Indigenous *Salvia* species - an investigation of the antimicrobial activity, antioxidant activity and chemical composition of leaf extracts

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

Johannesburg, 2005

Declaration

I, Vanessa Louise Fisher declare that this research report is my own work. It is being submitted for the degree of MSc (Med) Pharmacotherapy in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other university.

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Acknowledgements

I would like to thank the following:

My supervisors Prof. A. M. Viljoen and Ms S. F. van Vuuren.

Prof. Başer, Drs Betűl Demirci and Temel Őzek from the Medicinal and Aromatic Plant and Drug Research Centre, University of Anadolu, Turkey for their help with the GC-MS analysis during my visit to the Research Centre.

The staff at the Walter Sisulu Botanical Garden for access to plant material and Mr. J. Vlok and Dr. J. Manning for collecting and identifying plant material.

The National Research Foundation, University of the Witwatersrand Research Committee and Faculty of Health Sciences Research Endowment Fund for financial support.

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ABSTRACT

The genus *Salvia*, commonly known as the sages is a member of the mint family (Lamiaceae). In Latin, ‘sage’ means "to save" and the Romans called it "sacred herb". Throughout history it has been used for depression, fever, respiratory infections, women's complaints, sleep inducer, diuretic, gargles and sick room use. The essential oil is reported to have anti-inflammatory, antimicrobial, antioxidant, antiseptic, antispasmodic, astringent, diuretic, antihypertensive and insecticidal properties. Of the 900 species recorded worldwide, 30 are indigenous to South Africa where they are used extensively in traditional healing.

The aerial parts of twelve samples were hydrodistilled and the essential oil analysed by GC-MS. The essential oil composition varied quantitatively and qualitatively within the different *Salvia* species analysed. Linalool was the only compound that was present in all the essential oils. β-caryophyllene and caryophyllene oxide were present in all essential oil with the exception of *S. stenophylla.*

The essential oil as well as methanol and acetone extracts were tested for antimicrobial activity on a number of bacteria and fungi. No species showed activity against *Cryptococcus neoformans, Candida albicans* nor *Alternaria alternata*. All test samples studied demonstrated variable degrees of antibacterial activity with the exception of four test samples; *S. disermas* (methanol and acetone) from the Walter Sisulu Botanical Garden; *S. disermas* (methanol) from Mossel Bay and *S. lanceolata* (methanol). Gram-positive organisms were more sensitive to the test samples than the Gram-negative organisms. In general, the extracts were far more active than the essential oils.

Thin layer chromatography indicated that all methanol extracts possess antioxidant activity. All methanol extracts contain the antioxidant compound, rosmarinic acid. It is evident that, in addition to rosmarinic acid, other polar and non-polar compounds are present in all *Salvia* species that also act as antioxidants.

CHAPTER 1: INTRODUCTION

Salvia is a large and polymorphous genus of the Lamiacea family, comprising 900 species with a cosmopolitan distribution (Ulubelen, 1994). The genus name *Salvia* is derived from the Latin *salvare* which means "to be saved" or "to cure". This name was corrupted to *sauge* in French and *sawge* in Old English and eventually to sage, as we commonly know it today (Blumenthal *et al*., 1998). *Salvia* has been prized since ancient times and has enjoyed a reputation for being a panacea. The Romans referred to it as the "sacred herb" and the Spanish have referred to it as *ierba buena* or "good herb" (Baricevic and Bartol, 2000) in reference to its curative and healing properties. *Salvia* is an important genus that is widely cultivated and has been used for centuries in foods for flavouring and seasoning in addition to being used for the treatment of various medical conditions. Throughout history *Salvia* has been used for depression, fever, respiratory infections, women's complaints, sleep inducer, diuretic, gargles, night sweats, memory enhancer, disinfectant and sick room use. The essential oil is reported to have anti-inflammatory, antimicrobial, antioxidant, antiseptic, antispasmodic, astringent, anti-hypertensive and insecticidal properties.

The distribution of the genus extends all over northern Africa from west to east and southwards to the east African highlands. The genus is absent from most of western and central tropical Africa. This means that the species in southern Africa are geographically isolated from the species on the northern part of the continent. Few species are common to both northern and southern Africa (Jäger and van Staden, 2000). Southern Africa is home to 30 species of the genus *Salvia*. In South Africa, *Salvia* only occurs in sparse populations. A few species, such as *S. africana-lutea* and *S. chamelaeagnea* are relatively common in the Knysna and the Cape regions. *Salvia sclarea*, *S. coccinea*, *S. reflexa*, and *S. tiliifolia* have been introduced into southern Africa and are thus not endemic to the region. However, the latter three species have become naturalized in parts of South Africa. *S. verbenaca* is more than likely indigenous to the Mediterranean countries and the Canary Islands and has spread into Europe and Asia. If it is an introduced plant in our

Flora, it is now widely distributed and considered indigenous to South Africa (Codd, 1985).

In southern Africa traditional medicine plays an important role in health care. In South Africa it has been estimated that eighty percent of the total black population consults with traditional healers (Jäger and van Staden, 2000). These traditional healers and herbalists have centuries of experience and a vast knowledge in using plants for medicinal and healing purposes. Traditional usages of some indigenous *Salvia* species and their ethnomedicinal uses are summarised in Table 1. The table includes the uses of *Salvia* across all the ethnic groups in South Africa as well as the European settlers.

Table 1: Traditional uses of *Salvia* in southern Africa (summarised from Jäger and van Staden, 2000).

Species	**Medicinal use**
Salvia africana-caerulea	• Used as a remedy for coughs, colds and chest troubles • Prepared as a tea and used as a remedy for colic, diarrhoea, heartburn and indigestion
Salvia africana-lutea	• Used for colds and coughs • Used to treat a variety female ailments
Salvia chamelaeagnea	• Infusion of the dry leaf used for convulsions • Used for coughs, colds, fever, bronchitis, whooping cough, flu and general chest complaints • Also used for head-, ear- and stomach pains • Used for the treatment of burns • Helpful in the treatment of a variety of female ailments • Used in the treatment of diarrhoea • Has caused livestock death
Salvia coccinea	• Used in the treatment of kidney diseases • Used to relieve the cough associated with pulmonary tuberculosis

Species	Medicinal use
Salvia disermas	• In the form of a lotion it is used to treat sores • Used as an anti-hypertensive • Used to treat rheumatism
Salvia reflexa	• Has caused livestock death
Salvia repens	• Is added to the bath water for treating sores on the body • A decoction of the root is administered before meals for stomach ache and diarrhoea • The smoke of the burning plant is used to disinfect houses
Salvia runcinata	• A decoction of the root, leaf and stem taken for the relief of urticaria • The smoke of the burning plant is used to disinfect houses • A paste of the leaf is used as a purgative in infants
Salvia scabra	• A paste of the leaf is made with mother's milk and given as the first medicine to infants • A water extract of the roots is also given daily to infants until they are two months old
Salvia sclarea	• Used to treat diarrhoea and stomach aches • A milk decoction is used for the alleviation of a sore throat • A water decoction is used to treat sores and swellings
Salvia stenophylla	• The smoke of the burning plant is used to disinfect houses

Very little phytochemical work has been done on indigenous *Salvia* species (Jäger and van Staden, 2000). Diterpenoids are the main compounds present in the roots of *Salvia* plants whilst aerial parts usually contain flavonoids, which as in many Lamiaceae, are flavones substituted in the 6-position. Triterpenoids, derived from ursane (chiefly ursolic acid) as well as essential oils with volatile compounds such as monoterpenoids (Baricevic and Bartol, 2000) are also present in the genus. *Salvia* is also an abundant source of phenolic acids. The majority of the phenolic acids found in *Salvia* are those of caffeic acid derivatives, which are unique to *Salvia* except for rosmarinic acid and lithospermic

acid (Lu and Foo, 2002). Some of these compounds have been isolated and their structure elucidated, however, many compounds are still scientifically challenging and pharmacological studies with isolated constituents are still lacking. In addition to this, there is much variety in bioactivity within the genus due to the diversity of species present in the genus. More studies on structure-activity relationships within *Salvia* are needed in order to explain mechanisms of biological activity.

There has been a renaissance of interest in natural occurring therapeutic agents (Carson and Riley, 1995). The essential oils and extracts of many plant species have become the subjects of research in the past few years, and attempts to characterize their bioactive principles have recently gained momentum in many pharmaceutical and food processing applications (Vardar-Űnlű *et al*., 2003). This increased level of interest in natural product research can be attributed to the following factors; unmet therapeutic needs, the remarkable diversity of both chemical structures and biological activities of naturally occurring secondary metabolites, the utility of bioactive natural products as biochemical and molecular probes, the development of novel and sensitive techniques to detect biologically active natural products, improved techniques to isolate, purify, and structurally characterize these active constituents and advances in solving the demand for supply of complex natural products (Clark, 1996).

The potential use of higher plants as a source of new drugs is still poorly explored. Only a small percentage of the estimated plant species has been investigated phytochemically and an even smaller percentage has been properly studied in terms of their pharmacological properties. In most cases those plant species that have been investigated have only been screened or alternatively had preliminary studies carried out on them. About twenty five percent of the drugs prescribed worldwide come from plants, 121 such active compounds being in current use. Of the 252 drugs considered as basic and essential by the World Health Organisation (WHO), eleven percent are exclusively of plant origin and a significant number are synthetic drugs obtained from natural precursors (Rates, 2001). Natural compounds can be lead compounds, allowing the design and rational planning of new drugs, biomimetic synthesis development and the discovery of

new therapeutic properties not yet attributed to known compounds from plants. The negative side is that the research and development of these compounds from plant origin is a difficult and expensive task with each new drug requiring vast sums of money and many years of hard work.

Plants can be used as therapeutic resources in several ways. They can be used as herbal teas or other home made remedies when they are considered as medicinal plants. They can be used as crude extracts or standard enriched fractions in preparations such as tinctures, fluid extracts, powder, pills and capsules, when they are considered as phytopharmaceutical preparations or herbal medicines. Finally, the plants can be subjected to successive extraction and purification procedures to isolate compounds of interest, which can themselves be active and used directly as a drug or they can be precursors in the processes or synthesis of a drug (Rates, 2001). Traditionally, indigenous *Salvia* is used in the form of water and milk decoctions, extracts, tinctures, lotions, infusions, pastes and teas. This research report aims to determine the biological activity of methanol and acetone extracts as well as the essential oils of selected indigenous *Salvia* species. The biological activities investigated will include the assessment of the antimicrobial and antioxidant activity.

Essential oils are accepted and recognised in academia, in the chemical industry and, more recently, in everyday life. Essential oils are aromatic substances produced by certain plants. They have been termed essential because it was once thought that each oil represented the essence of the original plant (Nakatsu *et al*., 2000). Plant essential oils are a potentially useful source of antimicrobial compounds (Friedman *et al*., 2002) and their antimicrobial activities have been recognised, albeit empirically, for centuries (Faleiro *et al*., 2003). This antimicrobial activity could act as a chemical defense mechanism against plant pathogenic diseases. Damage or "wounding" of leaves, which are covered with volatile oil glands, results in the rupture of glands causing the oil to flow over the area or "wound" (Svoboda and Hampson, 1999). The existence of antimicrobial activity in the essential oil would be of substantial benefit to the plant in such circumstances. These antimicrobial compounds are therefore important in the control of plant and human

diseases of microbial origin. Antimicrobials are among the most important classes of therapeutic agents and have had an enormous impact on both life expectancy and the quality of life (Clark, 1996).

Natural antimicrobial compounds are needed and highly sought after as there is an increased need for safer antimicrobial food preservatives which are more acceptable than the present preservatives whose safety in food is sometimes questionable. Food processors, food safety researchers and regulatory agencies have been increasingly concerned with the growing number of food borne illness outbreaks caused by certain pathogens. Several reports on the antimicrobial effectiveness of essential oils in foods suggest that the use of these essential oils may improve food safety (Friedman *et al*., 2002).

Salvia is also of particular interest because it may be used for the production of raw materials or preparations that are in need of phytochemicals with significant antioxidant capacity (Exarchou *et al*., 2002). Antioxidants play a role in protecting the body against the damage caused by free radicals. The importance of free radicals in the aetiology of certain diseases has been increasingly recognised. In fact, free radicals have been implicated in more than one hundred diseases. They are produced in the body through normal metabolic pathways. Exogenous sources of free radicals include tobacco smoke, ionizing radiation, certain pollutants, organic solvents and even pesticides. When produced in excess, free radicals have the ability to cause tissue injury. Additional dietary supplies of natural antioxidants act as protective agents against damage by free radicals and can ultimately act as efficient scavengers of free radicals before any tissue damage is done.

The family Lamiaceae is considered to be a promising source of natural antioxidants (Hohmann *et al*., 1999). It is believed that the antioxidant properties of *Salvia* can be largely attributed to the rosmarinic acid, (Exarchou *et al*., 2002; Petersen and Simmonds, 2003) carnosic acid and carnosol content (Wang *et al*., 1999). In addition to these three main compounds, the antioxidant activity of *Salvia* may also be partly due to the presence

of other phenolic compounds such as flavonoids (Exarchou *et al*., 2002) as well as diterpenes and triterpenes (Wang *et al*., 1999). However, investigative studies have concluded that the flavonoids have a rather small contribution to the total antioxidant activity of extracts (Exarchou *et al*., 2002).

Within the Lamiaceae, rosmarinic acid is restricted to the sub-family Nepetoideae however, it has also been found in other plant families (Petersen and Simmonds, 2003). This distribution shows that the occurrence of rosmarinic acid cannot be used as a chemotaxonomical marker to differentiate among different plant families. Therefore extracts that are rich in rosmarinic acid have high radical scavenging activity. In saying this, *Salvia* is an ideal candidate to investigate phytochemically as a potential, useful, natural antioxidant.

The process of researching and developing a therapeutic compound or material species in South Africa from plant origin is a multi-disciplinary process. There is an integration of sciences including botany, pharmacology and chemistry which all need to be carefully and thoroughly investigated. The use of *Salvia* therefore, has been largely empirical. The aim of this report is to further investigate the antimicrobial and the antioxidant activities of *Salvia* species in South Africa and to scientifically validate the appropriate use of *Salvia* in African traditional healing and also in today's modern world.

Study Objectives:

- To analyse the composition of the essential oils using gas chromatography-mass spectroscopy.

- To determine the antimicrobial properties of the essential oils, methanol and acetone extracts of selected species.

- To determine the preliminary antioxidant properties of the methanol extracts of selected species.

- To scientifically validate the traditional use of *Salvia* especially in traditional herbal preparations.

CHAPTER 2: MATERIALS AND METHODS

2.1 Collection of plant material

Ten *Salvia* species (twelve samples) were collected from the wild or the Walter Sisulu Botanical Garden. Voucher specimens are housed in the Department of Pharmacy and Pharmacology, University of the Witwatersrand.

Table 2: Table of study samples with voucher number and locality.

Species	Locality	Voucher
S. africana-caerulea	West Coast – Dynefontein	AV 570
S. africana-lutea	West Coast – Dynefontein	AV 572
S. chamelaeagnea	Walter Sisulu Botanical Garden	AV584
S. disermas	Walter Sisulu Botanical Garden	AV 583
S. disermas	Mossel Bay	AV502
S. disermas	Kokerboomwoud	AV 250
S. dolomitica	Walter Sisulu Botanical Garden	AV 602
S. lanceolata	West Coast – Dynefontein	AV 571
S. namaensis	Swartberg Pass	AV 501
S. runcinata	Tarlton	AV 632
S. stenophylla	Lady Grey	AV 618
S. verbenaca	De Rust	AV 631

2.1.1 Essential oil isolation

Essential oils were extracted, from the aerial parts of individual *Salvia* species, by hydrodistillation in a Clevenger-type apparatus for three hours. (Figure 1) The plant material was weighed before distillation and the amount of oil weighed after distillation to determine the yield of oil. Due to the volatile nature of these essential oils, they were stored in tightly sealed amber vials and refrigerated. All oils were recovered without solvent and were less dense than water.

2.1.2 Preparation of methanol and acetone extracts

The oven dried (30 °C) and finely ground plant material was weighed out into five-gram samples. From each *Salvia* species a methanol and acetone extract was prepared. Extracts were extracted and filtered twice and reconstituted to 128.00 mg/ml, unlike the essential oils that were used without any dilution. Previous studies within the microbiology

laboratory Department of Pharmacy and Pharmacology at The University of the Witwatersrand have confirmed no antimicrobail activity for acetone independently. This is in support of literature (Eloff, 1998, NCCLS, 2003).

2.2 Analytical chemistry

2.2.1 Gas chromatography mass spectroscopy (GC/MS)

The analysis of the essential oil was performed using a Hewlett-Packard GCD system equipped with a HP-Innowax column (60 m x 0.25 mm ∅., with 0.25 μm film thickness). Temperature at the injection port was 250 °C. Column temperature was initially 60 °C for ten minutes then gradually increased to 220 °C at a 4 °C per minute rate. Temperatures were held at 220 °C for ten minutes until it was finally raised to 240 °C at a rate of 1 °C per minute. The time in the column added up to a total of 80 minutes. Helium was the carrier gas at a flow rate of 0.7 ml/minute. An electron ionization system with an ionization energy of 70 eV was used with a split ratio of 50:1. The mass range was between 35 and 425 m/z. 0.9 μl of hexane was added to 0.1 μl of the essential oil and was injected. Data was recorded after a 5 minute lag phase. Identification took place using the TBAM'S database libraries by matching both retention indices and mass spectral fragmentation patterns.

2.2.2 Thin layer chromatography

All thin layer chromatography was performed using silica plates (silica gel 60 with fluorescent indicator UV254. Layer: 0.20 mm. Macherey-Nagel, Germany). The mobile phase used:

1. Chloroform-ethyl acetate-benzene-formic acid (2,4:2:1:0,6) was used for the methanol extracts and a rosmarinic acid standard. (Figure 4)

After development the TLC plates (10 cm) were air-dried and detection was made possible with the use of a spray reagent, vanillin-sulphuric acid (10 % acid). Colour development took place after a short heating period at 100 ºC.

Rosmarinic acid (Fluka 435285/1 52902), in its pure form, was obtained and used as a standard. Rosmarinic acid was subjected to thin layer chromatography on silica gel plates along with the other methanol extracts of the test samples. The volume applied (spotted) for the methanol extract (of a 128.00 mg/ml dilution) and rosmarinic acid was 3 µl, in order to keep the same concentration of test samples for standardization. For identification and visulization of antioxidant properties the plates were sprayed with a DPPH (0.038/100 ml of methanol) spray reagent.

2.3 Antimicrobial studies

2.3.1 Microbial strains

Salvia has traditionally been used to treat diarrhoea, stomach complaints and other minor digestive disturbances, therefore *Escherichia coli, Bacillus cereus* and *Yersinia enterocolitica* were selected as reference test strains. Certain species have been used to treat kidney infections and gynecological ailments therefore *Candida albicans* and *Escherichia coli* were included in the study. Due to the fact that *Salvia* has been used as a remedy for general respiratory infections *Klebsiella pneumoniae* has been selected. In addition to this, *Salvia* has been used as an antiseptic and a remedy to treat skin infections and inflammation therefore *Staphylococcus aureus* has been selected. All antimicrobial tests were carried out under sterile conditions. All assays were undertaken in duplicate and where necessary confirmed with a third test.

Table 3: Table of test organisms included in the study.

Gram-negative test organisms	*Escherichia coli* *Yersinia enterocolitica* *Klebsiella pneumoniae*	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 90112 ATCC 10231
Fungal test organism	*Alternaria alternata*	Clinical strain

2.3.2 Disc diffusion method

The bacterial and fungal cultures were harvested from stock agar plates. The bacterial cultures were inoculated in Mueller Hinton (Oxoid) broth, whilst the fungal cultures were harvested from Sabourauds Dextrose (Oxoid) agar. Base layers of autoclaved Mueller Hinton (Oxoid) agar was prepared for bacterial cultures and Sabourauds Dextrose (Oxoid) agar was prepared for fungal and yeast cultures. This method was employed whereby yeast, bacterial or fungal spore suspensions yielding an approximate inoculum size of 1 x 10^6 CFU/ml was incorporated into Tryptone Soya (Oxoid) agar. With aseptic manipulation, sterile 6 mm discs with approximately 15-20 µl either plant essential oil, plant methanol extract or plant acetone extract (Figure 2) were introduced onto the seeded agar plate. Positive bacterial controls, in the form of antibiotic discs, were included in the assay. For the Gram-positive and Gram-negative bacteria Neomycin (30 µg, Oxoid) was used as a control and for the mould and yeasts, Nystatin (100 IU, Oxoid) was used as a control. The plates were initially placed in the refrigerator for one hour to allow diffusion of the disc contents into the agar with minimal bacterial or fungal growth, while this takes place. Thereafter the bacterial plates were incubated at 37 °C for 24 hours. The yeasts were also incubated at 37 °C but for 48 hours. *Alternaria alternata* was incubated at 25 °C for four days. The zones of inhibition were thereafter examined. Measurements were recorded from the edge of the disc to the end of the zone of inhibition and recorded in millimeters.

2.3.3 Determination of minimum inhibitory concentration (MIC)

A minimum inhibitory concentration (MIC) was determined for those test samples that displayed antimicrobial activity from the disc diffusion assay (NCCLS, 2003). The MIC was determined using the ρ-Iodonitrotetrazolium (INT) microplate method (Eloff, 1998). *Bacillus cereus* was inoculated in Tryptone Soya Broth and incubated for 24 hours at 37 °C. Two microplates were prepared (Figure 3) so as to cover the necessary test samples. An inoculum of 1 ml was transferred into 100 ml sterile broth to give a 1:100 stock culture. Methanol and acetone test samples were prepared starting at a concentration of 16.000 mg/ml in the first well. The series of dilutions were carried out so that a final concentration of 0.125 mg/ml was obtained in the eighth and final well. For the essential

oils, 51.200 mg of each essential oil was weighed out and 400 µl of acetone was added. This gave the desired concentration of 32.000 mg/ml in the first well, taking into account the sterile water and 100 µl culture added. The microplates were aseptically prepared whereby 100 µl of sterile water was added to each well in the microplates. An amount of 100 µl of extract / essential oil was added to the first row of wells and a series of dilutions (diluting 100 µl each time) was performed. An amount of 100 µl of prepared *Bacillus cereus* stock culture was added to all the wells. Both negative and positive controls were included. Ciprofloxacin (Oxoid) was included as a positive control. Stock solutions of 0.01 mg/ml were prepared and serially diluted as in assays performed with test samples.

The addition of the test samples to the wells and the serial dilutions took place under laminar airflow so as to minimize the risk of contamination. The microplates were sealed to prevent mixing or contamination of wells and were incubated at 37 °C. After 24 hours, INT solution was prepared at a concentration of 0.2 mg/ml and 40 µl INT was added to each well in both microplates. The compound INT is used to indicate biological activity because 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 if any bacterial growth was present. The microplates were visually examined after 6 hours and the MIC's were determined.

Figure 1: Process of hydrodistillation.

Figure 2: Preparing discs for disc diffusion assay.

Figure 3: Preparing microplate.

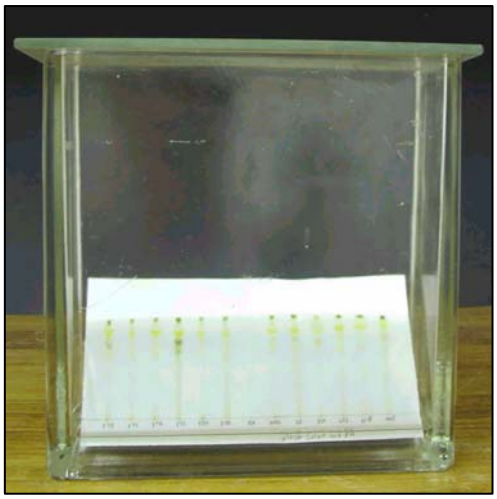


Figure 4: TLC performed on extracts and rosmarinic acid.

CHAPTER 3: MONOGRAPHS OF *SALVIA* SPECIES STUDIED

Salvia africana-caerulea

Salvia africana-lutea

Salvia chamelaeagnea

Salvia disermas

Salvia dolomitica

Salvia lanceolata

Salvia namaensis

Salvia runcinata

Salvia stenophylla

Salvia verbenaca

Salvia africana-caerulea L. E. Codd

1. Botanical description

Shrub 0,6 – 1,5 m tall, often branching at the base with several erect, usually sparingly branched stems. Leaves: blade simple, petiolate, greenish and vary in size and shape. Flowers often dense, light blue to bluish purple or pinkish in colour.

Figure 5: *S. africana-caerulea.*

2. Distribution

In coastal fynbos and on rocky slopes. Distributed from Vanrhynsdorp district to Cape Town and eastwards to Montagu and Caledon.

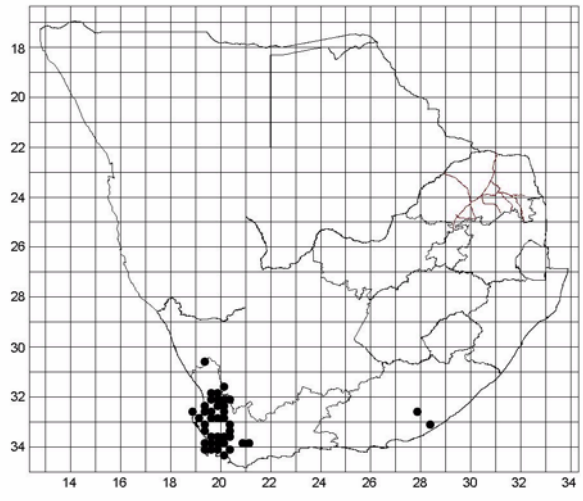


Figure 6: Geographical distribution of S. *africana-caerulea**

* All distribution maps have been purchased and included with permission from the South African National Biodiversity Institute.

3. Essential oil composition

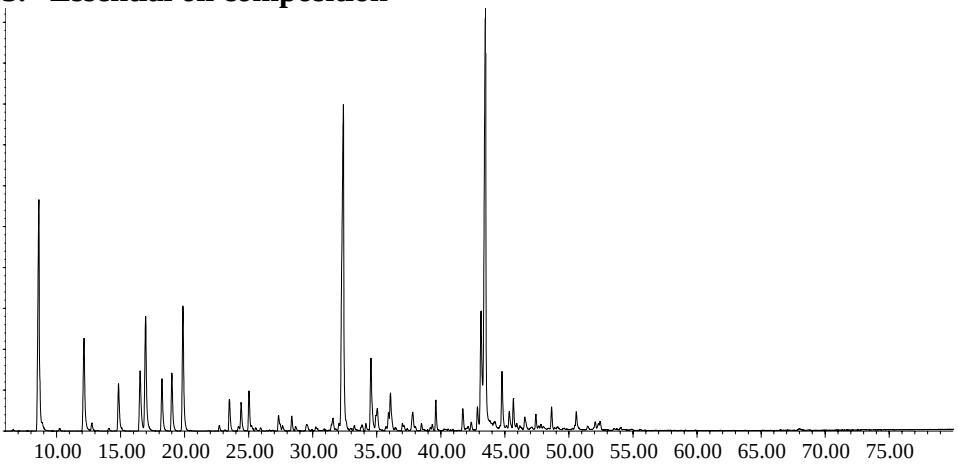


Figure 7: Gas chromatography profile of *S. africana-caerulea* collected on the West Coast, Dynefontein. For all chromatograms the X axis represents time in minutes and the Y axis the attenuation.

4. GC-MS results

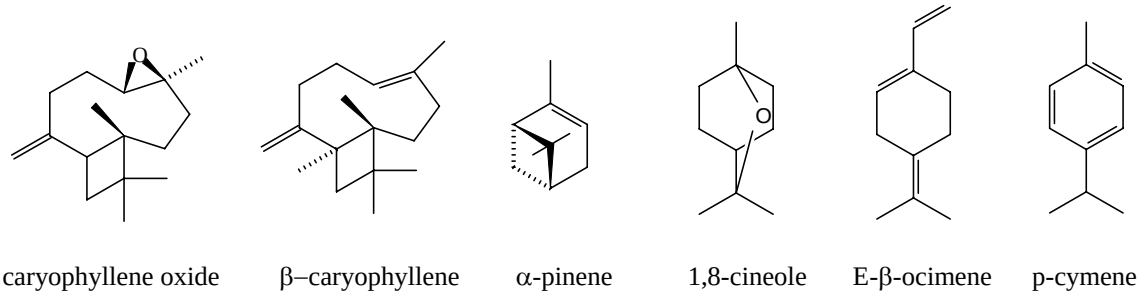
Table 4: GC-MS results of *Salvia africana-caerulea*.

RRI *	Retention time (minutes)	Area percentage	Compound
1032	**8.62**	**9.7**	**α-pinene**
1076	10.22	0.1	Camphene
1118	12.15	4.1	β-pinene
1132	12.74	0.3	Sabinene
1159	14.08	0.1	δ-3 carene
1174	14.84	2.0	Myrcene
1203	16.53	2.7	Limonene
1213	**16.95**	**5.2**	**1,8-cineole**
1246	18.23	2.0	(Z)-β-ocimene
1266	**19.00**	**5.2**	**(E)-β-ocimene**
1280	**19.87**	**5.2**	**ρ-cymene**
1348	22.70	0.2	6-methyl-5-hepten-2-one
1384	24.21	0.2	α-pinene oxide
1386	25.03	1.4	(Z)-3-hexen-1-ol
1452	26.88	0.3	1-octen-3-ol
1467	27.33	0.5	6-methyl-5-hepten-2-ol
1474	27.49	0.1	*trans*-sabinene hydrate
1476	27.64	0.2	(Z)-β-ocimene epoxide

RRI *	Retention time (minutes)	Area percentage	Compound
1498	28.36	0.5	(E)-β-ocimene epoxide
1497	28.67	0.2	α-copaene
1536	29.51	0.1	β-bourbonene
1532	29.51	0.1	Camphor
1553	30.24	0.1	Linalool
1571	30.86	0.04	*trans*-p-menth-2-en-1-ol
1589	31.47	0.1	Isocaryophyllene
1597	31.57	0.4	Bornylacetate
1594	31.73	0.7	*trans*-α-bergamotene
1602	32.04	0.1	6-methyl-3,5-heptadien-2-one
1612	**32.25**	**17.3**	**β-caryophyllene**
1648	33.26	0.2	Myrtenal
1661	33.75	0.1	*trans*-pinocarvyl acetate
1664	33.86	0.2	*trans*-pinocarveol
1668	34.16	0.2	(Z)-β-farnesene
1687	34.54	3.2	α-humulene
1698	34.93	0.3	Myrtenyl acetate
1729	35.72	0.2	*cis*-1,2-epoxy-terpin-4-ol
1727	35.90	0.5	7-epi-1,2-dehydrosesquicineole
1741	36.06	1.6	β-bisabolene
1773	37.00	0.2	δ-cadinene
1776	37.13	0.1	γ-cadinene
1796	37.81	0.8	Selina-3,7(11)-diene
1830	33.48	0.2	2,6-dimethyl.3(E),5(E),7-octatrien-2-ol
1845	38.98	0.02	*trans*-carveol
1853	39.18	0.1	*cis*-calamenene
1864	39.33	0.2	ρ-cymen-8-ol
1868	39.61	1.0	(E)-geranyl acetone
1900	40.57	0.04	Epicubebol
1969	42.12	0.03	*cis*-jasmone
1984	42.86	0.9	(E)-12-norcarophyll-5-en-2-one
2001	43.14	5.0	Isocarophyllene oxide
2008	43.48	18.7	Caryophyllene oxide
2071	44.78	2.0	Humulene epoxide II
2081	45.12	0.1	Humulene epoxide III
2104	45.65	0.9	Viridiflorol
2144	46.55	0.6	Spathulenol
2232	48.65	0.7	α-bisabolol
2324	50.58	0.6	Caryophylladienol II
2389	51.47	0.2	Caryophyllenol I
2392	52.42	0.3	Caryophyllenol II
	Total	**98.43**	

RRI* – relative retention indices calculated against n-alkanes

The major compounds in *Salvia africana-caerulea* are caryophyllene oxide (18.7%), β-caryophyllene (17.3%), α-pinene (9.7%), 1,8-cineole (5.2%), (E)-β-ocimene (5.2%) and p-cymene (5.2%).



caryophyllene oxide β–caryophyllene α-pinene 1,8-cineole E-β-ocimene p-cymene

Figure 8: Chemical structure of the major compounds identified in *Salvia africana-caerulea* essential oil.

Salvia africana-lutea L. E. Codd

1. Botanical description

Branched shrub growing up to 2 m tall. Stems densely leafy. Leaves are petiolate, blade simple. Flowers are large and golden brown, reddish brown, khaki or occasionally purplish in colour.

Figure 9: *S. africana-lutea.*

2. Distribution

Found on coastal sand dunes, adjoining hills and in arid fynbos on rocky slopes to 800 m in altitude. Distributed from Namaqualand to the Cape Peninsula and eastwards to Port Alfred.

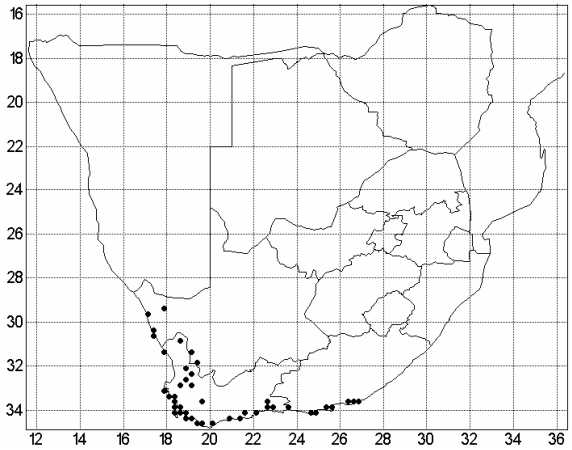


Figure 10: Geographical distribution of *S. africana-lutea.*

3. Essential oil composition

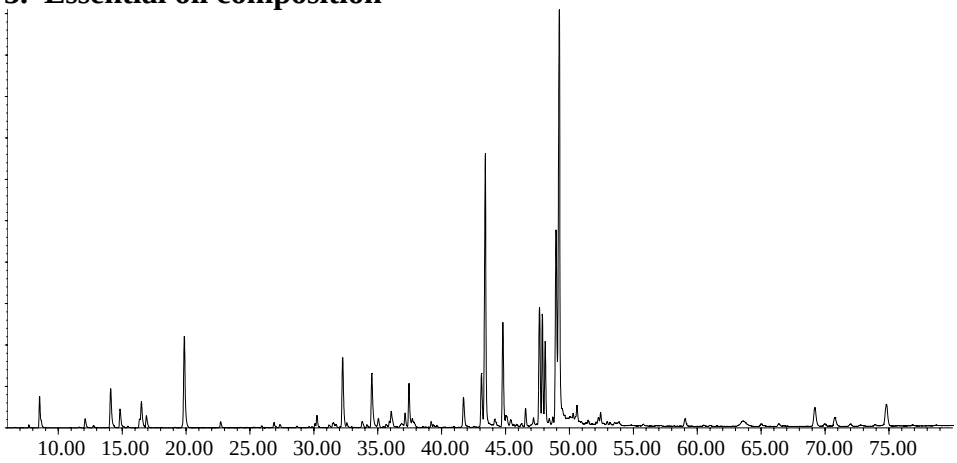


Figure 11: Gas chromatography profile of *S. africana-lutea* collected on the West Coast, Dynefontein.

3. GC-MS results

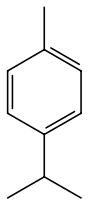
Table 5: GC-MS results of *Salvia africana-lutea.*

RRI *	Retention time (minutes)	Area percentage	Compound
1000	7.69	0.1	Decane
1032	8.62	1.2	α-pinene
1100	11.63	0.03	Undecane
1118	12.15	0.5	β-pinene
1132	12.74	0.1	Sabinene
1159	14.08	2.2	δ-3 carene
1174	14.84	0.9	Myrcene
1187	15.44	0.1	o-cymene
1188	15.59	0.1	α-terpinene
1205	16.35	0.2	Sylvestrene (m-mentha-1(6),8-diene)
1203	16.53	1.4	Limonene
1213	16.95	0.6	1,8-cineole
1280	**19.87**	**5.2**	**ρ-cymene**
1348	22.70	0.3	6-methyl-5-hepten-2-one
1429	25.89	0.03	Perillen
1452	26.88	0.3	1-octen-3-ol
1348	27.33	0.2	6-methyl-5-hepten-2-ol
1497	28.67	0.1	α-copaene
1544	29.95	0.1	α-gurjunene
1553	30.24	0.5	Linalool
1568	31.19	0.1	*trans*-α-bergamotene

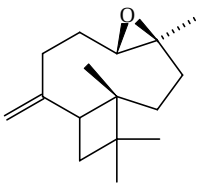
RRI *	Retention time (minutes)	Area percentage	Compound
1612	32.25	3.8	β-caryophyllene
1628	32.56	0.1	Aromadendrene
1661	33.77	0.4	Allo aromadendrene
1687	34.54	3.1	α-humulene
1704	35.05	0.3	γ-muurolene
1709	35.18	0.3	α-terpinylacetate
1727	35.90	0.2	7-epi-1,2-dehydrosesquicineole
1741	36.06	1.0	β-bisabolene
1743	37.13	0.5	γ–cadinene
1786	37.44	2.0	ar-curcumene
1853	39.18	0.2	*cis*-calamenene
1864	39.33	0.1	ρ-cymen-8-ol
1868	39.61	0.1	(E)-geranyl acetone
1949	41.70	0.8	Piperitone
2001	43.14	2.6	Isocaryophyllene oxide
2008	**43.48**	**13.3**	**Caryophyllene oxide**
2045	44.15	0.3	Humulene epoxide I
2071	**44.78**	**5.0**	**Humulene epoxide II**
2144	46.55	0.8	Spathulenol
2185	**47.61**	**5.8**	**γ-eudesmol**
2204	47.83	4.6	Eremoligenol
2232	48.65	0.3	α-bisabolol
2250	**48.90**	**10.1**	**α-eudesmol**
2257	**49.06**	**19.8**	**β-eudesmol**
2324	50.58	0.7	Caryophylladieneol II
2392	52.42	0.5	Caryophyllenol II
2923	74.79	0.9	Carissone
	Total	**91.86**	

RRI* – relative retention indices calculated against n-alkanes

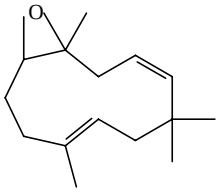
The major compounds in *Salvia africana-lutea* are p-cymene (5.2%), caryophyllene oxide (13.3%), humulene epoxide II (5.0%), γ–eudesmol (5.8%), α-eudesmol (10.1%) and β-eudesmol (19.8%).



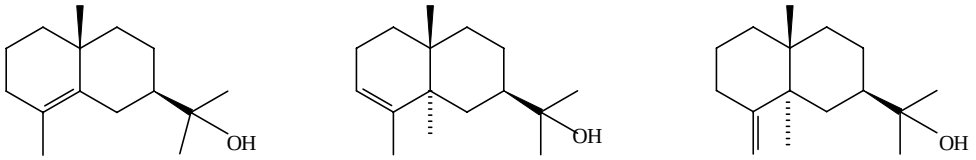
p-cymene



caryophyllene oxide



humulene epoxide II



γ–eudesmol α-eudesmol β-eudesmol

Figure 12: Chemical structure of the major compounds identified in *Salvia africana-lutea* essential oil.

Salvia chamelaeagnea L. E. Codd

1. Botanical description

Branched shrub 0,6-2 m tall. Leaves are petiolate, blade simple. Flowers are blue or purplish blue often with white on the lower lip.

Figure 13: *S. chamelaeagnea.*

2. Distribution

Distributed from Clanwilliam to Cape Town and eastwards to Ladysmith and Riversdale districts. Found in fynbos along watercourses, in sandy soil among rocks and along roadsides.

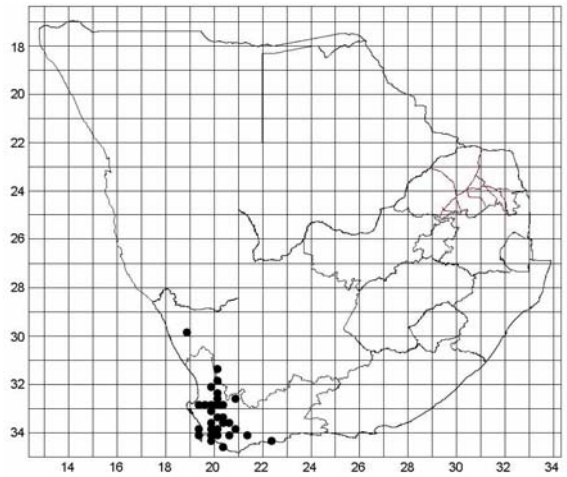


Figure 14: Geographical distribution of *S. chamelaeagnea.*

No essential oil was obtained for *Salvia chamelaeagnea.*

Salvia disermas L. E. Codd

1. Botanical description

Soft shrub, between 0,3 and 1,0 m tall with one or more stems from a woody rootstock. Leaves often crowded and larger near the base of the plant, petiolate or subsessile, blade ovate to oblong-lanceolate. Flowers are whitish, pale blue or mauve and denser above.

Figure 15: *S. disermas.*

2. Distribution

Distributed from the North-West Province to the Northern Cape Province through the western Free State, Karoo, Namaqualand and southwards to Uniondale and Swellendam. Found on sandy soil in water-courses, on limestone formations and rocky hillsides. Tends to spread as a weed along roadsides and overgrazed veld.

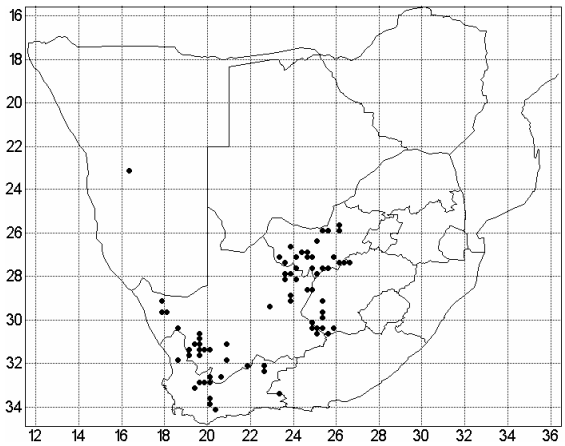


Figure 16: Geographical distribution of *S. disermas.*

3. Essential oil composition

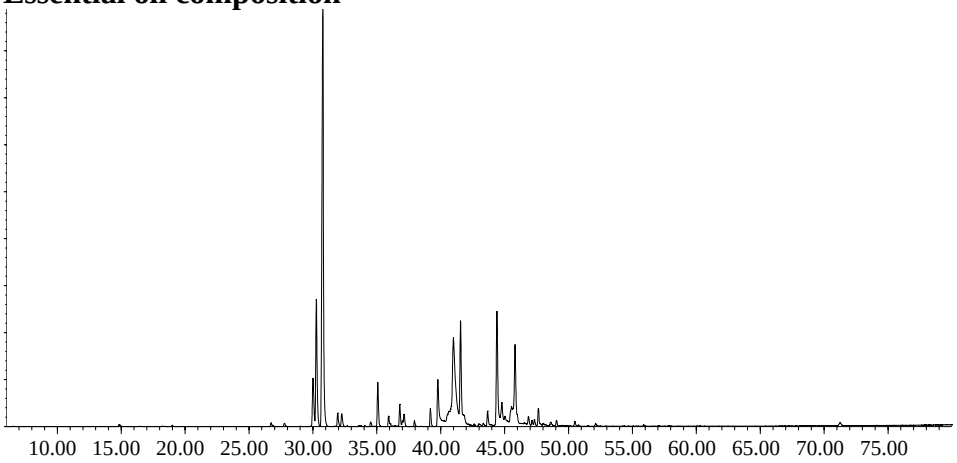


Figure 17: Gas chromatography profile of *S. disermas* collected at Mossel Bay.

4. GC-MS results

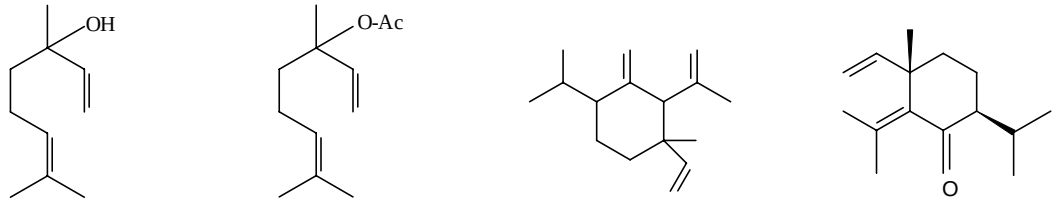
Table 6: GC-MS results of *Salvia disermas.*

RRI *	Retention time (minutes)	Area percentage	Compound
1174	14.84	0.2	Myrcene
1266	19.00	0.1	(E)-β-ocimene
1450	26.72	0.2	*trans*-linalool-oxide
1480	27.77	0.1	Nerol oxide
1544	30.01	3.7	α-gurjunene
1553	**30.24**	**8.9**	**Linalool**
1565	**30.78**	**34.5**	**Linalyl acetate**
1600	31.93	0.9	Achillene
1612	32.25	1.0	β-caryophyllene
1687	34.54	0.3	α-humulene
1682	35.07	2.8	α-terpineol
1733	35.91	0.7	Neryl acetate
1765	36.80	1.4	Geranylacetate
1773	37.00	0.3	δ-cadinene
1776	37.13	0.3	γ-cadinene
1808	37.95	0.3	Nerol
1857	39.20	1.1	Geraniol
1871	39.78	4.4	Diepishyobunone
1900	40.62	0.7	Isoshuyobunone
1916	**40.99**	**10.7**	**Shyobunone**
1931	**41.55**	**6.2**	**Epi-isoshyobunone**
1953	42.99	0.2	Shyobunol
2001	43.14	0.1	Isocaryophyllene oxide
2069	44.78	1.0	Germacrene D 4-ol

RRI *	Retention time (minutes)	Area percentage	Compound
2187	47.62	1.1	T-cadinol
2255	49.05	0.2	α−cadinol
2312	50.50	0.3	9-geranyl-p-cymene
2376	52.11	0.1	Manoyl oxide
	Total	**81.8**	

RRI* – relative retention indices calculated against n-alkanes

The major compounds in *Salvia disermas* are linalool (8.9%), linayl acetate (34.5), shyobunone (10.7%) and epi-isoshyobunone (6.2%).



linalool linayl acetate shyobunone epi-isoshyobunone

Figure 18: Chemical structure of the major compounds identified in *Salvia disermas* essential oil.

Salvia dolomitica L. E. Codd

1. Botanical description

Shrub 1-2 m tall, branched from the base. Leaves petiolate. Flowers are light pink or lilac with cream or yellow markings on the lower lip.

Figure 19: *S. dolomitica.*

2. Distribution

Restricted to the Northern Province and northern Mpumalanga. Usually found on dolomitic outcrops between 1000 and 1500 m in altitude.

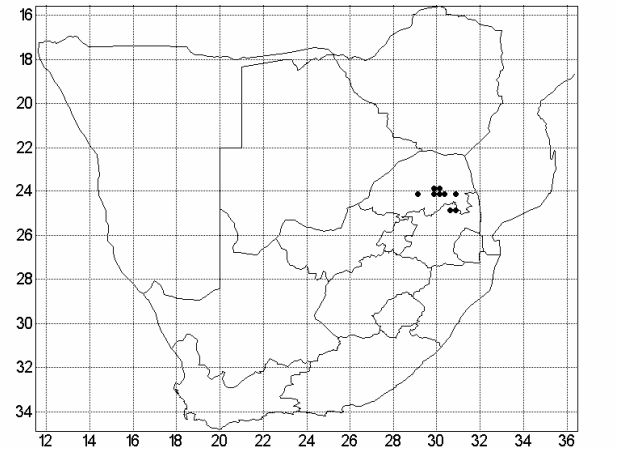


Figure 20: Geographical distribution of *S. dolomitica.*

3. Essential oil composition

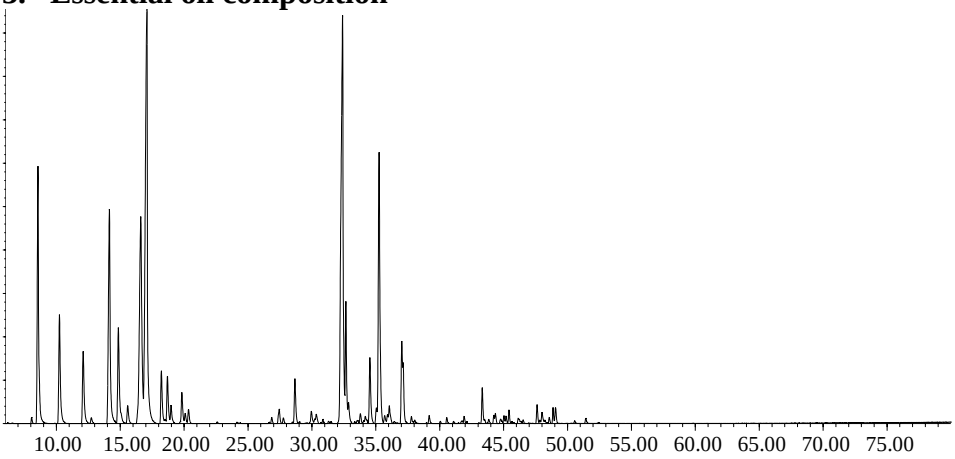


Figure 21: Gas chromatography profile of *S. dolomitica* collected at Walter Sisulu Botanical Garden.

4. GC-MS results

Table 7: GC-MS results of *Salvia dolomitica.*

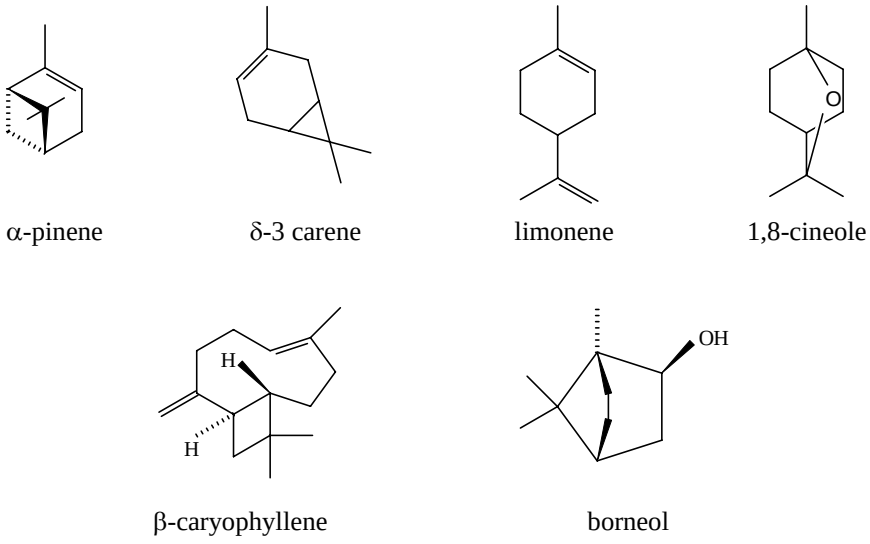
RRI *	Retention time (minutes)	Area percentage	Compound
1014	8.09	0.1	Tricyclene
1032	8.62	7.1	**α-pinene**
1076	10.22	3.3	Camphene
1118	12.15	2.3	β-pinene
1132	12.74	0.2	Sabinene
1159	14.08	7.5	**δ-3 carene**
1174	14.84	3.0	Myrcene
1188	15.58	0.5	α-terpinene
1203	16.53	9.7	**Limonene**
1213	16.95	17.6	**1,8-cineole**
1246	18.23	1.5	(Z)-β–ocimene
1255	18.7	1.4	γ-terpinene
1266	19.00	0.5	(E)-β-ocimene
1280	19.87	0.9	ρ-cymene
1286	20.08	0.3	Isoterpinolene
1290	20.35	0.4	Terpinolene
1382	24.13	0.02	*cis*-allo ocimene
1466	27.44	0.5	α-cubebene
1474	27.49	0.5	*trans*-sabinene hydrate
1468	27.76	0.1	Isoledene

RRI *	Retention time (minutes)	Area percentage	Compound
1497	28.67	1.2	α-copaene
1544	29.95	0.3	α-gurjunene
1553	30.24	0.1	Linalool
1556	30.35	0.3	*cis*-sabinene hydrate
1571	30.86	0.1	*trans*-p-menth-2-en-1-ol
1589	31.30	0.1	Aristolene
1589	31.47	0.04	Isocaryophyllene
1612	**32.25**	**17.4**	**β-caryophyllene**
1628	32.56	3.0	Aromadendrene
1617	32.84	0.6	Selina-5,11-diene
1634	33.33	0.02	Cadina-3,5-diene
1661	33.77	0.3	Allo aromadendrene
1674	34.04	0.03	γ–gurjunene
1677	34.17	0.1	Epizonarene
1687	34.54	1.9	α-humulene
1704	35.05	0.4	γ-muurolene
1719	**35.22**	**8.5**	**Borneol**
1740	35.91	0.2	Valencene
1740	36.05	0.5	α-muurolene
1773	37.00	2.1	δ-cadinene
1776	37.13	1.5	γ-cadinene
1799	37.78	1.2	Cadina-1,4-diene (cubenene)
1853	39.18	0.2	*cis*-calamenene
1900	40.57	0.1	Epicubebol
1941	41.47	0.02	α-calacorene
1957	41.90	0.1	Cubebol
1969	42.12	0.04	*cis*-jasmone
2008	43.48	0.8	Caryophyllene oxide
2033	43.84	0.1	Epiglobulol
2050	44.24	0.1	(E)-nerolidol
2057	44.40	0.2	Ledol
2071	44.78	0.1	Humulene epoxide II
2088	45.18	0.1	1-epi-cubenol
2098	45.44	0.3	Globulol
2104	45.65	0.03	Viridiflorol
2144	46.14	0.1	Rosifoliol
2144	46.55	0.08	Spathulenol
2219	47.62	0.4	T-cadinol
2204	47.83	0.08	Eremoligenol
2209	48.01	0.3	T-muurolol
2218	48.23	0.06	Torreyol
2228	48.58	0.1	Pogostol
2250	48.90	0.3	α-eudesmol
2257	49.06	0.4	β-eudesmol
2324	50.58	0.06	Caryophylladieneol II

RRI *	Retention time (minutes)	Area percentage	Compound
2389	51.47	0.1	Caryophyllenol I
	Total	**99.77**	

RRI* – relative retention indices calculated against n-alkanes

The major compounds in *Salvia dolomitica* are α-pinene (7.1%), δ-3 carene (7.5%), limonene (9.7%), 1,8-cineole (17.6%), β-caryophyllene (17.4%) and borneol (8.5%).



α-pinene δ-3 carene limonene 1,8-cineole

β-caryophyllene borneol

Figure 22: Chemical structure of the major compounds identified in *Salvia dolomitica* essential oil.

Salvia lanceolata L. E. Codd

1. Botanical description

Branched shrub 1-2 m tall, stems often reddish brown. Leaves: blade simple, petiolate, thick textured. Flowers vary in colour from dull rose to brownish crimson or grey-blue.

Figure 23: *S. lanceolata*.

2. Distribution

Distributed from Namaqualand to the Cape Peninsula and eastwards to Montagu. Found mainly in coastal sandveld or arid fynbos, on sandy soil and rocky hillsides at altitudes of 0-300m.

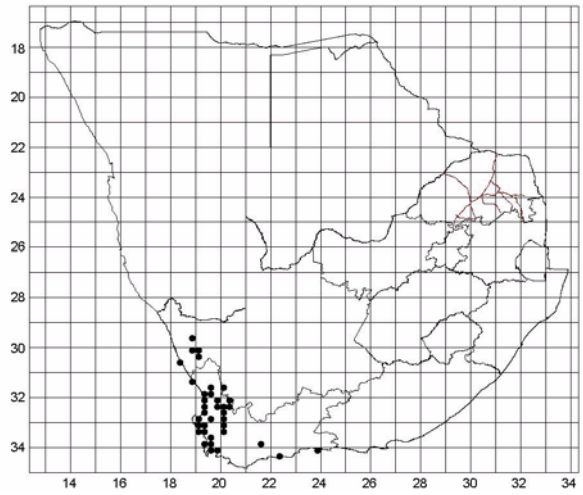


Figure 24: Geographical distribution of *S. lanceolata.*

3. Essential oil composition

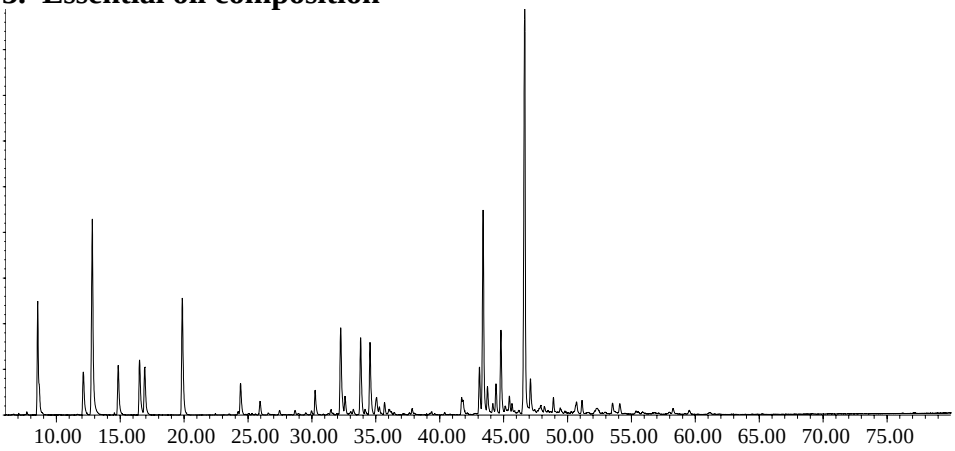


Figure 25: Gas chromatography profile of *S. lanceolata* collected on the West Coast, Dynefontein.

3. GC-MS results

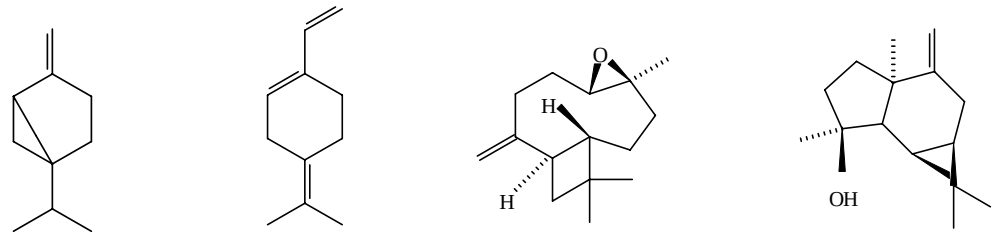
Table 8: GC-MS results of *Salvia lanceolata.*

RRI *	Retention time (minutes)	Area percentage	Compound
1000	7.69	0.1	Decane
1032	8.62	4.6	α-pinene
1035	8.70	Present	α-thujene
1118	12.15	2.4	β-pinene
1132	**12.74**	**11.4**	**Sabinene**
1174	14.84	2.6	Myrcene
1203	16.53	3.0	Limonene
1213	16.95	2.5	1,8-cineole
1266	**19.00**	**6.2**	**(E)-β-ocimene**
1384	24.21	0.1	α-pinene oxide
1429	25.89	0.7	Perillen
1400	26.59	0.1	Tetradecane
1474	27.49	0.2	*trans*-sabinene hydrate
1497	28.67	0.1	α-copaene
1544	29.95	3.7	α-gurjunene
1553	30.24	1.1	Linalool
1612	32.25	4.7	β-caryophyllene
1628	32.56	0.9	Aromadendrene
1596	33.03	0.1	α-guaiene
1648	33.26	0.2	Myrtenal
1661	33.77	3.9	Allo aromadendrene
1668	34.16	0.2	(Z)-β-farnesene

RRI *	Retention time (minutes)	Area percentage	Compound
1687	34.54	3.6	α-humulene
1706	35.07	1.1	α-terpineol
1709	35.18	1.1	α-terpinylacetate
1708	35.28	0.3	Ledene
1755	36.03	0.2	Bicyclogermacrene
1751	36.45	0.1	Carvone
1804	37.83	0.3	Myrtenol
1864	39.33	0.1	ρ-cymen-8-ol
2001	43.14	2.1	Isocaryophyllene oxide
2008	**43.48**	**9.4**	**Caryophyllene oxide**
2045	44.15	0.4	Humulene epoxide I
2057	44.40	1.3	Ledol
2071	44.78	3.8	Humulene epoxide II
2081	45.12	0.1	Humulene epoxide III
2098	45.44	0.6	Globulol
2104	45.65	0.3	Viridiflorol
2144	**46.55**	**20.9**	**Spathulenol**
2247	48.90	0.5	α-bergamotol
2389	51.47	0.5	caryophyllenol I
	Total	**95.5**	

RRI* – relative retention indices calculated against n-alkanes

The major compounds in *Salvia lanceolata* are sabinene (11.4%), E-β-ocimene (6.2%), caryophyllene oxide (9.4%) and spathulenol (20.9%).



sabinene E-β-ocimene caryophyllene oxide spathulenol

Figure 26: Chemical structure of the major compounds identified in *Salvia lanceolata* essential oil.

Salvia namaensis L. E. Codd

1. Botanical description

Bushy shrub 0,3-1,2 m tall, often herbaceous above and woody below. Leaves shortly petiolate. White, mauve or blue flowers that are more crowded above.

Figure 27: *S. namaensis.*

2. Distribution

Found on rocky slopes, in watercourses and on surface limestone. Distributed in northern and central Cape as far south as Oudtshoorn and Willowmore, and western Free State.

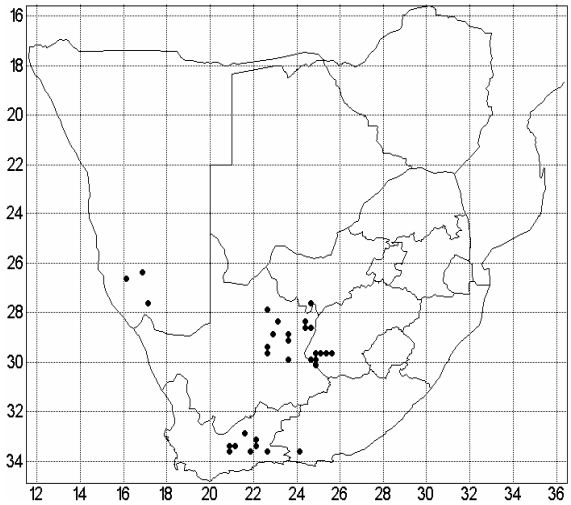


Figure 28: Geographical distribution of *S. namaensis.*

3. Essential oil composition

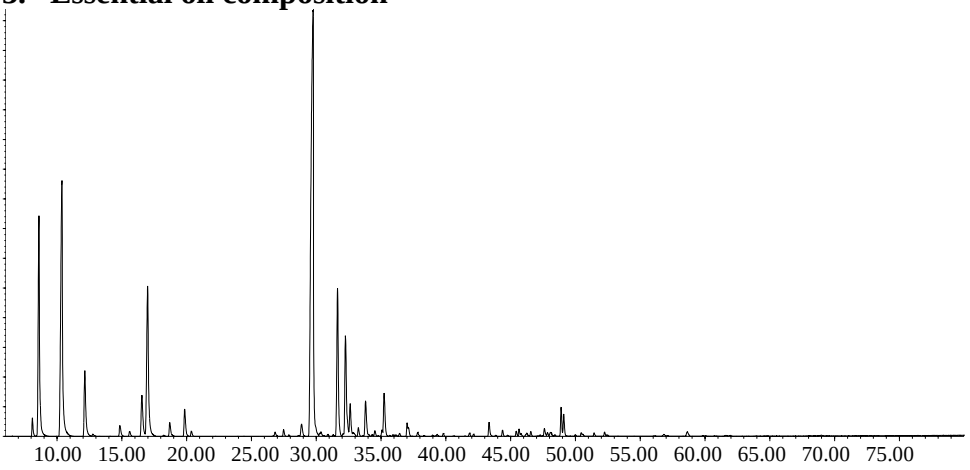


Figure 29: Gas chromatography profile of *S. namaensis* collected at Swartberg Pass.

4. GC-MS results

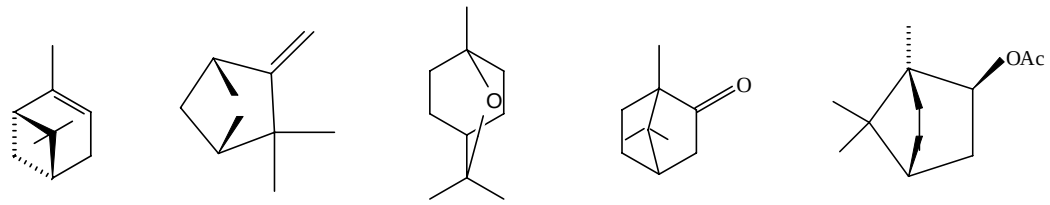
Table 9: GC-MS results of *Salvia namaensis.*

RRI *	Retention time (minutes)	Area percentage	Compound
1014	8.09	0.6	Tricyclene
1032	**8.62**	**9.3**	**α-pinene**
1076	**10.22**	**14.7**	**Camphene**
1118	12.15	3.3	β-pinene
1132	12.74	0.1	Sabinene
1174	14.84	0.5	Myrcene
1203	16.53	2.2	Limonene
1213	**16.95**	**8.2**	**1,8-cineole**
1246	18.23	0.1	(Z)-β-ocimene
1255	18.70	0.6	γ-terpinene
1280	19.87	1.3	ρ-cymene
1290	20.35	0.2	Terpinolene
1452	26.66	0.02	α,p-dimethylstyrene (p-cymenene)
1451	26.82	0.2	β-thujone
1474	27.49	0.3	*trans*-sabinene hydrate
1435	28.63	0.02	α-campholene aldehyde
1497	28.67	0.6	α-copaene
1532	**29.51**	**33.5**	**Camphor**
1553	30.24	0.1	Linalool
1556	30.35	0.1	*cis*-sabinene hydrate
1571	30.86	0.1	*trans*-p-menth-2-en-1-ol

RRI *	Retention time (minutes)	Area percentage	Compound
1586	31.29	0.1	Pinocarvone
1597	**31.57**	**6.8**	**Bornylacetate**
1612	32.25	4.8	β-caryophyllene
1628	32.56	1.2	Aromadendrene
1617	32.84	0.02	Selina-5,11-diene
1648	33.26	0.3	Myrtenal
1661	33.77	1.7	Allo aromadendrene
1682	34.31	0.1	δ-terpineol
1687	34.54	0.2	α-humulene
1700	34.78	0.02	Limonen-4-ol
1706	35.07	0.2	α-terpineol
1719	35.22	2.1	Borneol
1725	35.66	0.02	Verbenone
1755	36.03	0.1	Bicyclogermacrene
1773	37.00	0.5	δ-cadinene
1804	37.83	0.1	Myrtenol
1804	38.98	0.02	*trans*-carveol
1864	39.33	0.1	ρ-cymen-8-ol
1882	39.81	0.1	*cis*-carveol
1953	41.80	0.1	Palustrol
1948	42.14	0.1	*trans*-jasmone
2008	43.48	0.6	Caryophyllene oxide
2057	44.40	0.2	Ledol
2071	44.78	0.1	Humulene epoxide II
2098	45.44	0.1	Globulol
2104	45.65	0.2	Viridiflorol
2144	46.14	0.02	Rosifoliol
2144	46.55	0.2	Spathulenol
2198	47.27	0.03	Thymol
2185	47.61	0.3	γ-eudesmol
2204	47.83	0.1	Eremoligenol
2239	48.41	0.1	Carvacrol
2250	48.90	1.1	α-eudesmol
2257	49.06	0.8	β-eudesmol
2304	50.44	0.1	Cetylacetate
2389	51.47	0.1	Caryophyllenol I
2384	52.26	0.2	1-hexadecanol
	Total	**98.97**	

RRI* – relative retention indices calculated against n-alkanes

The major compounds in *Salvia namaensis* are α–pinene (9.3%), camphene (14.7%), 1,8-cineole (8.2%), camphor (33.5%) and bornylacetate (6.8%).



α–pinene camphene 1,8-cineole camphor bornylacetate

Figure 30: Chemical structure of the major compounds identified in *Salvia namaensis* essential oil.

Salvia runcinata L. E. Codd

1. Botanical description

Perennial erect herb 0,15 - 0,5 m tall with several stems from a taproot or less often a creeping rootstock. Leaves shortly petiolate. Flowers denser above and more widely spaced below, white or pale blue to mauve or purplish with the lower lip slightly longer.

Figure 31: *S. runcinata.*

2. Distribution

Variable species extending from northern Gauteng to the Free State. Also widespread throughout the northern, eastern and southern Cape as far as Bredasdorp district but rare in Kwazulu-Natal and Lesotho. Found in a variety of habitats, usually on heavy soils and locally common on disturbed places or overgrazed veld.

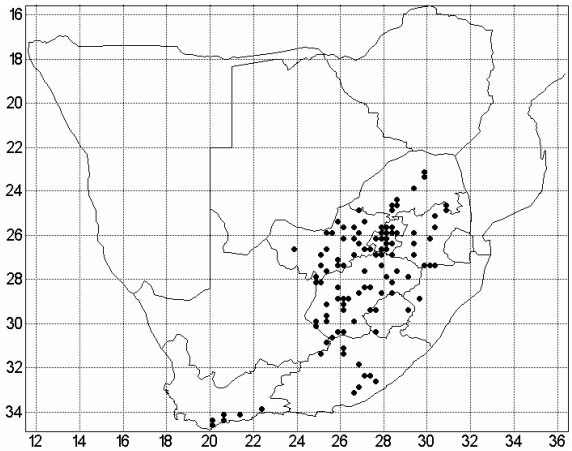


Figure 32: Geographical distribution of *S. runcinata.*

3. Essential oil composition

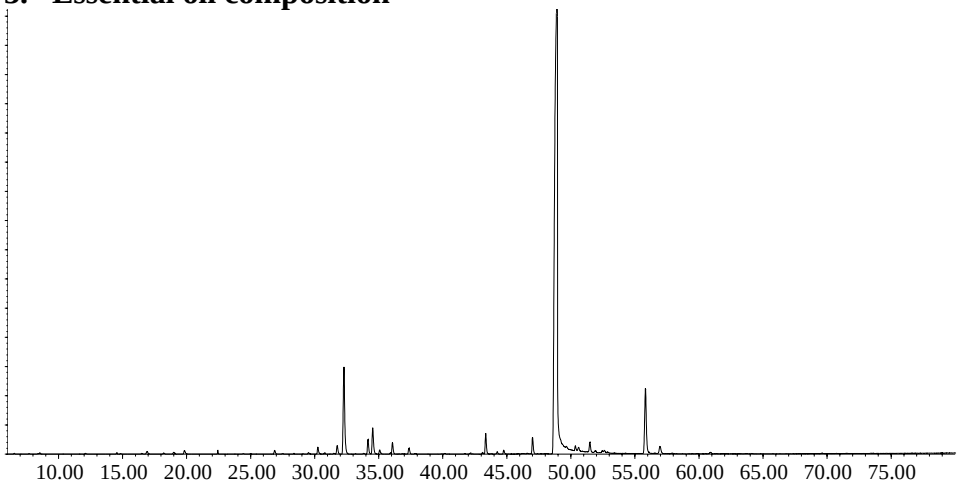


Figure 33: Gas chromatography profile of *S. runcinata* collected at Tarlton.

4. GC-MS results

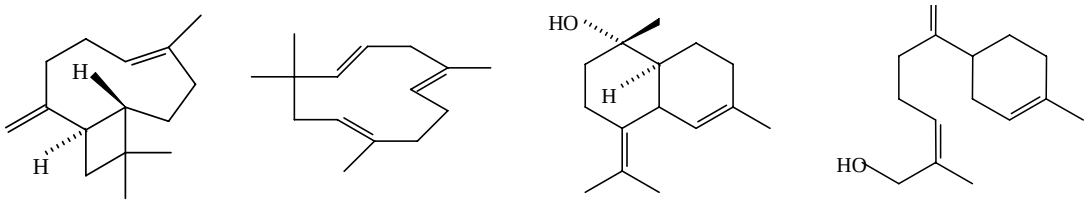
Table 10: GC-MS results of *Salvia runcinata.*

RRI *	Retention Time (minutes)	Area percentage	Compound
1032	8.62	0.1	α-pinene
1118	12.15	0.1	β-pinene
1174	14.84	0.04	Myrcene
1203	16.53	0.1	Limonene
1213	16.95	0.2	1,8-cineole
1246	18.23	0.1	(Z)-β-ocimene
1266	19.00	0.1	(E)-β-ocimene
1280	19.87	0.3	ρ-cymene
1400	25.02	0.04	Nonanal
1553	30.24	0.5	Linalool
1612	**32.25**	**7.6**	**β-caryophyllene**
1668	34.16	1.1	(Z)-β-farnesene
1687	**34.54**	**2.1**	**α-humulene**
1706	35.07	0.3	α-terpineol
1727	35.90	0.1	7-epi-1,2-dehydrosesquicineole
1741	36.06	0.8	β-bisabolene
1783	37.36	0.5	β -sesquiphellandrene
2008	43.48	1.5	Caryophyllene oxide
2050	44.24	0.2	(E)-nerolidol
2071	44.78	0.3	Humulene epoxide II
2161	47.01	1.3	α-bisabolol oxide
2232	**48.65**	**71.7**	**α-bisabolol**

RRI *	Retention Time (minutes)	Area percentage	Compound
2324	50.58	0.3	Caryophylladienol
2518	**55.80**	**6.2**	***cis*-lanceol**
	Total	**95.58**	

RRI* – relative retention indices calculated against n-alkanes

The major compounds in *Salvia runcinata* are β-caryophyllene (18.7%), α-humulene (2.1%), α-bisabolol (71.7%) and *cis*-lanceol (6.2%).



β-caryophyllene α-humulene α-bisabolol *cis*-lanceol

Figure 34: Chemical structure of the major compounds identified in *Salvia runcinata* essential oil.

Salvia stenophylla L. E. Codd

1. Botanical description

Perennial, erect, bushy herb 0,2-0,4 m tall, usually branched from a woody taproot. Leaves shortly petiolate or subsessile. Flowers are pale blue or mauve in colour.

Figure 35: *S. stenophylla.*

2. Distribution

Distributed from Gauteng, Free State and Lesotho to northern, north-eastern and eastern Cape. Rarely found in Kwazulu Natal. Found in grassland, open woodland and semi-arid shrub, often on calcareous or brackish soil, sandy soil in watercourses or damp areas. Sometimes considered a semi-weed of disturbed places.

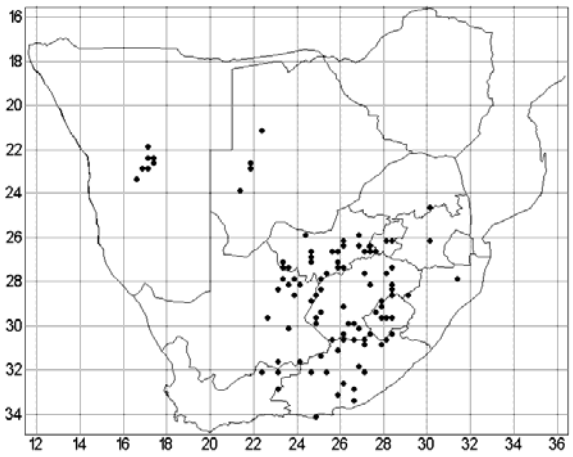


Figure 36: Geographical distribution of *S. stenophylla.*

3. Essential oil composition

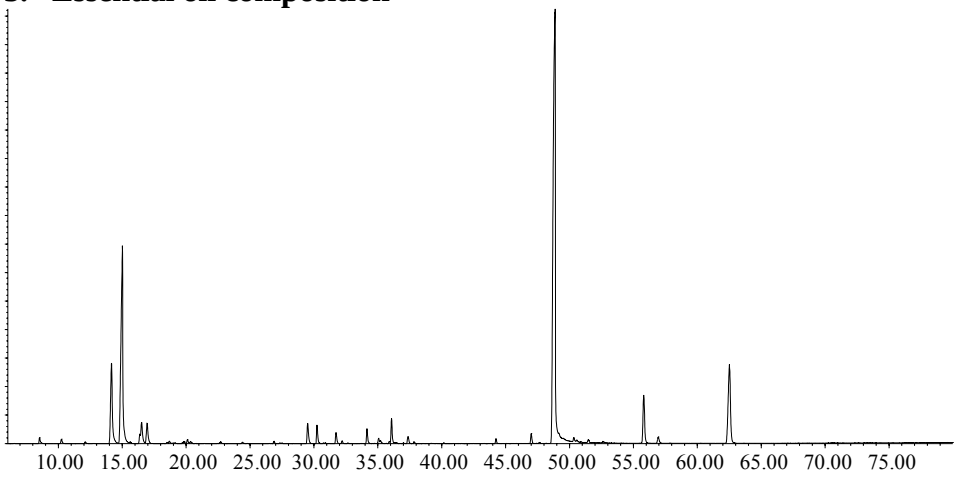


Figure 37: Gas chromatography profile of *S. stenophylla* collected at Lady Grey.

4. GC-MS results

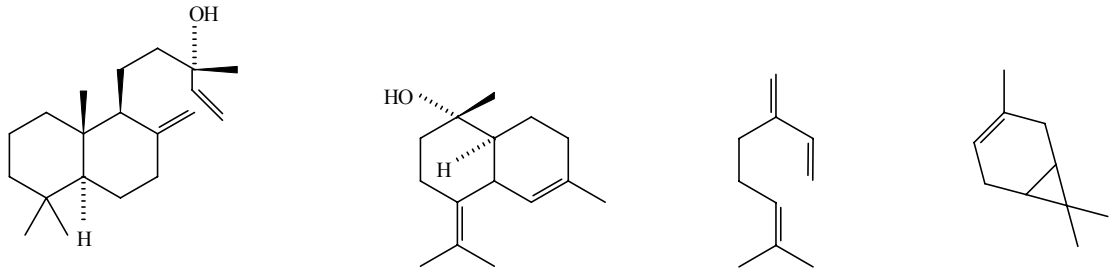
Table 11: GC-MS results of *Salvia stenophylla.*

RRI *	Retention Time (minutes)	Area percentage	Compound
1032	8.62	0.3	α-pinene
1076	10.22	0.3	Camphene
1118	12.15	0.1	β-pinene
1159	**14.08**	**7.0**	**δ-3 carene**
1174	**14.84**	**18.8**	**Myrcene**
1205	16.35	0.5	Sylvestrene (m-mentha-1(6),8-diene)
1203	16.53	1.6	Limonene
1218	16.95	1.7	β-phellandrene
1246	18.23	0.1	(Z)-β-ocimene
1255	18.70	0.2	γ-terpinene
1265	19.14	0.1	5-methyl-3-heptanone
1278	19.82	0.1	m-cymene
1280	19.87	0.2	ρ-cymene
1290	20.35	0.1	Terpinolene
1348	22.70	0.1	6-methyl-5-hepten-2-one
1532	29.51	1.4	Camphor
1553	30.24	1.1	Linalool
1568	31.73	0.7	*trans*-α-bergamotene
1611	32.20	0.3	Terpinen-4-ol
1668	34.16	0.9	(Z)-β-farnesene
1682	35.07	0.3	α-terpineol
1719	35.22	0.2	Borneol
1727	35.90	0.1	7-epi-1,2-dehydrosesquicineole

RRI *	Retention Time (minutes)	Area percentage	Compound
1755	36.03	1.4	Bicyclogermacrene
1784	37.36	0.5	(E)-bisabolene
1804	37.83	0.1	Myrtenol
2050	44.24	0.2	(E)-nerolidol
2161	47.01	0.6	α-bisabolol oxide
2219	47.62	0.03	T-cadinol
2232	**48.65**	**47.6**	**α-bisabolol**
2518	55.80	0.2	*cis*-lanceol
2544	56.96	0.2	Bisabola-2,7,10-trien-13-ol
2676	**62.51**	**8.6**	**Manool**
	Total	**95.63**	

RRI* – relative retention indices calculated against n-alkanes

The major compounds in *Salvia stenophylla* are δ-3 carene (7.00%), mycrene (18.8%), α-bisabolol (47.6%) and manool (8.6%).



manool α-bisabolol mycrene δ-3 carene

Figure 38: Chemical structure of the major compounds identified in *Salvia stenophylla* essential oil.

Salvia verbenaca L. E. Codd

1. Botanical description

Perennial, short-lived shrub with stems arising from a woody taproot. Stems erect, 0,15-0,4 m tall. Leaves shortly petiolate to subsessile. Flowers are spaced below and denser above and are range from light blue to purple in colour, lower lip usually slightly shorter.

Figure 39: *S. verbenaca.*

2. Distribution

Salvia verbenaca is an introduced plant in our Flora area. It is indigenous in the countries around the Mediterranean and on the Canary Islands and has also spread further afield in Europe and Asia. It has also become naturalized in Australia and the United States. In South Africa it is now widely distributed, mainly in the drier, western half of the country, in northern, central and western Cape and the western Free State with fewer plants reaching the south western Cape.

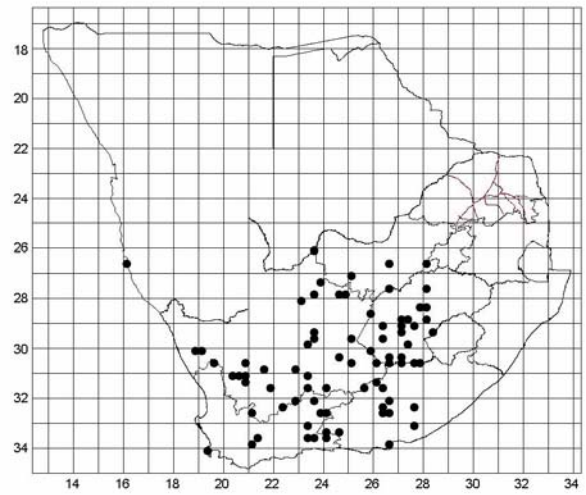


Figure 40: Geographical distribution of *S. verbenaca.*

Salvia verbenaca does not produce essential oil and therefore the analysis with the aid of GC-MS was not possible.

CHAPTER 4: RESULTS AND DISCUSSION

4.1 Analytical chemistry

The GC-MS results have been included under the monographs of each species and the major compounds of the individual species have been identified. *S. verbenaca* does not produce any essential oil and the analysis with the aid of GC-MS was therefore not possible. No essential oil was obtained for *S. chamelaeagnea.* The species that were analysed with the aid of GC-MS are included in Table 12.

Table 12: *Salvia* species analysed by of GC-MS and the percentage oil yield (wet weight) after distillation.

Species	Plant material weight	Percentage Yield
S. africana-caerulea	335g	0.17%
S. africana-lutea	167g	0.32%
S. disermas (Mossel Bay)	369g	1.24%
S. dolomitica	463g	1.02%
S. lanceolata	314g	0.17%
S. namaensis	249g	1.64%
S. runcinata	90g	0.15%
S. stenophylla	398g	0.79%

The majority of the compounds identified by GC-MS are common to all the species studied with the exception of a few. Camphor was only identified in three species. In *S. africana-caerulea* and *S. stenophylla* it is found in very small concentrations whereas in *S. namaensis* it exists as a major compound with a concentration of 33.50%. Camphene, also present in low concentrations in a few species and is another major compound in *S. namaensis*. The compounds β-caryophyllene and caryophyllene oxide are present in all species with the exception of *S. stenophylla*. Terpinen-4-ol is present in low concentrations in *S. stenophylla*. Spathulenol is found in high concentrations in *S. lanceolata* when compared to the other species. Carvacrol is only present in *S. namaensis*, in very low concentrations. α-Bisabolol is present as a major compound, in *S. runcinata* and *S. stenophylla*. α-Eudesmol and β-eudesmol are both found in high

concentrations in *S. africana-lutea*. *S. stenophylla* is the only species to contain the compound manool.

The compounds present in *S. disermas* vary considerably when compared with the other species. *S. disermas* lacks compounds such us α-pinene and limonene that are present in the other species. *S. disermas* also contains certain compounds that are not present in other species. These include compounds such as linalyl acetate (34.50%), diepishyobunone (4.40%), isoshuyobunone (0.70%), shyobunone (10.70%), epi-isoshyobunone (6.20%) and shyobunol (0.20%).

Table 13: The major compounds identified in the *Salvia* species.

Species	Major Compounds
Salvia africana-caerulea	α-pinene, 1,8-cineole, (E)-β-ocimene, ρ-cymene, β-caropyllene.
Salvia africana-lutea	ρ-cymene, caropyllene oxide, humulene epoxide II, γ-eudesmol, α-eudesmol, β-eudesmol.
Salvia disermas	linalool, linalyl acetate,shyobunone, epi-isoshyobunone.
Salvia dolomitica	α-pinene, δ-3 carene, limonene, 1,8-cineole, β-caryophyllene, borneol
Salvia lanceolata	sabinene, (E)-β-ocimene, caryophyllene oxide, spathulenol.
Salvia namaensis	α-pinene, camphene, 1,8-cineole, camphor, bornylacetate.
Salvia runcinata	β-caryophyllene, α-humulene, α-bisabolol, *cis*-lanceol.
Salvia stenophylla	δ-3 carene, myrcene, α-bisabolol, manool.

Little phytochemical work has been done on the *Salvia* species occurring in southern Africa and thus there is not an abundance of literature on the chemical constituents of indigenous *Salvia* species. However, the essential oil of *S. stenophylla* collected on the 'High Veld' of the Free State has been investigated by GC-MS (Jäger and van Staden, 2000). It was found that the compounds mainly responsible for the persistent wood odour of *S. stenophylla*, α-bisabolol and manool, were the most abundant. 1,8-Cineole and camphor were present at very low levels and the species lacked α-thujone and β-thujone. A similar study on *S. stenophylla* has been undertaken (Jäger and van Staden, 2000). Most of the compounds were well known natural substances. The oil contained 29.80% α-bisabolol, which was isolated by column chromatography and identified as (+)-epi-α-bisabolol. This was the first time this isomer had been shown to occur in nature. The

results from these two independent studies are congruent with the results obtained in this study.

Thin layer chromatography was employed on the methanol extracts, essential oils and on rosmarinic acid, which was included as a standard. Chromatography is an analytical method widely used for the separation, identification and determination of the components in complex mixtures. The application of various chromatographic techniques for the scientific validation of traditional medicine is inevitable. A specific solvent system is used as a mobile phase, which allows separation of different components present in the "mixture" based on their polarity. This system is not as sensitive as high performance liquid chromatography, but its strength lies in the relatively cheap and rapid assessment of the quality of extracts and actives present. For isolation, quantifying the purity of the sample and evaluating the concentration of compounds in a sample, high performance liquid chromatography provides an accurate method.

Literature states that the main compound responsible for the anti-oxidant activity of *Salvia* is rosmarinic acid. Rosmarinic acid was subjected to thin layer chromatography on silica gel plates along with the other methanol extracts of the test samples (Figure 41). When the methanol extracts are compared to the rosmarinic acid standard a common compound with Rf = 0.38 is observed. *S. namaensis*, *S. chamelaeagnea*, *S. dolomitica* and *S. stenophylla* all have a common non-polar compound (Rf = 0.91). Various other polar and non-polar compounds are present in the methanol extracts.

The antioxidant assay involves using a stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) with a dark violet colour, whereby antioxidants are allowed to react with the stable radical in methanol solution. Antioxidant compounds donate electrons to DPPH, resulting in decolourization which is stoichiometric with respect to the number of electrons captured by DPPH. The reduction in the concentration of the DPPH radical is followed by monitoring the decrease in its absorbance at a characteristic wavelength during the reaction. In its radical form, DPPH absorbs at 515 nm, but upon reduction by antioxidant or a radical species, the absorption disappears. Results show that the

methanol extracts have both polar and non-polar compounds, in addition to rosmarinic acid that are responsible for the antioxidant activity.

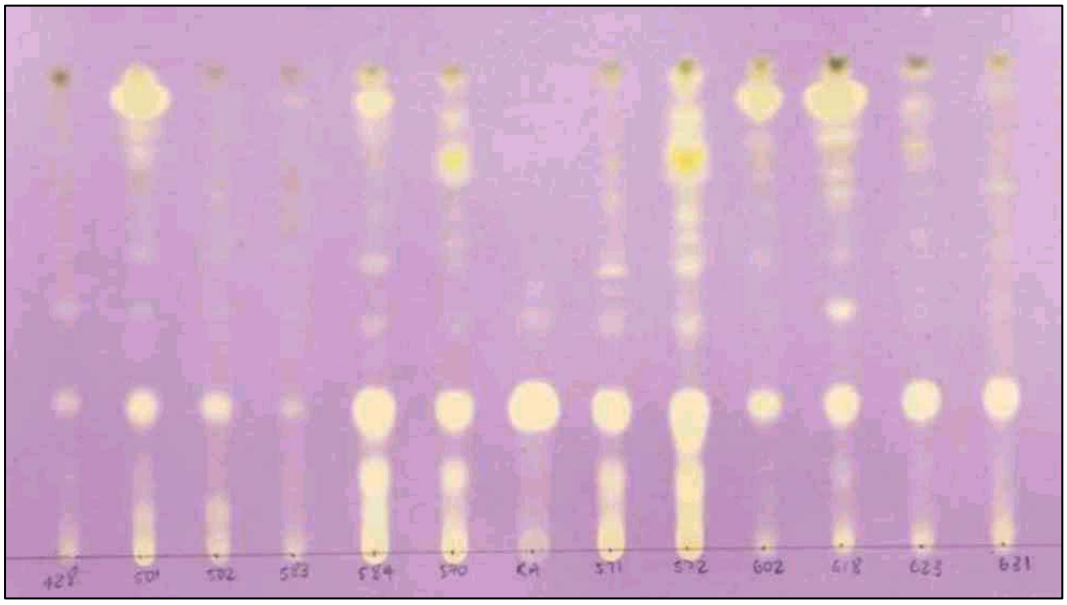


Figure 41: TLC plate of *Salvia* methanol extracts and rosmarinic acid after been sprayed with DPPH reagent. White spots are indicative of compounds showing antioxidant activity.

1 *Salvia rigida*
2 *Salvia namaensis*
3 *Salvia disermas*
(Mossel Bay)
4 *Salvia disermas*
(Walter Sisulu Botanical Garden)
5 *Salvia chamelaeagnea*
6 *Salvia africana-caerulea*
7 Rosmarinic acid
8 *Salvia lanceolata*
9 *Salvia africana-lutea*
10 *Salvia dolomitica*
11 *Salvia stenophylla*
12 *Salvia runcinata*
13 *Salvia verbenaca*

4.2 Antimicrobial activity

The disc diffusion method is useful for the preliminary examination of the antimicrobial properties of the test samples. The lack of reproducibility of the disc diffusion method is, however, a distinct disadvantage and it should therefore only be used as a preliminary screening. Extensive literature on the antimicrobial and antifungal potency of the *Salvia* genus reveals a broad variability with regard to micro-organisms sensitivity as well as to the efficiency (measured as MIC) of tested compounds, when different species within the genus are considered (Baricevic and Bartol, 2000).

Table 13: Antibacterial screening results from the disc diffusion assay. Results expressed in millimeters (mm) from disc edge.

Test Sample	***S. aureus* ATCC12600**	***B. cereus* ATCC11778**	***E. coli* ATCC11775**	***Y. enterocolitica* ATCC23715**	***K. pneumoniae* ATCC13883**
S. africana-caerulea Essential oil	2.0	<1.0	R	1.0	<1.0
S. africana-caerulea Methanol extract	<1.0	4.0	R	R	R
S. africana-caerulea Acetone extract	3.0	5.0	R	R	R
S. africana-lutea Essential oil	R	2.0	R	R	R
S. africana-lutea Methanol extract	R	2.0	R	R	R
S. africana-lutea Acetone extract	1.0	4.0	R	R	R
S. chamelaeagnea Methanol extract	4.0	5.0	R	R	R
S. chamelaeagnea Acetone extract	6.0	7.0	R	R	R
S. disermas (WSBG) Methanol extract	R	R	R	R	R
S. disermas (WSBG) Acetone extract	R	R	R	R	R
*S. disermas (*Mossel Bay) Essential oil	<1.0	3.0	R	R	R
S. disermas (Mossel Bay) Methanol extract	R	R	R	R	R
S. disermas (Mossel Bay) Acetone extract	R	1.5	R	R	R

Test Sample	*S. aureus* ATCC12600	*B. cereus* ATCC11778	*E. coli* ATCC11775	*Y. enterocolitica* ATCC23715	*K. pneumoniae* ATCC13883
S. disermas (Kokerboom-woud) Essential oil	2.0	2.0	R	R	R
S. dolomitica Essential oil	R	1.0	R	R	R
S. dolomitica Methanol extract	4.0	7.0	R	R	R
S. dolomitica Acetone extract	7.0	7.0	<1.0	R	R
S. lanceolata Essential oil	<1.0	R	R	R	R
S. lanceolata Methanol extract	R	R	R	R	R
S. lanceolata Acetone extract	<1.0	R	R	R	R
S. namaensis Essential oil	<1.0	<1.0	<1.0	<1.0	<1.0
S. namaensis Methanol extract	5.0	7.0	R	R	R
S. namaensis Acetone extract	6.0	8.0	R	R	R
S. runcinata Essential oil	R	1.0	R	R	R
S. runcinata Methanol extract	<1.0	4.0	R	R	R
S. runcinata Acetone extract	4.0	8.0	R	R	R
S. stenophylla Essential oil	<1.0	2.0	R	R	R
S. stenophylla Methanol extract	6.0	8.0	R	R	R
S. stenophylla Acetone extract	7.0	9.0	R	R	R
S. verbenaca Methanol extract	<1.0	<1.0	R	R	R
S. verbenaca Acetone extract	R	2.0	R	R	R
Control – Neomycin	6.0	6.0	2.0	4.5	5.0

R = Resistant

The results of the disc diffusion assay are summarised in Table 13. No zones of inhibition were evident on any of the fungal test organisms used in this assay and they are therefore not included in the table. Measurements were recorded from the edge of the disc to the end of the zone of inhibition and recorded in millimeters (Figure 42 and 43). All test samples studied demonstrated variable degrees of antibacterial activity with the exception of four test samples; *S. disermas* methanol and acetone extracts from the Walter Sisulu Botanical Garden; *S. disermas* methanol extract from Mossel Bay and the *S. lanceolata* methanol extract, which showed no activity.

The acetone extract of *S. africana-caerulea* showed more activity than the essential oil. Antibacterial activity was noted against *S. aureus*, *B. cereus* and interestingly the essential oil was one of two test samples that showed minimal activity against *Y. enterocolitica* and *K. pneumoniae*. *Salvia africana-lutea* showed activity against *B. cereus* but to a much lesser extent when compared with the activity of *S. africana-caerulea* extract. The acetone extract of *S. africana-lutea* also showed little activity against *S. aureus*. The extracts of *S. chamelaeagnea* showed good activity against both *S. aureus* and *B. cereus*.

In general, *S. disermas* and *S. lanceolata* exhibited poor antibacterial activity. Both the essential oils of *S. disermas* showed activity against *S. aureus* and *B. cereus* and only one extract showed activity against *B. cereus,* the acetone extract from Mossel Bay. The essential oil and acetone extract of *S. lanceolata* showed slight activity against *S. aureus* and the methanol extract showed no activity.

Both methanol and acetone extracts of *S. dolomitica* demonstrated substantial activity against *B. cereus* and *S. aureus* whilst the essential oil showed slight activity only against *B. cereus*. *S. dolomitica* acetone extract showed minimal activity against *E. coli*. The essential oil of *S. namaensis* showed activity, albeit very weak, against all test organisms studied. In addition to this, both extracts showed significant activity against *S. aureus* and *B. cereus.* The essential oil of *S. runcinata* exhibited little activity against *B. cereus*. The

acetone extract of *S. runcinata* showed activity against *S. aureus* and *B. cereus* and the methanol extract demonstrated similar activity but to a lesser degree.

The methanol extract of *S. verbenaca* showed minimal activity against *S. aureus* and *B. cereus* whilst the acetone extract only inhibited the growth of *B. cereus*. The methanol and acetone extracts of *S. stenophylla* showed excellent activity against *B. cereus* and *S. aureus*. The essential oil showed activity against the same bacteria but to a much lesser degree. The acetone extract of *S. stenophylla* had the largest zone of inhibition of 9.00 mm against *B. cereus.*

The microplate bioassay method was chosen to determine the MIC of selected test samples. Two microplates were prepared. The Gram-positive organism *B. cereus* was chosen in this assay as a result of the high antimicrobial activity shown by test samples in the disc diffusion assay towards this organism. A positive control, ciprofloxacin, was included to ensure the antimicrobial sensitivity of the organism. *Bacillus cereus* with no test samples or antimicrobial is included as a negative control. The MIC results have been summarized in Table 14.

The minimum inhibitory concentration is the lowest concentration of an antimicrobial, and in this case our test sample, found to inhibit the growth of a particular test organism, namely *B. cereus*. These results reflect that the concentration of *S. disermas* (Mossel Bay-essential oil) and *S. africana-lutea* (acetone) needed to inhibit the growth of *B. cereus* is 8.00 mg/ml. The concentration of *S. disermas* (Kokerboomwoud - essential oil, *S. dolomitica* (essential oil), *S. africana-lutea* (essential oil) and *S. stenophylla* (essential oil) are all required at a concentration of 4.00 mg/ml to inhibit *B. cereus.*

Table 14: Minimum Inhibitory Concentration (MIC) of *Salvia* against *B. cereus.*

Microplate	Test Sample	MIC (mg/ml)
A1	*S. disermas* (Mossel Bay) essential oil	8.00
A2	*S. disermas* (Mossel Bay) acetone extract	1.00
A3	*S. disermas* (Kokerboomwoud) essential oil	4.00
A4	*S. chamelaeagnea* methanol extract	< 0.50
A5	*S. chamelaeagnea* acetone extract	< 0.50
A6	*S. dolomitica* essential oil	4.00
A7	*S. dolomitica* methanol extract	1.00
A8	*S. dolomitica* acetone extract	< 0.50
A9	*S. verbenaca* acetone extract	< 0.50
A10	*S. namaensis* methanol extract	1.00
A11	*S. namaensis* acetone extract	< 0.50
B1	*S. africana-lutea* essential oil	4.00
B2	*S. africana-lutea* methanol extract	1.00
B3	*S. africana-lutea* acetone extract	8.00
B4	*S. africana-caerulea* methanol extract	1.00
B5	*S. africana-caerulea* acetone extract	1.00
B6	*S. runcinata* essential oil	2.00
B7	*S. runcinata* methanol extract	2.00
B8	*S. runcinata* acetone extract	< 0.50
B9	*S. stenophylla* essential oil	4.00
B10	*S. stenophylla* methanol extract	< 0.50
B11	*S. stenophylla* acetone extract	< 0.50

The MIC for the essential oil and methanol extract of *S. runcinata* is 2.00 mg/ml. For the following test samples a concentration of 1.00 mg/ml is required to inhibit the growth of *B. cereus*: *S. disermas* (Mossel Bay – acetone), *S. dolomitica* (methanol), *S. namaensis* (methanol), *S. africana-lutea* (methanol) and *S. africana-caerulea* (methanol and acetone). It can thus be seen that in general the essential oils are required at much higher concentrations than the extracts to inhibit *B. cereus*.

For those test samples that showed no colour change with INT solution we can conclude that *B. cereus* is inhibited at all of the above concentrations and the MIC is smaller than the last dilution. In order to obtain the exact MIC of such test samples, a series of

dilutions would have to be performed that are smaller than the ones already used at the particular range identified.

A MIC of less than 0.50 mg/ml was determined for the following test samples: *S. chamelaeagnea* (methanol and acetone), *S. dolomitica* (acetone), *S. verbenaca* (acetone), *S. namaensis* (acetone), *S. runcinata* (acetone) and *S. stenophylla* (acetone and methanol).

S. chamelaeagnea (acetone), *S. dolomitica* (methanol and acetone), *S. namaensis* (methanol and acetone), *S. runcinata* (acetone) and *S. stenophylla* (methanol and acetone) all had relatively low MIC values with corresponding large zones of inhibition in the disc diffusion assay. Although *S. verbenaca* had a small zone of inhibition (2 mm), the MIC value was very low. It would have been anticipated that the MIC value would have been larger.

In general, there seemed to be an overall agreement between the size of inhibition zones obtained in the disc diffusion assay and the minimum inhibitory concentration values. The larger zones of inhibition correlated with lower minimum inhibitory concentration values and vice versa however, some variations did occur.

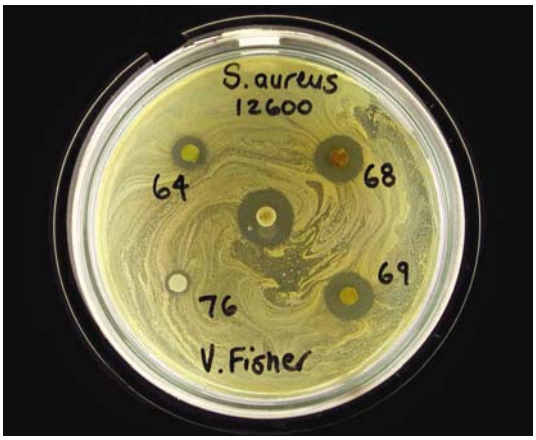


Figure 42: Disc diffusion plate of *S. aureus*
64 - *S. dolomitica* (methanol);
68 - *S. namaensis* (acetone);
69 - *S. namaensis* (methanol);
76 - *S. africana-lutea* (essential oil).
Neomycin (control) in middle of plate.

Figure 43: Disc diffusion plate of *S. aureus*
78 - *S. africana-caerulea* (acetone);
81 - *S. runcinata* (acetone);
83 - *S. stenophylla* (methanol);
84 - *S. stenophylla* (acetone).
Neomycin (control) in middle of plate.

The extracts and essential oils were screened against a varied but small selection of bacterial and fungal organisms. It should be noted that those test samples that showed little or no antimicrobial activity may be active against other yeasts, bacteria or moulds, which were not included in this particular study. All test samples lacked activity against the fungal organisms tested. Due to the fact that the controls for the fungal tests demonstrated inhibition it is clear that the failure of the test samples was due to a lack of potency and activity, and not as a result of other internal or external factors such as media composition, incubation conditions or organism resistance.

There has been extensive literature published on the anti-fungal properties of the genus *Salvia* and various oil components have been identified as being responsible for the activity. Viollon and Chaumont (1994) studied the anti-fungal properties of a variety of essential oils and their main components upon *Cryptococcus neoformans*. The conclusion was clear that among the phenolic compounds, thymol and carvacrol are the most fungitoxic. Velickovic *et al*., (2002) expanded the list of fungitoxic oil constituents to include α-bisabolol and Tzakou *et al*., (2001) demonstrated that 1,8-cineole and α-pinene are particularly active against *Candida albicans*. Peana *et al*., (1999) concluded that α-terpineol and linalool have the strongest candidicidal activity. When Baricevic and Bartol (2000) measured the antimicrobial property of the essential oil of *Salvia* against *Alternaria alternata* and *Aspergillus parasiticus*, a strong fungistatic effect was observed. The antifungal activity in that study was thought to be due to the high content of thujone in the essential oil samples that were tested. In comparison with these findings the lack of anti-fungal activity for all test samples is therefore surprising. Thymol, carvacrol and thujone are found in very small concentrations in *S. namaensis* and are possibly of little significance. According to the GC-MS results for *S. stenophylla* and *S. runcinata*, α-bisabolol has an area percentage of 47.6 % and 71.7 % respectively and yet they both show no anti-fungal activity. In addition to this 1,8-cineole, α-pinene, α-terpineol, and linalool are present in many other test samples in varying and often quite substantial concentrations and yet no anti-fungal activity is evident. It is likely that test samples may be required in much higher concentrations to produce similar results to that found in the literature. Alternatively, the concentrations of identified compounds may be needed in

higher concentrations. It could also be possible that antagonism may be taking place between compounds within a particular species.

In contrast to the anti-fungal properties it is clear that certain *Salvia* species definitely possess antimicrobial activity. Although research is lacking on indigenous *Salvia* species, much time and research has gone into studies on the antimicrobial properties of *Salvia* worldwide. Promising results have been documented and in one study *Salvia* has even shown to be the most potent inhibitor of oral bacteria when compared to peppermint and tea tree essential oils (Shapiro *et al*., 1994).

Generally, Gram-negative bacteria proved not to be sensitive or in some cases, only slightly sensitive when compared with the sensitivity of Gram-positive bacteria. This is in agreement with the observation of Baricevic and Bartol (2000). Because of the difference in cell wall structure between Gram-positive and Gram-negative bacteria, it was anticipated that the Gram-positive bacteria would be more sensitive to the test samples. Gram-negative bacteria have an outer membrane which makes penetration of any antibiotic, and in this case the test samples, a difficult task (Velickovic *et al*., 2002). However, certain test samples did demonstrate a small amount of inhibition against some Gram-negative organisms and it may be probable that, for realization of antimicrobial action, larger quantities of oils may be required to inhibit Gram-negative bacteria.

The methanol and acetone extracts were far more active than the essential oils. This is probably due to the lipophillic and hydrophilic properties of the essential oils and extracts respectively. The disc diffusion method depends on the aqueous solubility of the test samples in order to facilitate diffusion through the solid medium, the agar. Essential oils are generally hydrophobic and do not readily diffuse or penetrate through an aqueous medium as well as the methanol and acetone extracts do. Therefore, the potency of the essential oils may be decreased due to decreased permeability. It is because of this that the prevalence of false negatives or reduced activity might be anticipated in a disc diffusion assay (Nakatsu *et al*., 2000). However, in practice, the hydrophobicity of essential oils enables them to partition into the lipids of the cell membrane and

mitochondria, rendering them permeable and leading to leaking of cell contents. Physical conditions that improve the activity of the essential oils are low pH, low temperature and low oxygen levels (Burt, 2004). Theoretically, it would therefore be desirable to develop a system that would allow lipophilic materials to disperse in an aqueous environment using a solvent that is safe for consumption, lacks toxicity and does not interfere with antimicrobial activity of the test sample.

A significant difficulty in the study of biologically active essential oils is that the biological activity does not depend on only one active compound or constituent. In addition, the essential oils are usually mixtures of structurally very similar compounds and, therefore, the biological activity of essential oils cannot easily be explained with a simple, single molecule or compound. In fact, the action is possibly the result of the combined effect of both active and inactive compounds. The nature as well as the proportion of the individual compounds will have a direct influence on the antimicrobial activity (Pattnaik *et al.*, 1997). In some instances, the inactive compounds may influence resorption, rate of reactions and the bioavailability of the active compounds either positively or negatively (Svoboda and Hampson, 1999). However, the ultimate goal is to identify as accurately as possible the active compounds, the structure activity relationships of compounds and the synergism that take place between these compounds in order to be able to use this information in developing future medicinal compounds.

Literature studied on essential oils from a variety of plant species show similarities in antimicrobial activity with the compounds found in the majority of the *Salvia* species.

The essential oil of *Melaleuca alternifolia,* or tea tree oil, has been used medicinally, for its antimicrobial activity for many years. A study was done by Carson and Riley (1995) on the antimicrobial activity of the major components of tea tree oil. The components identified in the essential oil and tested included: 1,8-cineole, 1-terpinen-4-ol, ρ-cymene, linalool, α-terpinene, γ-terpinene, α-terpineol and terpinolene. The test organisms included *B. subtilis, C. albicans, E. coli, P. aeruginosa* and *S. aureus.* It was found that terpinen-4-ol was active against all the test organisms while linalool and α-terpineol were

active against all organisms with the exception of *P. aeruginosa. S. stenophylla* contains terpinen-4-ol in low concentrations. Linalool is present as a major compound in *S. disermas* and as a minor compound in every species. α-Terpineol is present as a minor compound in more than fifty percent of the species studied. In another study by Carson *et al.*, (2002) the mechanism of action of tea tree oil and three of its components, 1,8-cineole, terpinen-4-ol and α-terpineol, against *S. aureus* was investigated. Loss of 260-nm-absorbing material occurred after treatment with 1,8-cineole and α-terpineol. When the agents were tested at one-half the MIC, only 1,8-cineole significantly reduced the tolerance of *S. aureus* to sodium chloride. *Salvia africana-caerulea, S. namaensis* and *S. dolomitica* (methanol and acetone) all inhibited *S. aureus* to some degree and all contain 1,8-cineole. The antimicrobial activity of linalool and 1,8-cineole has been demonstrated separately and it has also been observed that the antimicrobial activity is increased when they are used in combination (Faleiro *et al*., 2003).

Ulubelen *et al*., (1994) studied the terpenoids from *Salvia sclarea.* The diterpenoids and the sesquiterpenoids were tested for antimicrobial activity against standard bacterial strains. Manool, spathulenol and caryophyllene oxide were found to be active against *S. aureus*. *Salvia stenophylla* is the only species that has manool present, in addition to this it is present as a major compound. *Salvia stenophylla* showed very good activity against *S. aureus* in the disc diffusion assay in this report. Spathulenol is present as a major compound in *S. lanceolata* and is present as a minor compound in some of the other species. *S. lanceolata* exhibited weak activity against *S. aureus.* Caryophyllene oxide is present in all species with the exception of *S. stenophylla*, all of which showed some degree of inhibition towards *S. aureus*. No direct relationship can be found between caryophyllene oxide and the test samples.

The composition and antimicrobial activity of the essential oil of *Hypericum rumeliacum* have been researched by Couladis *et al*., (2003). The major compounds reported to be responsible for the antimicrobial activity are: α-pinene, β-pinene and α-copaene. α-pinene and β-pinene are present in all species except *S. disermas*. In this research report *S. disermas* showed negligible activity.

Űnlű *et al*., (2002) have researched the compositions and the antimicrobial activities of the essential oils of *Achilla setacea* and *Achilla teretifolia*. The essential oils exhibited antimicrobial activity against *C. perfringes*, *S. aureus*, *S. pneumoniae* and *B. cereus*. The major compounds identified were camphor, 1,8-cineole and borneol and can be considered as the antimicrobial compounds of the essential oils. Borneol is present in *S. namaensis*, *S. stenophylla* and *S. dolomitica*. Camphor is present as a major compound in *S. namaensis*. When comparing their data with the results generated in this study it can be seen that these three species show excellent antimicrobial activity towards *B. cereus*, both in the disc diffusion assay and the microplate assay. The antimicrobial activity observed in these three species that contain borneol is actually greater than the neomycin control included in this assay.

Camphene has been identified in the literature as an antimicrobial compound. In a study by Tirillini *et al*., (1996) on the chemical composition and the antimicrobial activity of the essential oil of *Piper angustifolium*, camphor and camphene were identified as the main compounds. Conclusions were drawn that the oil inhibited the growth of *E. coli*, among others, due to the presence of camphene and camphor. Camphene is present as a major compound in *S. namaensis* and as minor compounds in *S. stenophylla*, *S. africana-lutea* and *S. dolomitica*. Although very weakly, *S. namaensis* is the only essential oil that inhibited the growth of all organisms including *E. coli*. *Salvia dolomitica* has the second highest concentration of camphene present and is the only other essential oil to inhibit the growth of *E. coli*. *S. africana-lutea* and *S. stenophylla* do not inhibit the growth of *E. coli* in this study but they do show remarkable antimicrobial activity toward *S. aureus* and *B. cereus* and they contain camphene in small concentrations.

The bacterial susceptibility of *E. coli*, *S. aureus, B. subtilis* and *K. pneumoniae* to the essential oils of *Thymus kotschyanus* and *Thymus persicus* was investigated by Rasooli and Mirmostafa, (2003). The results from their study showed that carvacrol, thymol and ρ-cymene contributed to more than 75 % of the chemical composition of thyme oils with strong antibacterial properties. ρ-cymene is present in all species besides *S. lanceolata*

and *S. disermas*. It is found in concentrations of 5.20 % in both *S. africana-lutea* and *S. africana-caerulea. K. pneumoniae* was slightly inhibited by the essential oil of *S. africana-caerulea* but not by *S. africana-lutea*.

The chemical composition of the essential oil of *Salvia desoleana* from Italy was analysed by Peana *et al., (*1999). *S. desoleana* had a high content of monoterpenic esters including linalyl acetate and α-terpinyl acetate. Weak antimicrobial activity was noted against *S. aureus, E. coli* and *S. epidermidis*. However, inhibition increased progressively with increased contact time. *Salvia disermas* showed poor antibacterial activity. It is the only species in this study to have the compound linalyl acetate in a concentration of 34.50 %. α-terpinyl acetate is found in small concentrations in three different species. The contact time of our test samples and the organism was only 24 hours, if the parameters of this study were altered and the contact time increased different results may have been noticed.

Costantin *et al.,* (2001) has identified the main compounds of *Piper cernuum* essential oil as being bicyclogermacrene (21.88 %) and β-caryophyllene (20.69 %). The main compounds of *Piper regnellii* essential oil have been identified as mycrene (52.60 %) and linalool (15.89 %). Both essential oils presented growth inhibitory activities against *S. aureus.* Mycrene is present in all species and is of particular importance in *S. stenophylla* as it is present in a concentration of 18.80 %. *S. stenophylla* shows good inhibitory activity toward *S. aureus* in this study. Besides *S. stenophylla*, β-caryophyllene is present in all species. The concentrations of β-caryophyllene within each species does not correspond to the degree of inhibition exhibited towards *S. aureus* in the disc diffusion assay.

The chemical composition and antibacterial activity tests were carried out on the essential oil of *Senecio graveolens* by Perez *et al*., (1999). The essential oil proved that it has antibacterial effects on oxacillin-sensitive and oxacillin-resistant *S. aureus*. The chemical composition of the essential oil was analysed by GC-MS and some of the compounds identified were: α-pinene, α-terpinene, ρ-cymene, sabinene, terpinen-4-ol and α- and β-

eudesmol. Sabinene is found in many of the species and present as a major compound in *S. lanceolata*. Our results are not in agreement with this study because *S. lanceolata* showed very weak antibacterial activity. The compounds α- and β- eudesmol are present in large concentrations in *S. africana-lutea.* This species showed moderate activity in the disc diffusion assay.

A fact that may seem paradoxical, but may very well explain the lack of anticipated activity from certain *Salvia* species, is the interaction that may take place between two constituents within a species. While two major constituents in a particular species would demonstrate and have antimicrobial activity by themselves, combining the two would eliminate their activity. This has been observed with the compounds eugenol and linalool, both active singularly, but show an antagonistic effect toward one another. Many combinations of compounds may give rise to this phenomenon that may have not, as yet, been researched (Nakatsu *et al*., 2000).

Published studies have also indicated the high degree of variability in quantity and composition of essential oils within species that have been collected from different locations. This geographical variation was proved in a study on *Salvia fruticosa* collected from different parts on the island of Crete, Greece (Skoula *et al*., 2000). The three samples, in this study, exhibited significant differences in essential oil yield and composition. Variability in yield and content of essential oils in the Lamiaceae is known to be affected by environmental factors such as light, nutrient availability and season.

This study has not taken in to account the enantiomers that exist for different compounds. Demirci *et al*., (2002) discusses the enantiomeric distribution of some monoterpenes in the essential oils of certain *Salvia* species found in Turkey. It is known that both enantiomers of camphor are found in nature, but the (-) – form is said to be less common than the (+) – form. (-)-Camphor has been shown to have antibacterial activity whilst (+)-camphor is inactive. The antimicrobial activity of the enantiomers of borneol have been studied by Tabanca *et al*., (2001). Both enantiomers showed antibacterial activity towards all bacteria included in the study however, the MIC values varied.

The methanol and acetone extracts displayed promising antibacterial activity against Gram-positive bacteria. This is consistent with a screening programme undertaken by Izzo *et al.,* (1995) in which it was found that plant extracts and essential oils readily inhibit Gram-positive rather than Gram-negative bacteria. It is possible that the antibacterial properties of the extracts in this study could be attributed to the presence of carnosol derivatives that are reported to have strong antibacterial activity as well as antioxidant properties. In a study undertaken by Lee *et al*., (1999) it was found that the ability of the compounds to generate superoxide radicals also inhibited bacterial growth by non-selective inhibitory action on DNA, RNA and protein synthesis.

4.3 Antioxidant activity

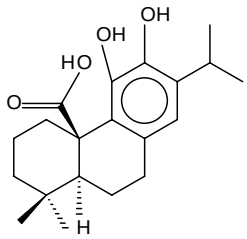
Within the last decade, the importance of free radicals in the aetiology of disease has been increasingly recognized and has lead to the development of new approaches in biochemical evaluation of events associated with mutagenisis, tumorigenesis and / or cancer promotion (Baricevic and Bartol, 2000).

The reason that antioxidants are important to human physical well being comes from the fact that oxygen is a potentially toxic element since it can be transformed by metabolic activity into more reactive forms such as superoxide, hydrogen peroxide, singlet oxygen and hydroxyl radicals, collectively known as active oxygen. These molecules are formed in living cells by various metabolic pathways. Specific molecules, pollution from tobacco smoke and burning of fossil fuels, together with UV radiation and pollutants such as ozone, nitrogen oxide and sulphur dioxide, add to the formation of free radicals. Superoxide is converted by an enzyme superoxide dismutase, into H_2O_2. This compound is able to cross all biological membranes. Much of the damage done by these compounds is thought to be due to the conversion to highly reactive oxidants such as the hydroxyl radical. This radical is very reactive as it combines with almost all molecules found in living cells. Proteins, lipids, carbohydrates and DNA in living cells represent oxidizable substrate. The secondary events include changes in membrane structure, permeability and fluidity, lysomal destabilization and stimulation of apoptosis. Lipid peroxidation finally

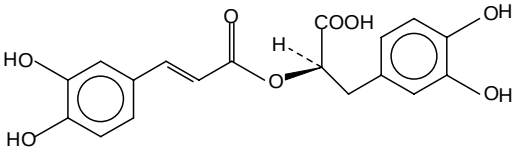
leads to loss of membrane function and integrity leading to cell necrosis and death (Svoboda and Hampson, 1999).

The antioxidant activity of *Salvia* has been extensively studied. The antioxidant properties of water extracts obtained from herbs of the species Lamiaceae, was investigated by Triantaphyllou *et al*., (2001). The extracts were examined for their effect against lipid oxidation in comparison to a tea (water) extract. *Salvia* was one of three extracts found to have a remarkable capacity in retarding lipid oxidation. Examination by thin layer chromatography showed that the extracts were rich in compounds such as hydroxycinnamic acids, flavonoids, caffeic acids and rosmarinic acid. The antioxidant activity of a synthetic antioxidant and *Salvia* has also been compared (Bandoniene *et al*., 2001) and the data obtained showed that all the *Salvia* extracts had a very strong antioxidant activity.

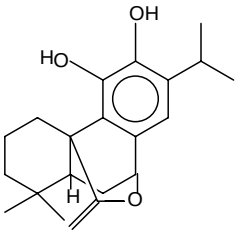
An antioxidant screening study in Yugoslavia (Malencic *et al*., 2000) on the wild growing *Salvia* species isolated rosmarinic acid as the dominant naturally occurring compound. Wang *et al*., (1999) reports that the antioxidative effects of *Salvia* relate to the presence of carnosic acid, carnosol and rosmarinic acid (Figure 44). Exarchou *et al*., (2002) found that the major component of all *Salvia* ethanol extracts was rosmarinic acid, determined by thin layer chromatography and nuclear magnetic resonance spectroscopy. Furthermore, the extracts that are rich in rosmarinic acid were found to have a higher radical scavenging activity. It was concluded that the flavanoids have a rather small contribution to the total antioxidant activity of the extracts and the main ingredient is rosmarinic acid, which is indeed a strong radical scavenger.



carnosic acid



rosmarinic acid



carnosol

Figure 44: Chemical structures of the major antioxidant compounds in *Salvia.*

Rosmarinic acid is an ester of caffeic acid and 3,4-dihydroxyphenyllactic acid. It is commonly found in species of the Boraginaceae and the subfamily Nepetoideae of the Lamiaceae. However, it is also found in other higher plant families and in some fern and hornwort species (Petersen and Simmonds, 2003). Rosmarinic acid is the most abundant caffeic acid dimer found in *Salvia* species and has been reported to be the major phenolic compound responsible for the high antioxidant activity of *Salvia* species (Lu and Foo, 2002); (Baricevic and Bartol, 2000). When measuring the radical scavenger effect on 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical, the antioxidant effect of rosmarinic acid has been shown to be comparable to that of ascorbic acid (Baricevic and Bartol, 2000). In plants, rosmarinic acid is believed to act as a preformed constitutively accumulated defence compound. Rosmarinic acid has a number of interesting biological activities that include antiviral, anti-inflammatory and antioxidant properties. (Petersen and Simmonds, 2003). It has been found that plant cell cultures accumulate rosmarinic acid in amounts much higher than in the plant itself (up to 36% of the cell dry weight). For this reason a biotechnological production of rosmarinic acid with plant cell cultures has been proposed (Petersen and Simmonds, 2003).

Interestingly, the results of an experimental study by Liu *et al.*, (2001) indicate that certain *Salvia* species have anti-tumor potential. This potential is thought to be mediated through dual mechanisms, one of which is the antioxidant property of *Salvia*. Certain *Salvia* species investigated by Du *et al*., (1997) showed results that indicate that there were protective effects on learning and memory impairment caused by cerebral ischemia reperfusion and these results may be related to its antioxidant activity. There is also the possible efficacy for the application of the oils from such medicinal plants, through their antioxidant capacities, in the prevention of age-related macular degeneration (Nakatsu *et al*., 2000). Many reports on the relationship between the skin aging induced by UV-irradiation and the active oxygen suggest that the prevention of skin aging can be achieved by active-oxygen scavengers. *Salvia* is reported to contain chain-breaking antioxidants, which are able to inhibit peroxidation chain reactions (Masaki *et al*., 1995).

Oxygen free radicals appear to be an important factor in chronic inflammatory joint disease such as rheumatoid arthritis. There are several known reactions of oxygen centered free radicals which are relevant to tissue injury in inflamed joint conditions. Traditionally *S. disermas* has been used to treat such inflammatory conditions especially rheumatism.

The conclusion that *Salvia* has antioxidant properties is a result of a qualitative screening of certain indigenous *Salvia* species. It is desirable that this is followed up with a quantitative assay. Although certain compounds have been pointed out to contribute largely to the antioxidant activity, further studies are required to reveal whether *Salvia* contains other antioxidant compounds that may contribute to their activity. Further work on the characterization of specific components needs to be performed in order to establish the connection between antioxidant activity and chemical composition.

The importance of researching traditional uses of *Salvia* is clear. It is therefore beneficial and desirable to obtain natural, non-toxic and easily consumable dietary supplements because these natural antioxidants will act as protective agents against free radicals and can ultimately, in sufficient amounts, act as efficient scavengers of free radicals before any tissue damage occurs (Baricevic and Bartol, 2000).

CHAPTER 5: CONCLUSION

- The essential oil composition varied quantitatively and qualitatively within the different *Salvia* species analysed. Some species lacked certain compounds that were present as major chemical compounds in other species. This observation was illustrated by GC-MS analysis of the essential oils.

- Many *Salvia* species demonstrated varied antibacterial activity although no antifungal activity was observed in this study. In general, *S. chamelaeagnea*, *S. dolomitica*, *S. namaensis*, *S. runcinata* and *S. stenophylla* showed good antimicrobial activity. *S. disermas* and *S. lanceolata* exhibited poor antimicrobial activity. Gram-positive bacteria were more sensitive than Gram-negative bacteria.

- The methanol and acetone extracts were more active than the essential oils.

- The chemical compounds found in the highest concentrations in the essential oils are not necessarily responsible for the antimicrobial activity. The possibility of synergy and / or antagonism existing between compounds within a species should be considered. Geographical variation and the enantiomeric distribution of chemical compounds are also factors that should be considered in a study of this nature.

- The antimicrobial activity exhibited by the investigated species, especially their greater activity against *B. cereus* and *S. aureus* may provide a scientific basis for the ethnomedicinal use of the indigenous *Salvia* species. Traditionally *Salvia* has been used to treat a host of infections, including skin infections.

- The antioxidant properties if *Salvia* can be largely attributed to the presence of rosmarinic acid, carnosic acid and carnosol. Rosmarinic acid was found to be present in all *Salvia* methanol extracts.

- The antioxidant activity of *Salvia* species may provide a scientific basis for the ethnomedicinal use of the indigenous *Salvia* species, especially treating conditions such as rheumatism.

- Therefore it can be concluded that the antimicrobial and antioxidant therapeutic applications for *Salvia* are supported based on its history of use in well established systems of traditional medicine and the assays carried out in this study.

RECOMMENDATIONS:

- It is recommended that further studies be carried out on the extracts due to the fact that they are far more active than the essential oils.

- Antimicrobial studies can be carried out incorporating more Gram-positive bacteria.

- Autobiographic assays can be carried out to determine the actual compounds responsible for the antimicrobial activity.

- Qualitative screening demonstrated that *Salvia* possesses antioxidant properties, this should be followed up with quantitative studies.

- The DPPH assay is one of several antioxidant assays. It was used as a qualitative assay in this study as it can be easily visualised on TLC. Other in vitro assays (lipid peroxidase, ABTS) should be carried out on the leaf extracts.

CHAPTER 6: REERENCES

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