purchased and included with permission from the South African National Biodiversity Institute)

4.2.2 Essential oil composition:

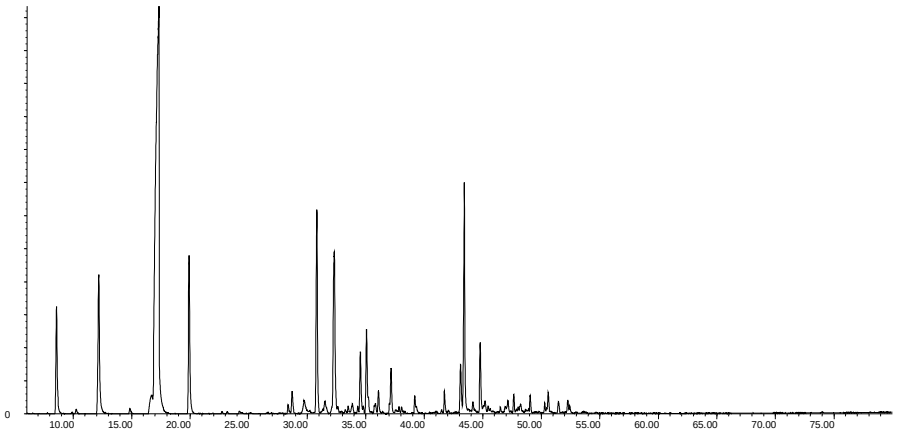


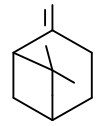
Figure 4.4: GC-MS chromatogram of *H. callicomum* essential oil.

Table 4.2: GC-MS results: Essential oil composition of *H. callicomum.*

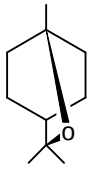
RRI	Retention time (minutes)	Compound	Area Percentage
1032	8.5	α-Pinene	3.6
1076	10.3	Camphene	0.2
1118	12.2	β-Pinene	6.2
1174	14.9	Myrcene	0.2
1203	16.8	Limonene	1.2
1213	17.3	1,8-Cineole	34.6
1244	18.1	2-Pentylfuran	tr
1280	19.9	*p*-Cymene	6.2
1348	22.7	6-Methyl-5-hepten-2-one	0.1
1386	23.2	1-Hexanol	0.1
1384	24.3	α-Pinene oxide	0.1
1391	24.4	(*Z*)-3-Hexen-1-ol	tr
1452	26.7	*p*-Cymenene = α,*p*-dimethylstyrene	tr
1479	27.6	Furfural	tr
1493	28.4	α-Ylangene	0.3
1497	28.8	α-Copaene	0.8
1525	29.8	Decanone	1.0
1568	30.8	1-Methyl-4-acetyl-cyclohex-1-ene	6.6
1586	31.3	Pinocarvone	0.1
1482	31.5	Fenchylalcohol	0.7
1594	31.7	*trans*-β-Bergamotene	0.2
1612	32.3	β-Caryophyllene	8.4
1648	33.3	Myrtenal	0.1
1664	33.9	*trans*-Pinocarveol	0.4
1682	34.3	δ-Terpineol	0.2
1687	34.5	α-Humulene	1.9
1729	35.7	*cis*-1,2-Epoxy-terpin-4-ol	0.1
1740	35.8	Valencene	0.3
1741	36.1	β-Bisabolene	0.9
1740	36.1	α-Muurolene	0.8
1751	36.5	Carvone	0.1
1773	37.0	δ-Cadinene	0.2
1776	37.1	γ-Cadinene	1.4
1804	37.9	Myrtenol	0.2
1810	38.1	3,7-Guaiadiene	0.2
1845	38.9	*trans*-Carveol	tr
1853	39.2	*cis*-Calamenene	0.5
1864	39.3	*p*-Cymen-8-ol	0.2
1941	41.5	α-Calacorene	0.1
1984	42.7	γ-Calacorene	0.1
2001	43.1	*iso* Caryophyllene oxide	1.6
2008	43.4	Caryophyllene oxide	7.7
2045	44.1	Humulene epoxide I	0.3
2071	44.8	Humulene epoxide II	2.3
2080	45.2	Cubenol	0.6
2098	45.4	Globulol	0.2
2104	45.6	Viridiflorol	0.1

RRI	Retention time (minutes)	Compound	Area Percentage
2113	45.8	Cumin alcohol	1.0
2131	46.5	Hexahydro-famesyl acetone	0.2
2187	47.6	*T*-Cadinol	0.5
2203	47.8	Copaborneol	0.1
2209	48.0	*T*-Muurolol	0.1
2204	48.2	Torreyol	0.3
2239	48.4	Carvacrol	0.1
2255	49.1	α-Cadinol	0.5
2264	49.5	4,7-Dimethyl-1-tetralone	tr
2316	50.5	Caryophylladienol I	0.1
2324	50.6	Caryophylladienol II	0.6
2390	52.2	10-Hydroxy calamenene	0.4
2392	52.4	Caryophyllenol II	0.3
2404	52.9	4-Isopropyl-6-methyl-1-tetralone	0.1
Total area percentage			**60.8**

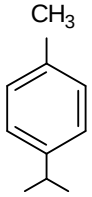
The major compounds in the essential oil of *H. callicomum* are β-pinene (6.2 %), 1,8-cineole (34.6 %), *p*-cymene (6.2%), β-caryophyllene (8.4%) and caryophyllene oxide (7.7 %).



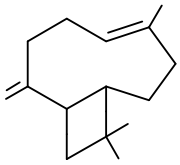
β-pinene



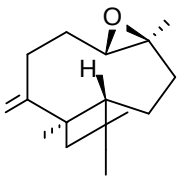
1,8-cineole



p-cymene



β-caryophyllene



caryophyllene oxide

4.3 *Helichrysum cymosum* subsp. *cymosum* (L.) D.Don

4.3.1 Botanical description:

Straggling, thin woolly shrub up to 1 m tall. Leaves are linear to elliptical, thinly silky or white felted beneath. Flower heads crowded in flat-topped clusters, without ray florets, surrounded by yellow papery flower heads (Manning *et al*., 2001).

4.3.2 Distribution:

In damp, sandy slopes. Distributed along the eastern coastline from KwaZulu-Natal through to the western Cape (Manning *et al*., 2001).

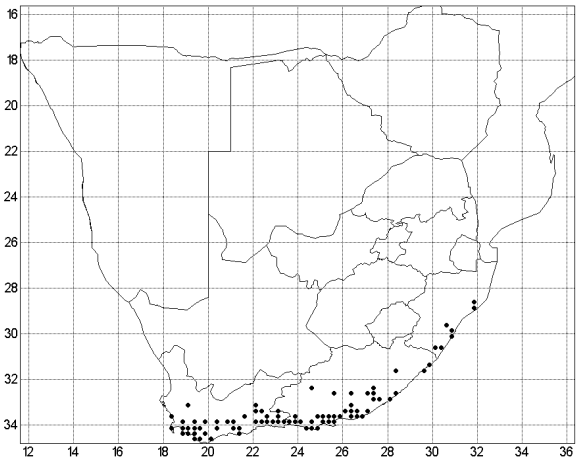


Figure 4.5: Geographical distribution of *H. cymosum* subsp. *cymosum.*
(All distribution maps have been purchased and included with permission from the South African National Biodiversity Institute)

4.3.3 Essential oil composition:

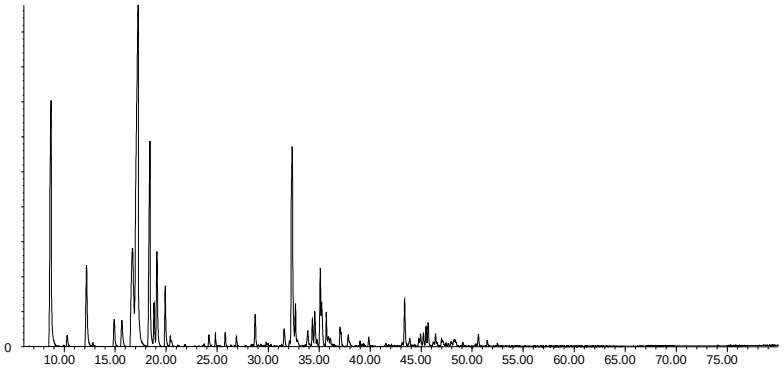


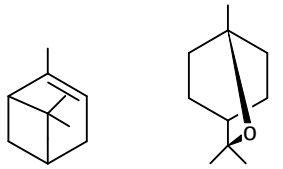
Figure 4.6: GC-MS chromatogram of *H. cymosum* subsp. *cymosum.*

Table 4.3: GC-MS results: Essential oil composition of *H. cymosum* subsp. *cymosum.*

RRI	Retention time (minutes)	Compound	Area Percentage
1032	8.5	α-Pinene	12.4
1072	9.9	α-Fenchene	0.1
1076	10.3	Camphene	0.4
1118	12.2	B-Pinene	3.7
1132	12.8	Sabinene	0.2
1174	14.9	Myrcene	1.2
1188	15.7	α-Terpinene	1.2
1203	16.8	Limonene	7.2
1213	17.3	1,8-Cineole	20.4
1246	18.2	(*Z*)-β-Ocimene	9.5
1255	18.7	γ-Terpinene	1.4
1266	19.0	(*E*)-β-Ocimene	3.6
1280	19.9	*p*-Cymene	2.4
1290	20.4	Terpinolene	0.4
1320	21.8	2-Heptanol	0.1
1386	23.2	1-Hexanol	tr
1382	24.2	*cis*-Allocimene	0.4
1391	24.4	(Z)-3-Hexen-1-ol	0.1
1398	24.8	2-Nonanone	0.4
1412	25.8	2-Octanol	0.5
1452	26.9	1-Octen-3-ol	0.3
1493	28.4	α-Ylangene	0.1
1497	28.8	α-Copaene	1.2
1510	29.3	2-Nonanol	0.1
1535	29.6	β-Bourbonene	0.6
1541	29.7	Benzaldehyde	0.1
1544	30.0	α-Gurjunene	0.1
1553	30.3	Linalool	0.1
1586	31.3	Pinocarvone	0.1
1591	31.5	Fenchylalcohol	0.7
1612	32.3	β-Caryophyllene	10.8
1628	32.6	Aromadendrene	1.5
1632	32.9	Selina-5,11-diene	0.3
1664	33.9	*trans*-Pinocarveol	0.6
1682	34.3	δ-Terpineol	0.9
1687	34.5	α-Humulene	1.3
1694	34.8	Limonen-4-ol	0.2
1706	35.1	α-Terpineol	2.6
1719	35.2	Borneol	1.8
1740	36.1	α-Muurolene	0.3
1741	35.8	δ-Guaiene	1.1
1773	37.0	δ-Cadinene	0.6
1804	37.9	Myrtenol	0.6
1838	38.6	2-Phenyl ethyl acetate	tr
1845	38.9	*trans*-Carveol	0.2
1853	39.2	*cis*-calamenene	0.6
1864	39.3	*p*-Cymen-8-ol	0.1

RRI	Retention time (minutes)	Compound	Area Percentage
1867	39.9	Benzylacetone	0.3
1896	40.4	cis-*p*-Mentha-1(7), 8-dien-2-ol	tr
1941	41.5	α-Calacorene	0.1
2001	43.1	*iso* Caryophyllene oxide	0.1
2008	43.4	Caryophyllene oxide	1.6
2033	43.9	Epiglobulol	0.3
2071	44.8	Humulene epoxide II	0.2
2073	44.9	β-Caryophyllene alcohol	0.4
2098	45.4	Globulol	0.6
2104	45.6	Viridiflorol	0.8
2144	46.2	Rosifoliol	0.2
2184	47.4	*cis-p*-Menth-3-en-1,2-diol	0.1
2238	48.2	Clovenol	0.1
2255	49.1	α-Cadinol	0.1
2316	50.5	Caryophylladienol I	tr
2324	50.6	Caryophylladienol II	0.4
2389	51.5	Caryophyllenol I	0.2
2392	52.4	Caryophyllenol II	0.1
Total area percentage			**98.1**

The major compounds in the essential oil of *H. cymosum* subsp. *cymosum* are α-pinene (12.4 %), 1,8-cineole (20.4 %), β-caryophyllene (10.8 %),



α-pinene 1,8- cineole β-caryophyllene

4.4 *Helichrysum dasyanthum* (Wild.) Sweet

4.4.1 Botanical description:

Straggling, grey woolly shrub up to 1.5 m. Leaves spreading, linear to elliptic-oblong, grey woolly margins. Flower heads are compact and straw-yellow hairy flower heads (Manning *et al*., 2001).

4.4.2 Distribution:

On sandy flats and slopes. Distributed from Namaqualand to the Baviaanskloof mountains and along the western Cape (Manning *et al*., 2001).

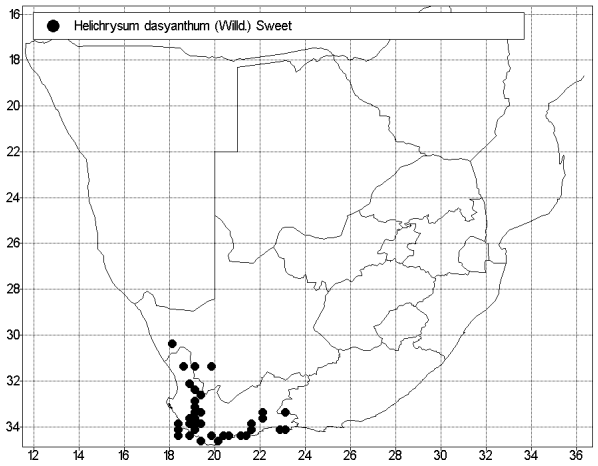


Figure 4.7: Geographical distribution of *H. dasyanthum.*
(All distribution maps have been purchased and included with permission from the South African National Biodiversity Institute)

4.4.3 Essential oil composition:

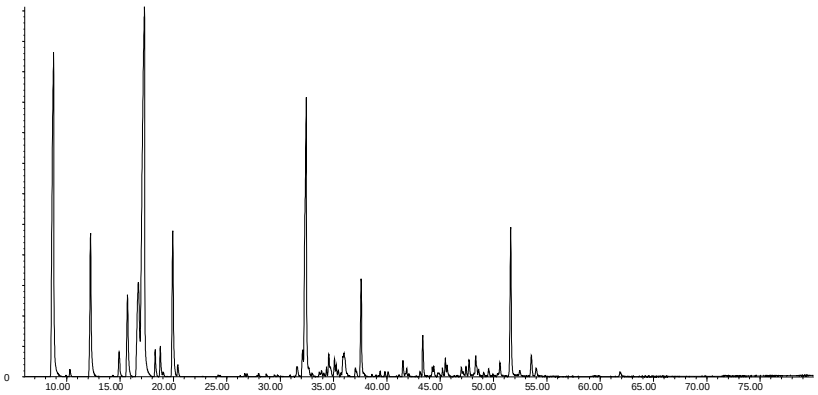


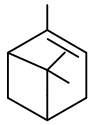
Figure 4.8: GC-MS chromatogram of *H. dasyanthum.*

Table 4.4: GC-MS results: Essential oil composition of *H. dasyanthum.*

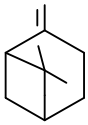
RRI	Retention time (minutes)	Compound	Area percentage
1032	8.5	α-Pinene	16.6
1072	9.9	α-Fenchene	0.1
1076	10.3	Camphene	0.3
1118	12.2	B-Pinene	6.2
1132	12.8	Sabinene	tr
1174	14.9	Myrcene	1.0
1188	15.7	α-Terpinene	3.5
1203	16.8	Limonene	6.1
1213	17.3	1,8-Cineole	20.6
1246	18.2	(*Z*)-β-Ocimene	0.9
1255	18.7	γ-Terpinene	3.5
1266	19.0	(*E*)-β-Ocimene	0.2
1280	19.9	*p*-Cymene	5.5
1290	20.4	Terpinolene	0.4
1348	22.7	6-Methyl-5-hepten-2-one	tr
1385	24.2	Heptylacetate	0.1
1386	24.4	1-Octenyl acetate	tr
1423	26.2	Hexyl-2-methyl butyrate	tr
1452	26.7	*p*-Cymenene = α,p-Dimethylstyrene	0.1
1452	26.9	1-Octen-3-ol	0.1
1482	27.7	Fenchylacetate	0.5
1497	28.8	α-Copaene	0.1
1532	29.5	Camphor	tr
1591	31.5	Fenchylalcohol	0.5
1612	32.3	β-Caryophyllene	13.3
1664	33.9	*trans*-Pinocarveol	0.2
1682	34.3	δ-Terpineol	0.2
1687	34.5	α-Humulene	0.6
1706	35.1	α-Terpineol	0.5
1719	35.2	Borneol	0.3
1596	35.7	α-Guaiene	0.1
1741	35.9	β-Bisabolene	1.5
1773	37.0	δ-Cadinene	0.2
1798	37.6	α-Dihydroagarofuran	3.4
1838	38.6	2-Phenyl ethyl acetate	0.1
1845	38.9	*trans*-Carveol	0.1
1853	39.2	*cis*-Calamenene	tr
1864	39.3	*p*-Cymen-8-ol	0.1
1889	40.1	Ascaridol	0.1
1941	41.5	α-Calacorene	0.5
1953	41.8	Palustrol	0.2
2001	43.1	*iso* Caryophyllene oxide	0.2
2008	43.4	Caryophyllene oxide	1.3
2050	44.3	(*E*)-Nerolidol	0.2
2057	44.4	Ledol	0.3
2073	44.9	β-Caryophyllene alcohol	0.1
2088	45.5	Ascaridol glycol	0.5

RRI	Retention time (minutes)	Compound	Area percentage
2145	46.6	Valeranone	tr
2184	47.4	*cis-p*-Menth-3-en-1,2-diol	0.3
2193	47.7	β-Betulenol	0.5
2238	48.2	Clovenol	0.1
2240	48.6	Pogostol	0.2
2256	49.1	Cadalene	0.1
2310	49.5	14-Acetoxy-β-caryophyllene	0.2
2324	50.6	Caryophylladienol II	0.5
2392	52.4	Caryophyllenol II	0.2
2438	53.5	Kaur-16-ene	0.7
Total area percentage			**93.12**

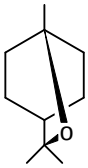
The major compounds identified in the essential of *H. dasyanthum* are α-pinene (16.6 %), β-pinene (6.2 %), 1,8-cineole (20.6 %), and β-caryophyllene (13.3 %).



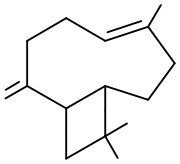
α-pinene



β-pinene



1,8-cineole



β-caryophyllene

4.5 *Helichrysum excisum* (Thunb.) Less

4.5.1 Botanical description:

Densely twiggy, closely leafy shrublet up to 45 cm with grey felted shoots. Leaves are silvery grey and felted and with yellow flower heads (Manning *et al*., 2001).

4.5.2 Distribution:

In sandstone slopes. Distributed in Bredasdorp and Little Karoo to Langkloof (Manning *et al*., 2001).

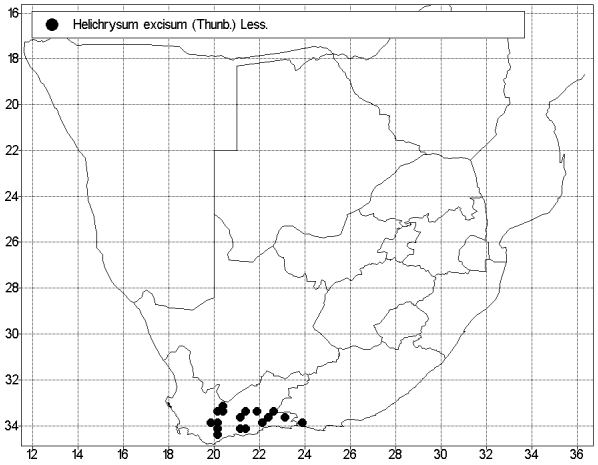


Figure 4.9: Geographical distribution of *H. excisum.*
(All distribution maps have been purchased and included with permission from the South African National Biodiversity Institute)

4.5.3 Essential oil composition:

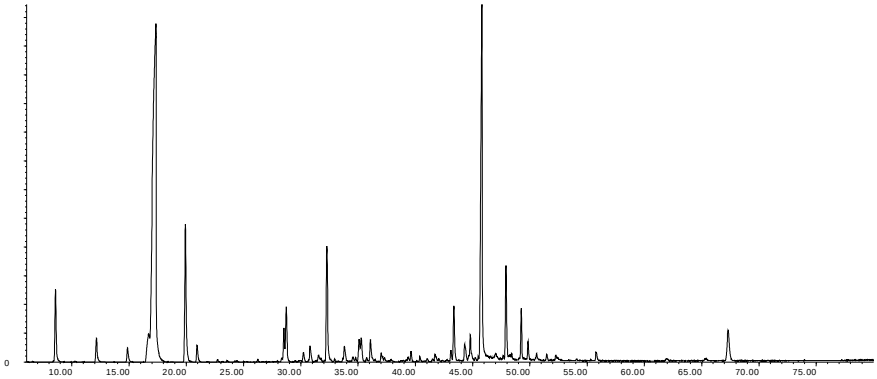


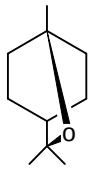
Figure 4.10: GC-MS chromatogram *H. excisum.*

Table 4.5: GC-MS results: Essential oil composition of *H. excisum.*

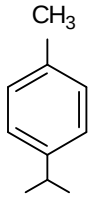
RRI	Retention time (minutes)	Compound	Area Percentage
1032	8.5	α-Pinene	2.8
1076	10.3	Camphene	tr
1118	12.2	β-Pinene	1.1
1132	12.8	Sabinene	0.2
1174	14.9	Myrcene	0.7
1203	16.8	Limonene	2.2
1213	17.3	1,8-Cineole	34.0
1280	19.9	*p*-Cymene	6.5
1286	20.9	2-Methyl butyl-3-methyl butyrate	0.7
1348	22.7	6-Methyl-5-hepten-2-one	0.1
1492	28.3	Cyclosativene	0.1
1495	28.5	α-Amorphene	1.3
1497	28.8	α-Copaene	2.5
1544	30.0	α-Gurjunene	0.1
1566	30.8	α-Santalene (β-gurjunene)	0.8
1586	31.3	Pinocarvone	0.1
1591	31.5	Fenchylalcohol	0.3
1594	31.7	*trans*-β-Bergamotene	0.1
1612	32.3	β-Caryophyllene	5.7
1638	33.0	*cis-p*-Menth-2-en-1-ol	0.1
1648	33.3	Myrtenal	0.1
1661	33.8	Alloaromadendrene	0.7
1687	34.5	α-Humulene	0.2
1683	34.5	*trans*-Verbenol	0.7
1706	35.1	α-Terpineol	0.8
1719	35.2	Borneol	1.2
1729	35.7	*cis*-1,2-Epoxy-terpin-4-ol	0.1
1758	36.5	*cis*-Piperitol	0.1
1773	37.0	δ-Cadinene	0.3
1776	37.1	γ-Cadinene	0.1
1797	37.6	*p*-Methylacetophenone	tr
1804	37.9	Myrtenol	0.1
1853	39.2	*cis*-Calamenene	0.1
1864	39.3	*p*-Cymen-8-ol	0.2
1868	39.6	(*E*)-Geranyl acetate	0.3
1918	41.1	β-Calacorene	0.2
1941	41.5	α-Calacorene	0.1
2001	43.1	*iso* Caryophyllene oxide	0.4
2008	43.4	Caryophyllene oxide	2.1
2050	44.3	(*E*)-Nerolidol	0.8
2088	44.8	6-Epi-cubenol	1.2
2080	45.2	Cubenol	0.1
2104	45.6	Viridiflorol	18.2
2187	47.6	*T*-cadinol	0.1
2203	47.8	Copaborneol	3.4
2239	48.4	Carvacrol	0.2

RRI	Retention time (minutes)	Compound	Area Percentage
2250	49.1	β-Eudesmol	0.1
2324	50.6	Caryophylladienol II	0.4
2389	51.5	Caryophyllenol I	0.2
2390	52.2	10-Hydroxy calamenene	0.2
2518	55.7	*cis*-Lanceol	0.4
Total area percentage			**92.26**

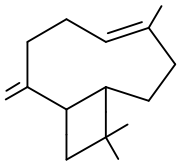
The major compounds identified in the essentail oil of *H. excisum* are 1,8-cineole (34 %), *p*-cymene (6.5 %), β-caryophyllene (5.7 %) and viridiflorol (18.2 %).



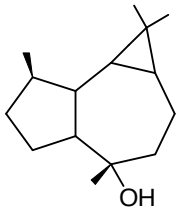
1,8-cineole



p-cymene



β-caryophyllene



viridiflorol

4.6 *Helichrysum felinum* Less.

4.6.1 Botanical Description:

Grey-woolly shrublet up to 80 cm with rod-like branches. Leaves spreading, hard to the touch, rough and felted beneath. Flower heads white or rosy with yellow centres enclosed in papery bracts (Manning *et al.*, 2001).

4.6.2 Distribution:

On sandstone slopes. Distributed from the Cape peninsula to KwaZulu-Natal (Goldblatt and Manning, 2000).

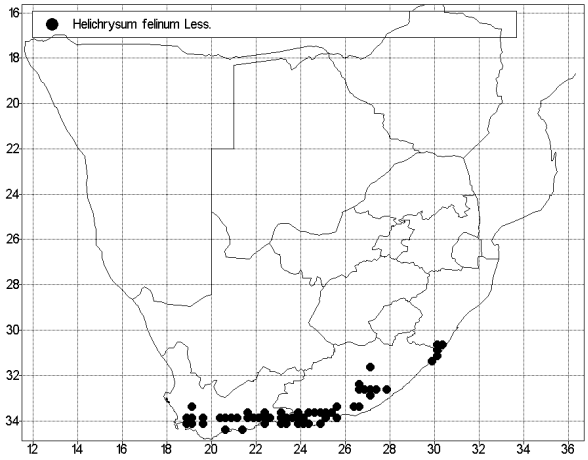


Figure 4.11: Geographical distribution of *H. felinum.*
(All distribution maps have been purchased and included with permission from the South African National Biodiversity Institute)

4.6.3 Essential oil composition:

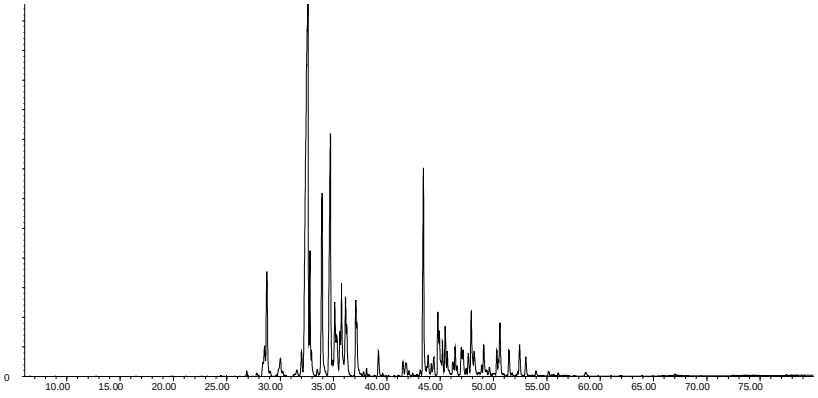


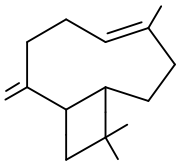
Figure 4.12: GC-MS chromatogram of *H. felinum.*

Table 4.6: GC-MS results: Essential oil composition of *H. felinum.*

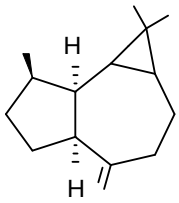
RRI	Retention time (minutes)	Compound	Area Percentage
1452	26.9	1-Octen-3-ol	0.1
1493	28.4	α-Ylangene	0.4
1495	28.5	α-Amorphene	1.3
1497	28.8	α-Copaene	4.0
1535	29.6	β-Bourbonene	tr
1544	30.0	α-Gurjunene	1.0
1553	30.3	Linalool	0.2
1594	31.7	*trans*-β-Bergamotene	0.9
1612	32.3	β-Caryophyllene	27.6
1628	32.6	Aromadendrene	2.2
1632	32.9	Selina-5,11-diene	0.7
1661	33.8	Alloaromadendrene	7.3
1674	34.0	γ-Gurjunene	0.1
1687	34.5	α-Humulene	9.4
1698	34.9	Myrtenyl acetate	0.8
1695	34.9	(*E*)-β-Farnesene	0.1
1704	35.0	γ-Muurolene	1.8
1740	35.8	Valencene	3.0
1773	37.0	δ-Cadinene	2.5
1776	37.1	γ-Cadinene	2.0
1810	38.1	3,7-Guaiadiene	0.2
1853	39.2	*cis*-Calamenene	0.8
1868	39.6	(*E*)-Geranyl acetate	0.1
1918	41.1	β-Calacorene	tr
1941	41.5	α-Calacorene	0.5
1984	42.7	γ-Calacorene	tr
2001	43.1	*iso* Caryophyllene oxide	0.2
2008	43.4	Caryophyllene oxide	6.9
2029	43.8	Perilla alcohol	0.2
2033	43.9	Epiglobulol	0.6
2045	44.1	Humulene epoxide I	0.3
2057	44.4	Ledol	0.6
2071	44.8	Humulene epoxide II	1.7
2073	44.9	β-Caryophyllene alcohol	1.4
2080	45.2	Cubenol	1.0
2098	45.4	Globulol	1.4
2104	45.6	Viridiflorol	0.7
2144	46.2	Rosifoliol	0.4
2144	46.6	Spathulenol	0.6
2187	47.6	*T*-cadinol	0.6
2203	47.8	Copaborneol	2.2
2200	48.2	Valerianol	0.9
2255	49.1	α-Cadinol	1.0
2316	50.5	Caryophylladienol I	0.2
2324	50.6	Caryophylladienol II	1.5
2389	51.5	Caryophyllenol I	0.8
2390	52.2	10-Hydroxy calamenene	0.1

RRI	Retention time (minutes)	Compound	Area Percentage
2392	52.4	Caryophyllenol II	1.0
2498	55.1	1-Heptadecanol	0.2
Total area percentage			**91.47**

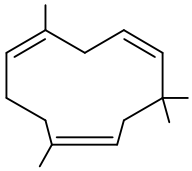
The major compounds identified in the essential oil of *H. felinum* are β-caryophyllene (27.6 %), alloaromadendrene (7.3 %), α-humulene (9.4%) and caryophyllene oxide (6.9%).



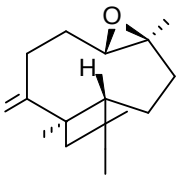
β-caryophyllene



alloaromadendrene



α-humulene



caryophyllene oxide

4.7 *Helichrysum odoratissimum* (L.) Sweet

4.7.1 Botanical description:

Straggling, much branched, aromatic, thinly white-woolly shrublet up to 50 cm. Leaves thinly to thickly woolly, base clinging, in long wings on stems. Flower heads matted with wool at base, outer pale brown, and inner yellow flower heads (Pooley, 1998).

4.7.2 Distribution:

On grassy or rocky slopes. Distributed from the Cape to Zimbabwe, Malawi and Mozambique (Pooley, 1998).

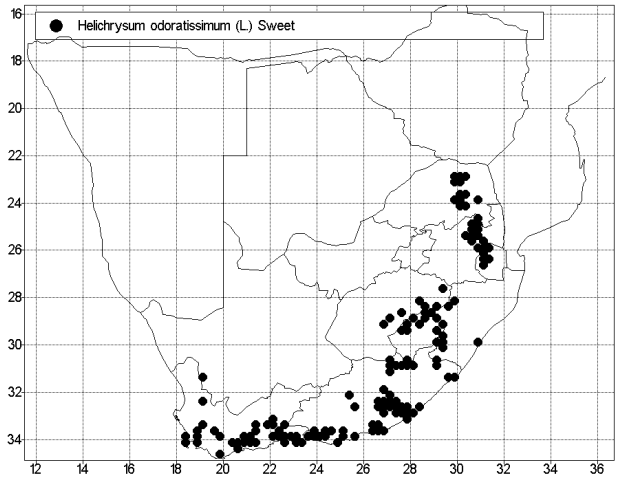


Figure 4.13: Geographical distribution of *H. odoratissimum*.
(All distribution maps have been purchased and included with permission from the South African National Biodiversity Institute)

4.7.3 Essential oil composition:

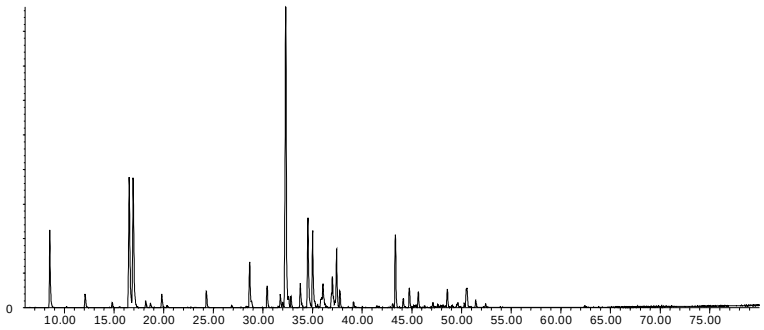


Figure 4.14: GC-MS chromatogram of *H. odoratissimum* (JEV 2437).

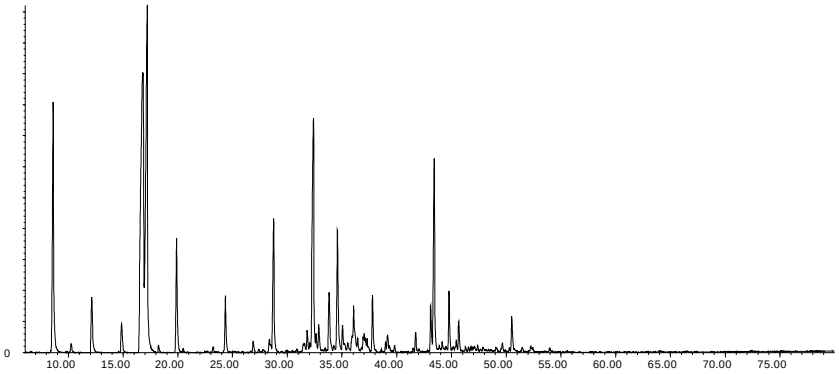


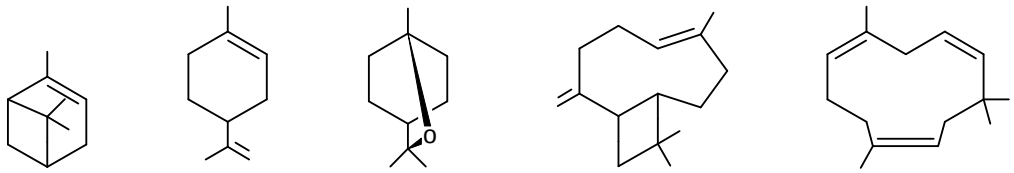
Figure 4.15: GC-MS chromatogram of *H. odoratissimum* (JEV 2440).

Table 4.7: GC-MS results: Essential oil composition of *H. odoratissimum* (JEV 2437).

RRI	Retention time (minutes)	Compound	Area Percentage
1032	8.5	α-Pinene	4.8
1118	12.2	β-Pinene	1.0
1174	14.9	Myrcene	0.4
1203	16.8	Limonene	11.6
1213	17.3	1,8-Cineole	11.2
1246	18.2	(*Z*)-β-Ocimene	0.5
1255	18.7	γ-Terpinene	0.3
1280	19.9	*p*-Cymene	1.0
1386	24.4	1-Octenyl acetate	1.1
1452	26.9	1-Octen-3-ol	0.1
1497	28.8	α-Copaene	3.2
1557	30.4	Italicene	1.5
1594	31.7	*trans*-β-Bergamotene	0.8
1612	32.3	β-Caryophyllene	25.2
1628	32.6	Aromadendrene	0.8
1632	32.9	Selina-5,11-diene	0.8
1661	33.8	Alloaromadendrene	1.7
1687	34.5	α-Humulene	7.2
1704	35.0	γ-Curcumene	5.4
1741	35.8	δ-Guaiene	0.4
1726	36.0	*cis*-α-Bisabolene	1.33
1771	36.9	γ-Bisabolene	0.6
1773	37.0	δ-Cadinene	2.0
1782	37.3	*cis*-Carvylacetate	0.1
1786	37.4	Ar-curcumene	3.9
1796	37.8	Selina-3,7-(11)-diene	1.1
1853	39.2	*cis*-Calamenene	0.2
1941	41.5	α-Calacorene	0.1
2001	43.1	*iso* Caryophyllene oxide	0.1
2008	43.4	Caryophyllene oxide	4.9
2045	44.1	Humulene epoxide I	0.5
2071	44.8	Humulene epoxide II	1.3

RRI	Retention time (minutes)	Compound	Area Percentage
2088	45.2	1-Epi-cubenol	0.1
2104	45.6	Viridiflorol	1.0
2170	47.1	β-Bisabolol	0.3
2187	47.6	T-Cadinol	0.2
2238	48.2	Clovenol	0.1
2239	48.4	Carvacrol	0.1
2269	49.6	Porosadienol	0.2
2308	50.5	*g*-Geranyl-*p*-cymene	0.7
2389	51.5	Caryophyllenol I	0.4
Total area percentage			**98.17**

The major compounds identified in the essential oil of *H. odoratissimum* (AV 587) are α-pinene (4.8 %), limonene (11.6 %), 1,8-cineole (11.2 %), β-caryophyllene (25.2 %), α-humulene (7.2 %).



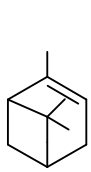
α-pinene limonene 1,8-cineole β-caryophyllene α-humulene

Table 4.8: GC-MS results: Essential oil composition of *H. odoratissimum* (JEV 2440).

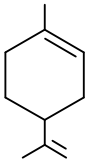
RRI	Retention time (minutes)	Compound	Area Percentage
1032	8.5	α-Pinene	8.3
1076	10.3	Camphene	0.3
1118	12.2	β-Pinene	1.9
1174	14.9	Myrcene	1.0
1203	16.8	Limonene	19.6
1213	17.3	1,8-Cineole	17.1
1246	18.2	(*Z*)-β-Ocimene	0.2
1280	19.9	*p*-Cymene	3.7
1282	20.5	Heptenylacetate	0.1
1348	22.7	6-Methyl-5-hepten-2-one	tr
1386	24.4	1-Octenyl acetate	1.7
1391	24.4	(*Z*)-3-Hexen-1-ol	0.1
1393	24.8	3-Octanol	tr
1413	25.2	Rosefuran	tr
1429	25.9	Perillen	tr
1452	26.7	*p*-Cymenene = α,p-dimethylstyrene	tr
1452	26.9	1-Octen-3-ol	0.4
1468/1469	27.4	*trans* 1,2-Limonene-epoxide	0.1

RRI	Retention time (minutes)	Compound	Area Percentage
1482	27.7	Fenchylacetate	0.1
1493	28.4	α-Ylangene	0.4
1497	28.8	α-Copaene	4.6
1544	30.0	α-Gurjunene	0.1
1557	30.4	Italicene	0.1
1568	30.8	1-Methyl-4-acetyl-cyclohex-1-ene	tr
1597	31.6	Bornyl acetate	0.2
1594	31.7	*trans*-β-Bergamotene	0.6
1612	32.3	β-Caryophyllene	9.3
1628	32.6	Aromadendrene	0.4
1632	32.9	Selina-5,11-diene	0.9
1648	33.3	Myrtenal	tr
1661	33.8	Alloaromadendrene	1.9
1678	34.3	*cis*-*p*-Mentha-2,8-diene-1-ol	0.1
1687	34.5	α-Humulene	4.0
1704	35.0	γ-Muurolene	0.6
1707	35.5	β-Chamigrene	0.1
1741	35.9	β-Bisabolene	0.4
1742	36.1	α-Selinene	1.7
1751	36.5	Carvone	0.4
1758	36.5	*cis*-Piperitol	0.1
1771	36.9	γ-Bisabolene	0.2
1773	37.0	δ-Cadinene	0.3
1776	37.1	γ-Cadinene	0.2
1782	37.3	*cis*-Carvylacetate	0.2
1796	37.9	Selina-3,7-(11)-diene	1.7
1819	38.6	2-Phenyl ethyl acetate	0.1
1845	38.9	*trans*-Carveol	0.2
1853	39.2	*cis*-Calamenene	0.5
1864	39.3	*p*-Cymen-8-ol	0.2
1868	39.6	(*E*)-Geranyl acetate	tr
1882	39.8	*cis*-Carveol	0.1
1941	41.5	α-Calacorene	0.1
2001	43.1	*iso* Caryophyllene oxide	1.3
2008	43.4	Caryophyllene oxide	5.9
2045	44.1	Humulene epoxide I	0.2
2071	44.8	Humulene epoxide II	1.7
2081	45.1	Humulene epoxide III	0.1
2104	45.6	Viridiflorol	0.8
2144	46.6	Spathulenol	0.1
2184	47.4	*cis*-*p*-Menth-3-en-1,2-diol	0.1
2187	47.6	*T*-Cadinol	0.1
2239	48.4	Carvacrol	Tr
2256	49.1	Cadalene	0.1
2269	49.6	Porosadienol	0.3
2308	50.5	*g*-Geranyl-*p*-cymene	1.1
2390	52.2	10-Hydroxy calamenene	0.2
Total area percentage			**96.305**

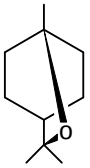
The major compounds identified in the essential oil of *H. odoratissimum* (JEV 2440) are α-pinene (8.3 %), limonene (19.6), 1,8-cineole (17.1 %), β-caryophyllene (9.3 %), α-humulene (4 %) and caryophyllene oxide (5.9%).



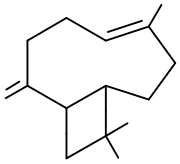
α-pinene



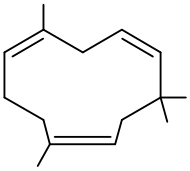
limonene



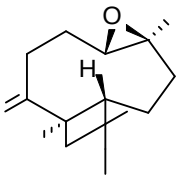
1,8-cineole



β-caryophyllene



α-humulene



caryophyllene oxide

4.8 *Helichrysum petiolare* Hilliard & B.L. Burtt

4.8.1 Botanical Description:

Straggling, grey-woolly shrublet or shrub. Leaves abruptly and conspicuously petiolated silvery grey in colour. Flower heads have creamy yellowish florets (Manning *et al*., 2001).

4.8.2 Distribution:

Sheltered slopes and forest margins. Cedarberg and Jonkershoek mountains to KwaZulu-Natal (Manning et al., 2001).

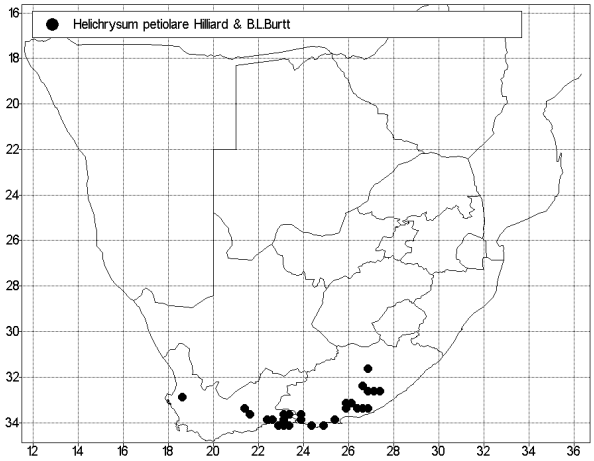


Figure 4.16: Geographical distribution of *H. petiolare*.
(All distribution maps have been purchased and included with permission from the South African National Biodiversity Institute)

4.8.3 Essential oil composition:

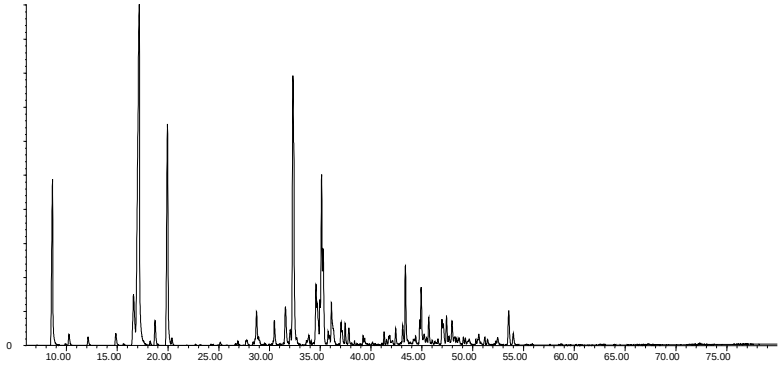


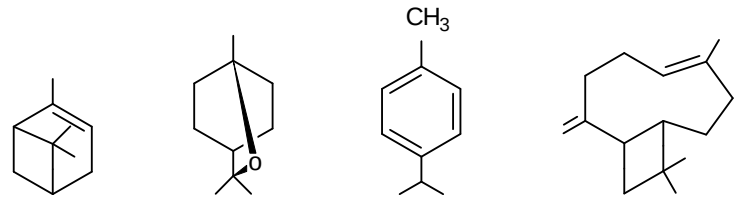
Figure 4.17: GC-MS chromatogram of *H. petiolare*.

Table 4.9: GC-MS results: Essential oil composition of *H. petiolare.*

RRI	Retention time (minutes)	Compound	Area Percentage
1032	8.5	α-Pinene	6.8
1072	9.9	α-Fenchene	0.7
1076	10.3	Camphene	0.4
1118	12.2	β-Pinene	0.4
1174	14.9	Myrcene	0.5
1188	15.7	α-Terpinene	0.1
1203	16.8	Limonene	3.1
1213	17.3	1,8-Cineole	22.4
1246	18.2	(*Z*)-β-Ocimene	0.1
1255	18.7	γ-Terpinene	1.0
1266	19.0	(*E*)-β-Ocimene	tr
1280	19.9	*p*-Cymene	9.8
1290	20.4	Terpinolene	0.3
1348	22.7	6-Methyl-5-hepten-2-one	tr
1386	23.2	1-Hexanol	tr
1384	24.3	α-Pinene oxide	tr
1406	25.1	α-Fenchone	0.1
1452	26.7	*p*-Cymenene = α,*p*-Dimethylstyrene	0.1
1452	26.9	1-Octen-3-ol	0.1
1449	27.1	1-Heptanol	tr
1497	28.8	α-Copaene	1.3
1506	29.3	Hexyl valerate	tr
1532	29.5	Camphor	0.1
1544	30.0	α-Gurjunene	0.1
1557	30.4	Italicene	1.0
1573	31.0	*iso*-Italicene	0.1
1591	31.5	Fenchylalcohol	1.5
1604	32.0	Thymol methyl ether	0.6
1612	32.3	β-Caryophyllene	14.0
1628	32.6	Aromadendrene	0.3
1664	33.9	*trans*-Pinocarveol	0.4
1674	34.0	γ-Gurjunene	0.1
1682	34.3	δ-Terpineol	tr
1687	34.5	α-Humulene	2
1704	35.0	γ-Muurolene	2.2
1706	35.1	α-Terpineol	5.1
1719	35.2	Borneol	3.3
1729	35.7	*Cis*-1,2-Epoxy-terpin-4-ol	0.4
1765	36.8	Geranyl acetate	0.1
1773	37.0	δ-Cadinene	0.7
1786	37.4	*ar*-Curcumene	0.6
1796	37.8	Selina-3,7-(11)-diene	0.6
1810	38.1	3,7-Guaiadiene	0.1
1845	38.9	*trans*-Carveol	0.1
1853	39.2	*cis*-Calamenene	0.3
1864	39.3	*p*-Cymen-8-ol	0.3

RRI	Retention time (minutes)	Compound	Area Percentage
1868	39.6	(*E*)-Geranyl acetate	0.1
1933	41.3	Nerylvalerate	0.3
1941	41.5	α-Calacorene	0.1
1953	41.8	Palustrol	0.3
2001	43.1	*iso* Caryophyllene oxide	0.6
2008	43.4	Caryophyllene oxide	2.5
2045	44.1	Humulene epoxide I	0.1
2050	44.3	(*E*)- Nerolidol	0.1
2057	44.4	Ledol	0.2
2071	44.8	Humulene epoxide II	0.7
2073	44.9	β-Caryophyllene alcohol	1.9
2098	45.4	Globulol	0.2
2104	45.6	Viridiflorol	0.9
2144	46.6	Spathulenol	0.2
2170	47.1	β-Bisabolol	0.8
2187	47.6	T-cadinol	0.2
2238	48.2	Clovenol	0.1
2239	48.4	Carvacrol	0.1
2255	49.1	α-Cadinol	0.2
2324	50.6	Caryophylladienol II	0.4
2346	51.2	Kaur-15-ene	0.3
2389	51.5	Caryophyllenol I	0.2
2392	52.4	Caryophyllenol II	0.3
2438	53.5	Kaur-16-ene	1.3
Total area percentage			**93.3**

The major compounds identified in the essential oil of *H. petiolare* include α-pinene (6.8%), 1,8-cineole (22.4%), *p*-cymene (9.8%), and β-caryophyllene (14 %).



α-pinene 1,8-cineole *p*-cymene β-caryophyllene

4.9 *Helichrysum rosum* (P.J. Bergius) Less. var. *rosum*

4.9.1 Botanical description:

Sprawling, thinly grey-woolly shrub to 2m, with milk-white tips. Leaves white woolly. Flower heads straw coloured (Manning *et al*., 2001).

4.9.2 Distribution:

In stony slopes and flats. Distributed through the eastern and western Cape (Manning et al., 2001).

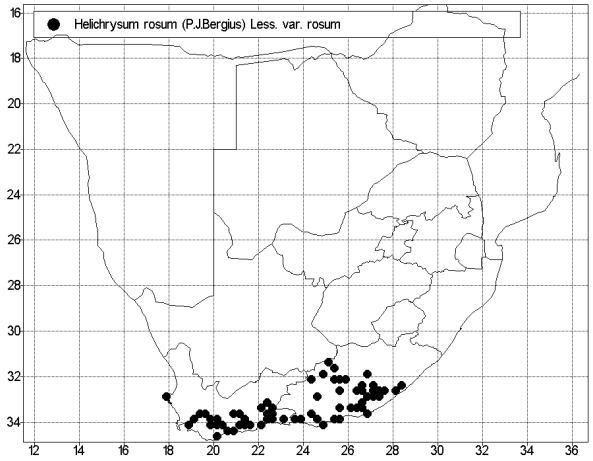


Figure 4.18: Geographical distribution of *H. rosum* var *rosum.*
(All distribution maps have been purchased and included with permission from the South African National Biodiversity Institute)

4.9.3 Essential oil composition:

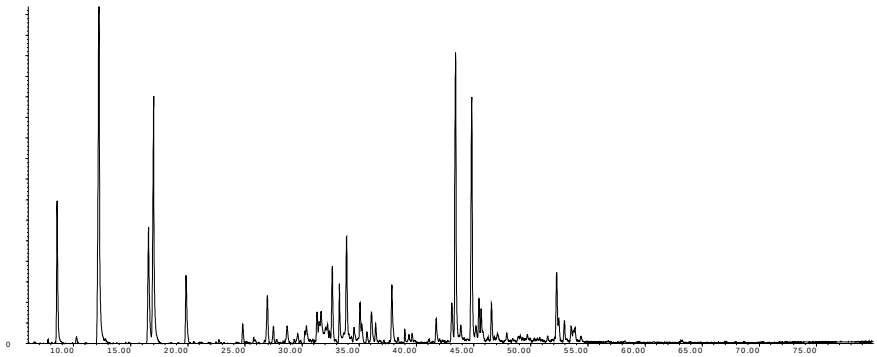


Figure 4.19: GC-MS chromatogram of *H. rosum* var. *rosum* essential oil.

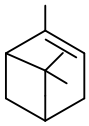
Table 4.10: GC-MS results: Essential oil composition of *H. rosum* var. *rosum.*

RRI	Retention time (minutes)	Compound	Area Percentage
1014	8.1	Tricyclene	Tr
1032	8.5	α-Pinene	4.7

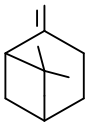
RRI	Retention time (minutes)	Compound	Area Percentage
1076	10.3	Camphene	0.2
1118	12.2	β-Pinene	16.1
1132	12.8	Sabinene	0.3
1174	14.9	Myrcene	tr
1203	16.8	Limonene	5.2
1213	17.3	1,8-Cineole	11.0
1280	19.9	*p*-Cymene	2.6
1282	20.5	2-Octanone	0.1
1348	22.7	6-Methyl-5-hepten-2-one	0.1
1393	24.8	3-Octanol	0.6
1398	24.8	2-Nonanone	1.8
1412	25.8	2-Octanol	0.2
1429	25.9	Perillene	0.1
1450	26.7	*trans*-Linalool oxide (furanoid)	0.1
1474	27.5	*trans*-Sabinene hydrate	0.6
1478	27.8	*cis*-Linalool oxide (furanoid)	0.1
1497	28.8	α-Copaene	0.7
1510	29.3	2-Nonanol	0.1
1535	29.6	β-Bourbonene	0.4
1553	30.3	Linalool	0.3
1556	30.4	*cis*-Sabinene hydrate	0.6
1586	31.3	Pinocarvone	0.9
1582	31.7	Nopinone	0.9
1611	32.2	Terpinen-4-ol	0.3
1628	32.6	Aromadendrene	2.5
1642	33.3	Thuj-3-ene-10-ol	2.1
1648	33.3	Myrtenal	0.1
1661	33.9	*trans*-Pinocarveol	3.1
1682	34.3	δ-Terpineol	0.1
1683	34.5	*trans*-Verbenol	0.7
1682	35.1	α-Terpineol	1.1
1719	35.2	Borneol	0.6
1725	35.7	Verbenone	0.4
1742	36.1	α-Selinene	1.3
1751	36.5	Carvone	0.5
1776	37.1	γ-Cadinene	0.1
1797	37.6	*p*-Methylacetophenone	0.1
1804	37.9	Myrtenol	2.0
1804	38.9	*trans*-Carveol	0.4
1864	39.3	*p*-Cymen-8-ol	0.4
1868	39.6	(*E*)-Geranyl acetate	0.3
1882	39.8	*cis*-Carveol	0.2
2001	43.1	Isocaryophyllene oxide	1.4
2008	43.4	Caryophyllene oxide	10.3
2033	43.9	Epiglobulol	0.5
2045	44.1	Humulene epoxide I	0.1
2071	44.8	Humulene epoxide II	8.5
2088	45.1	1-Epi-cubenol	0.4

RRI	Retention time (minutes)	Compound	Area Percentage
2098	45.4	Globulol	1.2
2104	45.6	Viridiflorol	0.5
2144	46.6	Spathulenol	1.1
2289	50.0	*oxo*-α-Ylangene	0.1
2324	50.6	Caryophylladienol II	0.1
2392	52.4	Caryophyllenol II	0.8
Total area percentage			**88.94**

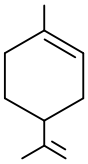
The major compounds identified in the essential oil of *H. rosum* var *rosum* are α-pinene (4.7%), β-pinene (16.1%), limonene (5.15%), 1,8-cineole (10.98%), caryophyllene oxide (10.3%)



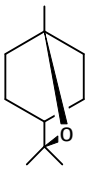
α-pinene



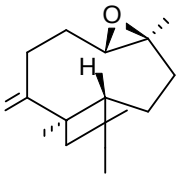
β-pinene



limonene



1,8-cineole



caryophyllene oxide

CHAPTER 5: CONCLUSIONS

It was postulated of the *Helichrysums* that the basis for the antimicrobial activity was due to the abundance of the essential oils. However the results of the antimicrobial assays revealed that only the essential oils of *H. excisum* exhibited antimicrobial activity against *B. cereus*. This could be attributed to the high concentration of viridiflorol (18.2%) present in the essential oil of *H. excisum.*

Better antimicrobial activity was observed for the phenolic extracts compared to the essential oils.

Antibacterial activity was observed only against Gram-positive bacteria.

The most active species against Gram-positive bacteria in the extracts was *H. dasyanthum.* The antimicrobial activity could be explained by the presence of kaurenoic derivatives. This had not been isolated from either *H. felinum* or *H. petiolare*, which share very similar phenolic hydroxyl containing groups, but lack significant activity against Gram-positive bacteria.

The species exhibited antibacterial activity against Gram-positive bacteria only.
The most active species against Gram-positive bacteria in both the essential oils and the extracts was *H. dasyanthum*.

H. dasyanthum produced the most significant antifungal activity in the acetone extract against *C. Neoformans, C. albicans,* and *A. alternate,* whilst the essential oils of *H. petiolare, H. felinum, H. asperum* and *H.callicomum* displayed antifungal activity against *C. albicans.*

The composition of the essential oils was dominated by the presence of monoterpenes. This correlated with results previously reported in other South African species (Lwanda *et al*., 1993; Ramanoelina *et al*., 1991).

The most significant compounds identified in the essential oils were 1,8 cineole (monoterpene hydrocarbon), α-pinene (monoterpene hydrocarbon), β-pinene

(monoterpene hydrocarbon), β-caryophyllene (sesquiterpene hydrocarbon), and caryophyllene oxide (oxygenated sesquiterpenes).

It is quite interesting to note that with the complex chemical composition of the essential oils, some chemical compounds present in certain members of the genus are totally absent in others. This variation in chemical composition is however not mirrored in the biological results, since all essential oils exhibited remarkably similar activities in the antimicrobial assays. Combinations of different chemical entities may thus result in similar biological activity. These *in-vitro* results provide some scientific validation for the widespread use of plants from this genus in traditional medicine.

This study of the identification of the chemical composition and the antimicrobial activity of the essential oils and plant extracts provides for the elucidation of a scientific rationale for the traditional use of the *Helichrysum* species in South Africa amongst the nine species studied.

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APPENDIX

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In vitro biological activity and essential oil composition of four indigenous South African *Helichrysum* species

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Abstract:
Helichrysum species are used widely to treat various medical conditions. In this study, the anti-microbial, anti-oxidant (DPPH assay) and anti-inflammatory activity (5-lipoxygenase assay) of *Helichrysum dasyanthum*, *Helichrysum felinum*, *Helichrysum excisum* and *Helichrysum petiolare* were investigated. The essential oil compositions of these species were determined. The acetone and methanol extracts as well as the essential oils exhibited activity against Gram-positive bacteria, while both the methanol and acetone extracts of all four species were active in the anti-oxidant assay. The essential oils, on the other hand, displayed activity in the 5-lipoxygenase assay, which was used as an indication of anti-inflammatory activity. Two extracts exhibited promising activity in the anti-microbial assay, the acetone extract of *Helichrysum dasyanthum* with a MIC value of 15.63 µg/ml and the methanol extract of *Helichrysum excisum* with a MIC value of 62.5 µg/ml. The acetone extract of *Helichrysum dasyanthum* was the most active free radical scavenger in the DPPH assay (IC_{50} of 9.53 µg/ml) while values for the anti-inflammatory activity of the essential oils ranged between 25 and 32 µg/ml. The essential oil compositions of three species (*Helichrysum dasyanthum*, *Helichrysum excisum* and *Helichrysum petiolare*) were dominated by the presence of monoterpenes such as a-pinene, 1,8-cineole and *p*-cymene. In the oil of *Helichrysum felinum*, monoterpenes were largely absent. Its profile consisted of a variety of sesquiterpenes in low concentrations with ß-caryophyllene dominating.