The antimicrobial properties and chemical composition of leaf extracts and essential oils of indigenous *Pteronia* species

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

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Table of Contents

DECLARATION

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

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_____ day of _____, 2007

I dedicate this research report to my wife Nazmeera, my daughter Yaseerah, son Yaaseen and parents Hoosen and Zaheda Coovadia, for all your love, support and understanding during the completion of this degree.

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Abstract

The genus *Pteronia* consists of approximately 80 species which are widely distributed in southern Africa. For hundreds of years the indigenous people of southern Africa have turned to the earth in order to provide healing for their people. The genus *Pteronia* has been amongst the first species to be used by the San and Khoi-San people for treating infections and stomach ailments.

Ten species were selected for the purpose of this report. The essential oils were isolated by using a Clevenger-type apparatus while the non-volatiles were extracted with acetone and methanol. The essential oils and extracts were assessed for antimicrobial activity. The disc diffusion assays included three Gram-negative bacteria; *Escherichia coli, Yersinia enterocolitica* and *Klebsiella pneumoniae*, three Gram-positive bacteria; *Staphylococcus aureus, Bacillus subtilis* and *Bacillus cereus* as well as one yeast; *Candida albicans*. Results indicated that the species were primarily active against Gram-positive organisms. The minimum inhibitory concentration of the ten most active species (essential oils and extracts) were determined using the microdilution method. The most promising activity was noted for *P. fasiculata* which had a MIC of 0.22 mg/ml against *S. aureus*, 0.39 mg/ml against *B. cereus* and 2.08 mg/ml against *B. subtilis*. The essential oils analysis by GC/MS revealed two chemotypes. In *Pteronia pallens*, *P. empetrifolia* and *P. flexicaulis* rare compounds, such as presilphiperfolol-7-ene, 7-α-(H)-silphiperfol-5-ene, 7-β-(H)-silphiperfol-5-ene, α-campholene aldehyde, silphiperfol-5-ene, camaroonan-7-α-ol, silphiperfol-7- β -ol, presilphiperfolan-9- α -ol and presilphiperfolan-8-ol (a major compound in *Pteronia pallens*) were recorded.

A cluster analysis of the essential oil data indicated that individual collections of *P. camphorata* within a population were tightly clustered. Similarly, *P. pallens* sampled from three different localities were also united in the cluster analysis. These results suggest minimal within and between population variations for some of the species studied.

List of tables

List of figures

CHAPTER 1: Introduction

Plants were once the primary source to treat all ailments. This vast resource that is available to all of mankind has been largely under-utilized. Natural products today represent approximately fifty percent of all clinically used drugs. Well-known examples of these products include morphine (analgesic), codeine (analgesic and cough suppressant, a derivative of morphine from the poppy plant) and more recently anticancer drugs such as the *Vinca* alkaloid and taxanes from *Taxus* species, which have known medicinal value (Rates, 2001).

Medicinal plants have been used in virtually all cultures as a source of medicine. The widespread use of herbal remedies and healthcare preparations, as those described in ancient texts such as the Vedas, the Quraan and the Bible, and obtained from commonly used traditional herbs and medicinal plants, has been traced to the occurrence of natural products with medicinal properties. Africa is a rich source of medicinal plants. Perhaps, a well-known species is *Phytolacca dodecandra*. Extracts of the plant, commonly known as 'endod', are used as an effective molluscicide to control schistosomiasis. Herbal preparations are popular and are of significance in primary healthcare in Belgium, France, Germany and the Netherlands. About 1400 herbal preparations are used widely for various applications, according to a recent survey in Member States of the European Union (Patwardhan *et al.* 2004).

For hundreds of years the indigenous people of Southern Africa have turned to the earth in order to provide healing for their people. In recent years several plants have been used in African traditional medicine for their antimicrobial, antimycobacterial, spasmolytic and anti-inflammatory activity. The selection of these plants to be tested was mainly based upon treatment of the corresponding diseases by traditional historically based measures in the treatment of diseases. *Pteronia* species have been amongst the first plants to be used for their anti-infective principles by the Hottentot's and Khoi-San people. Given its extensive historic use, it is ironic that the genus remains poorly explored (Shearing, 1997). Some species of *Pteronia* known as "boegoebossie" and "laventelbossie" have been recorded as ethnomedicinals in the treatment of stomach ailments and as a natural cosmetic by the 'Khoi-San' people (Shearing, 1997).

One of the most widely studied therapeutic applications of essential oils is their antimicrobial activity (Iwu *et al.* 1999). The capacity of essential oils to neutralize bacteria is irrefutable. Studies were undertaken as early as 1887 by Chamberland. In his book Aseptic Essentials published in 1938, René-Maurice Gattefosse described that there was as early as 1938 a considerable advancement of research in essential oil studies. Although the main consumers of medicinal plants have until recently been the local population, the field of ethnopharmacognosy attracts a number of local as well as foreign researches who have discovered the value of traditional healing (Lawrence, 1993).

1.1 Economic benefit

Many of the pharmacologically interesting medicinal plant species (those that could have a possible commercial value) currently in use by people around the world are targeted by researchers for possible bio-pharmaceutical research and economic benefit. This interest is a result of factors such as, the patient's belief that natural products are safer and far superior than conventional medicines, the consumer's move to the use of traditional or herbal medicines as well as the concerns of increasing heath care costs.

It is estimated that there are approximately 500 000 species of plants on earth (Jobling, 2000). Many natural compounds isolated from plants have been shown to have biological activities and the essential oils from aromatic and medicinal plants are particularly interesting. The prospect of an increased use of natural plant products in food as preservatives, pharmaceutical industries as antimicrobials, antioxidants or anti-inflammatory agents or as natural pesticides, may improve the application of new antimicrobial agents that are safe for man and his environment and are obtainable from available as well as renewable resources, with vast economic benefits to individuals or companies that market the product.

The natural products industry in Europe and the United States of America is equally interested in traditional medicines. In Europe and the United States of America the phytomedicine industry is training many scientists in the methodology of how to extract potential drugs from medical plants in a purified form for treatment and presentation of all kinds of diseases. We are at a stage where traditional medicine is considered more for its use of lead compounds in the research of new pharmaceutical products, rather than a separate treatment entity for use in the medical world.

1.2 Why essential oil research?

Essential oils of aromatic plants indigenous to the Eastern Cape / Karoo region of South Africa have been reported not only to have antimicrobial activities against both Gram-positive, Gram-negative bacteria and yeasts but also have unique fragrance properties (Muyima *et al.* 2002). This combination of properties allows the plants to be used in the active cosmetic sector. Advantages of this approach will include the offering of a pleasant aroma (essential oils are mostly pleasantly aromatic) enhancing the ornamental value, assuring protection against micro-organisms and in some instances enhancing the dermato-cosmetic properties as well as a preservative in the final product. Currently there are approximately 300 natural products used in the flavour and fragrance industry, and with each product containing many essential oils, this is largely an untapped market (Patwardhan *et al.* 2004)

Certain essential oils widely used in the flavour and fragrance industries, have also long been reputed to repel insects (e.g. citronella oil). Recent investigations in several countries confirm that some essential oils not only deter insects, but also have contact fungal and insecticidal actions (Mangena and Muyima, 1999). There is also an increase in public concern over the level of pesticide residues in food. This has lead to the booming industry of organic foods, however at a considerable higher cost to both manufacturer (farmer) and consumer. This concern has encouraged researchers around the world to look for other alternatives that are generally regarded as safe (organic / natural) compounds.

Naturally occurring biologically active compounds extracted from plants e.g. essential oils or plant phenolic extracts obtained from washing the surface of the plants are examples of these. These plant extracts are generally assumed to be more acceptable and less hazardous than synthetic compounds (Biavati *et al.* 1999). This also means that essential oils that have already been registered with the food and drug administration (FDA) or equivalent body in a country as being food grade, could be used as an alternative antifungal, antibacterial or as an insecticidal for fresh produce. The potential for these types of plants is considerable, and is a resource that has not fully been explored by any means what so ever.

1.3 Distribution and morphology of *Pteronia* species

Pteronia (L.) L. (Asteraceae) as a genus has approximately 80 species, which are primarily found in South Africa with the exception of one species being found in Zimbabwe. The species are most abundant in the southern Karoo and Namaqualand although 12 species occur in the Cape biome (Goldblatt and Manning, 2000). Members of this species are often the dominant taxa in the plant communities in which they are found.

The morphology of the genus is diverse with regards to size, flowers and leaf shape. They are mainly perennial and woody shrubs ranging from 0.3 to 1.5 meters in height. Flowering normally occurs in the spring months (September to November), with a few species flowering in December. The flower colours range from white, to yellow and pink buds depending on the species. The leaf texture varies from smooth, lacking any hair and bristles (glabrous) to hairy, with leaves either opposite or alternate to each other (Shearing, 1997). These species normally grow in sandy areas, with clay type soils on a flat or gentle incline (Figure 1).

Figure 1: A typical habitat where *Pteronia* species are found.

1.4 Traditional uses

According to the World Health Organization (WHO), the definition of a traditional medicine may be summarized as the sum total of all the knowledge and practical experience, whether explicable or not in the diagnosis, prevention and elimination of physical, mental or social imbalance and relying exclusively on practical experience and observation handed down from generation to generation, whether verbally or in writing.

Africa has a long and impressive list of medicinal plants based on local knowledge and ancestral experience. For instance, *Securidaca longipedumalata* is a typical plant found everywhere in Africa. The aerial parts and roots are used in Tanzania as a purgative for nervous system disorders. One cup of a root decoction is administered daily for two weeks which induces a purgatory response in the emptying of the bowel (http://darwin.nap.edu/books/0309054303/html/25.html).

The versatility of the use of traditional medicine is vast in nature. Throughout East Africa, the same leaves of *Securidaca longipedumalata* are used for the treatment of different ailments such as wounds, sores, coughs, venereal diseases and snakebites. In Malawi however, the leaves are used to treat headaches. In Nigeria, the dried leaves are used to treat various skin diseases including eczema and dermatitis amongst others. The dried root is used as an aphrodisiac in Angola. The same dried roots and leaves have religious significance in Guinea-Bissau and are understood to have psychotropic effects whilst the root bark is used for epilepsy in Ghana. (http://www.africanconservation.org/dcforum/DCForumID27/9.htm). This just manifests how a single plant can be used for different purposes in different areas of the continent.

Pteronia species have been amongst the first plants to be used for their anti-infective principles by the Hottentot's and is also used as a "buchu remedy" i.e. treatment of stomach ailments from cramps to nausea by the Khoi-San people (Shearing, 1997). Given its extensive historic use, it is ironic that the phytochemistry and antimicrobial properties of *Pteronia* remains poorly researched (http://www.msb.unm.edu/herbarium/pteronia).

Another well documented characteristic of various different species of *Pteronia*, is that it is highly toxic to ungulate herbivores, so much so that it is deadly in cattle especially *Pteronia*

pallens. The species is on the whole is not well grazed on as it is palatable to the sheep and cattle population. The species have also shown to possess properties of an insecticide (Shearing D, 1997).

1.5 New legislation?

In Southern Africa many aromatic plants have been used by traditional healers. New legislation that is currently being drafted by the health department in South Africa would require all traditional medicines to be registered with the relevant statutory department, in order to formally regulate the industry. This would require scientific evidence for the use of the products, proving efficacy, stability and safety of the products. Knowledge of the chemical profile and biological activity are used in the registration of these products, and as many of these products are used in traditional medicine, this would possibly preempt the process.

The aims of this study are as follows:

- To chromatographically record, for the first time, the essential oil composition of selected *Pteronia* species.
- To determine the antimicrobial properties of the essential oils and leaf extracts of selected *Pteronia* species.
- To provide a scientific basis for the ethnobotanical use of *Pteronia* species.

CHAPTER 2: Material and Methods

2.1 Species studied

Ten species (20 samples) of the genus *Pteronia* were collected from various areas in the eastern and southern Cape of South Africa (Table 1). Where possible, more than one individual in a population was sampled and multiple populations were sampled for some species. All plants were identified and photographed by Mr J Vlok, who also assisted in preparing the voucher specimens which are housed in the Department of Pharmacy and Pharmacology (University of the Witwatersrand).

Table 1: List of species included in this study with voucher and locality information. A, B and C denote individual plants within a single population.

Species	Voucher number	Locality of harvest	Samples and quantity
Pteronia adenocarpa	Vlok 2822	Frinsgeword	A (741.7g), B (686.4g)
Pteronia camphorata	Vlok 2804	Montagupass near Herold	A (586.5g), B (304.7g), C (167.5g)
Pteronia elongata	Vlok 2819	Prins Albert on Klaarstroom road	A (660.5g), B (474.3g)
Pteronia empetrifolia	Vlok 2818	Prins Albert	A (702.9g)
Pteronia fasiculata	Vlok 2814	Bakenshoogte	A (1019.3g), B (866.9g)
Pteronia flexicaulis	Vlok 2805	Southern Hills near Daskop	A (1029.6g), B (1140.5g)
Pteronia glomerata	Vlok 2824	West of Klaarstroom	A (574.9g), B (627.6g)
Pteronia pallens	Vlok 2811	Lategansvlei	A (158.8g)
Pteronia pallens	Vlok 2815	Volmoed	B (1227.7g)
Pteronia pallens	Vlok 2816	Between Oudtshoorn and Volmoed	B (200.9g)
Pteronia paniculata	Vlok 2813	Bakenshoogte	A (596.9g)
Pteronia viscosa	Vlok 2817	Prins Albert	A (554.0g), B (389.9g)

2.2 Preparation of essential oils and phenolic extracts

The plant material was air-dried and inserted into a Clevenger apparatus (Figure 2) together with 500 ml of water. The essential oils were isolated by hydro-distillation over a four hour period. The resultant oils were then stored in a refrigerator at temperatures of between 2°C and 8°C.

Figure 2: Plants being hydro-distillated using a Clevenger apparatus.

The essential oils were then subjected to antimicrobial studies, thin-layer chromatography as well as GC/MS (gas chromatography coupled to mass spectroscopy).

The phenolic extracts (termed phenolic as the medium used for extraction was acetone and methanol, which are both phenols) were prepared firstly by powdering the specimens, then soaking specimens in either acetone or methanol at room temperature. The extracts were then transferred into a petridish and placed in a fume cupboard where they were allowed to evaporate for 2-3 days. The extracts were then reconstituted in acetone or methanol to yield a concentration of 50 mg/ml, stored in the refrigerator between 2 °C and 8 °C and then subjected to further antimicrobial assays. Upon completion of the minimum inhibitory concentration (MIC), it was assessed from the results that some solutions had to be tested at a lower concentration of 5 mg/ml.

2.3 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/min. 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 Başer Library of Essential Oil Constituents and commercial libraries by matching both retention indices and mass spectral fragmentation patterns.

2.4 Thin-layer chromatography

One part essential oil was diluted with seven parts of hexane. About 2 µl of the mixture was applied to a silica gel plate (Alugram Sil G/UV254) and developed in a solvent system comprising toluene/ethyl acetate (93:7). The plate was then sprayed with either vanillin (1% alcoholic vanillin and 10% sulphuric acid) or anisaldehyde (sulphuric acid) spray and heated in an oven for a few minutes at 100 ^{o}C for improved visualization.

For the non-volatile extracts, 2 µl of the 50 mg/ml solution applied to the silica gel plates (Alugram Sil G/UV254) and developed in a solvent system comprising toluene:dioxane:acetic acid (90: 25: 5). The plates were sprayed using natural spray A (1% methanolic diphenylborinic acid/ 2-aminoethyl ester 98%) and natural spray B (5% polyethylene glycol in water). These spray reagents are used for the detection of flavanoids (Wagner and Blatt, 1996).The plates were allowed to dry before observing them under UV 254 nm and UV 366 nm.

2.5 Antimicrobial studies

The antimicrobial component will be divided into two separate microbiological assays:

- Disc diffusion assays
- Minimum inhibitory concentration (MIC) micro-plate method

2.5.1 Disc diffusion assays

Antimicrobial assays were performed on the essential oils and solvent extracts using the following organisms in order to obtain a broad spectrum antimicrobial activity, i.e. gram-positive; *Staphylococcus aureus* (ATCC 12600), *Bacillus cereus* (ATCC 17778), *Bacillus subtilis* (ATCC 6051), gram-negative; *Escherichia coli* (ATCC 11715), *Yersinia entercolitica* (ATCC 23715), *Klebsiella pneumoniae* (ATCC 13883) and yeast cultures; *Candida albicans* (ATCC 10231) and *Cryptococcus neoformans* (ATCC 90112). The disc diffusion method was employed, whereby suspensions of these organisms at approximately 1 x 10^6 CFU/ml were introduced into the agar. Essential oils and the phenolic extracts (at a starting concentration of 50 mg/ml) were placed aseptically onto discs and placed onto the inoculated agar (Figure 3). Each disc diffusion assay was performed in triplicate. Zones of inhibition were thereafter examined after 24 hours for bacterial cultures, after 48 hours for the yeast cultures and after seven days for the mould culture. Zones of inhibition were measured from the edge of the disc to where there was culture growth. Neomycin at a concentration of 50mg/ml and Nystatin at a concentration of 100IU was used as controls for the disc diffusion assays.

Figure 3: Samples being prepared for the disc diffusion assay.

2.5.2 Minimum inhibitory concentration (MIC) microplate method

Once the antimicrobial activities of the samples were assessed by disc diffusion, a minimum inhibitory concentration was determined using the *p*-iodonitrotetrazolium violet INT microplate method (Eloff, 1999). Plant extracts i.e. the phenolic extracts at a starting concentrations of 50 mg/ml as well as the essential oils at a starting concentrations of 128 mg/ml, were transferred into the first well of a micro-titre plate. Serial dilutions are performed so that the phenolic extract concentrations of 12.5, 6.25, 3.12, 1.56, 0.78, 0.39, 0.19 and 0.09 mg/ml are obtained as well as essential oil concentrations of 32, 16, 8, 4, 2, 1, 0.5 and 0.25 mg/ml are obtained. *Bacillus subtilis*, *B. cereus* and *S. aureus* were selected as test pathogens as they proved to be the most sensitive in the disc diffusion assay.

A fixed bacterial culture yielding an inoculum size of approximately 1 x 10^6 CFU/ml was added to all wells and incubated at 37 °C for 24 hrs. A 0.2 mg/ml *p*-iodonitrotetrazolium violet (INT) solution was prepared and 40 µl transferred to all inoculated wells. The plates are examined to determine a colour change in relation to concentration of microbial growth after 6 hours and the MIC was then calculated accordingly. The MIC's were determined in triplicate.

2.6 Cluster analysis

The percentage composition of the essential oils was used to determine the similarity between the different essential oil samples by cluster analysis using the NTSYS-pc software (version 2.02, Exeter Software, Setauket, New York) developed by Rohlf (1992). Correlation was selected as a measure of similarity, and the unweighted pair-group method with arithmetic average (UPGMA) was used for cluster definition.

CHAPTER 3: Results and Discussion

3.1 Thin-layer chromatography

The essential oils and non-volatile extracts were examined by TLC to assess patterns of variation and similarity. Figure 4 represents the TLC plate developed using the non-volatile compounds and viewed at 365 nm. The three lanes indicated by 2, 3 and 4 in figure 4 and 5 represents three individuals of *P. camphorata* var. *camphorata* collected from the same population. The results indicate that there is little inter-plant variation as the three TLC plots do not show any obvious differences. The three individuals of *P. pallens* from different population indicated by 15, 16 and 17 in figure 4 and 5 also show negligible differences.

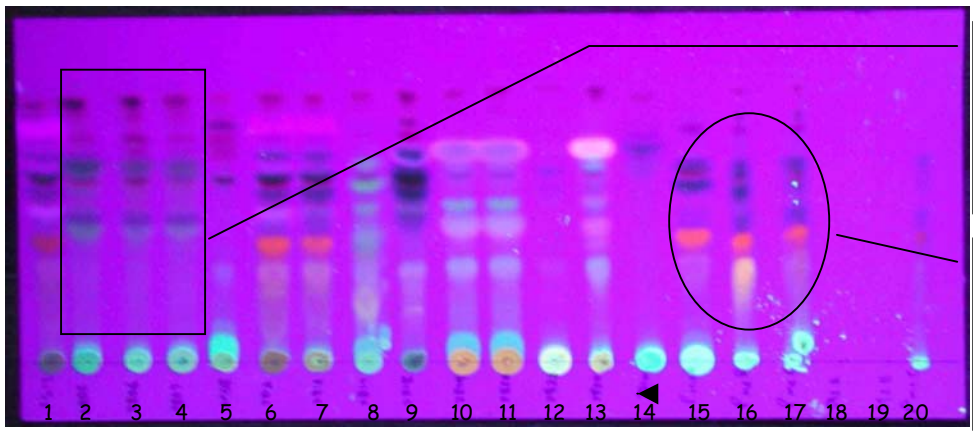


Figure 4: TLC plate of the essential oils viewed at 365nm

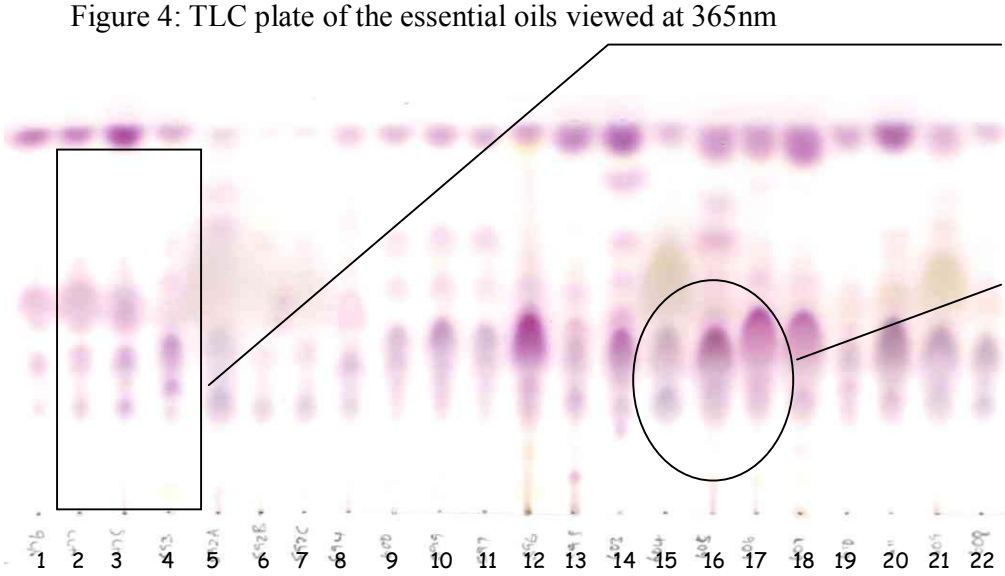


Figure 5: TLC plate of the essential oils sprayed with vanillin reagent.

Figure 4 and 5 lanes are: 1. *P. incana*, 2. *P. camphorata* A, 3. *P. camphorata* B, 4. *P. camphorata* C, 5. *P.incana,* 6. *P. incana,* 7. *P. flexicaulis,* 8. *P. glauca*, 9. *P. hutchensonia*, 10. *P. fasiculata*, 11. P. *paniculata* (Bakenshoogte), 12. *P. viscosa*, 13. *P. paniculata* (Prins Albert), 14. *P. paniculata* (Oudtshoorn), 15. *P. pallens* (Volmoed), 16. *P. pallens* (Oudtshoorn), 17. *P. pallens* (Lategansvlei), 18. *P. paniculata* (Oudtshoorn) 19. *P. membranacea* 20. *P. glomerata*, 21. *P. adenocarpa* and 22. *P elongata*

3.2 Disc diffusion assays

The results of the disc diffusion assays are shown in Table 2. In general, the bacteria displayed higher sensitivity against the methanol and acetone extracts rather than to the essential oils, except for *Pteronia pallens* where only the essential oil was the active and not the extracts.

The species tested showed minimal activity against the Gram-negative strains and no activity against the yeast. However most of the species tested exhibited antimicrobial activity against the Gram-positive organisms, especially *B. cereus* and *S. aureus* (figure 6).

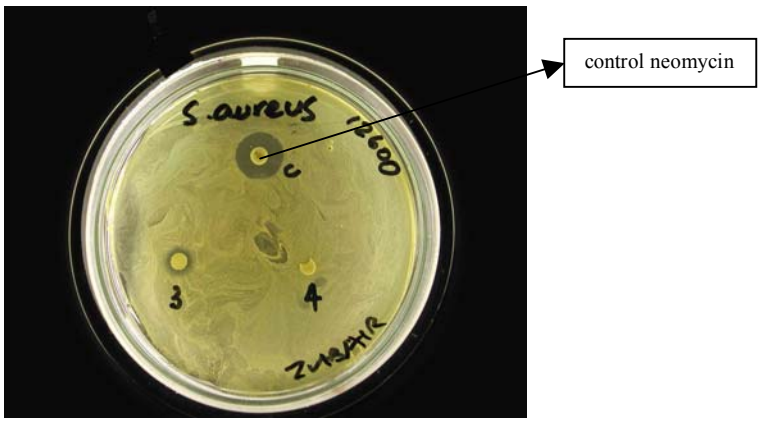


Figure 6: Zones of inhibition of *P. flexicaulis* acetone extract (3) and *P. fasiculata* acetone extract (4) against *S. aureus*.

Samples of *Pteronia adenocarpa, P. elongata*, *P. fasiculata* and *P. flexicaulis* (methanol and/or acetone extracts) exhibited zones of inhibition against *S. aureus*, *B. cereus* and *B. subtilis*. Interestingly however, the essential oils of each of the aforementioned plants displayed minimal or no antimicrobial activity.

Table 2: Disc diffusion data for phenolic extracts and essential oils.

Sample	**Extract**	*E. coli*	*S. aureus*	*B. cereus*	*B. subtilis*	*Y. entercolitica*	*K. pneumoniae*	*C. albicans*	*C. neoformans*
P. adenocarpa (A)	Acetone	0	**2**	**4**	**3**	0	0	0	0
P. adenocarpa (A)	Methanol	0	0	0	0	0	0	0	0
P. adenocarpa (B)	Essential oil	0	**2**	0	0	0	**2**	**2**	0
P. camphorata (A)	Acetone	0	0	**2**	0	0	0	0	0
P. camphorata (A)	Essential oil	0	0	0	**1**	0	**2**	**1**	0
P. camphorata (B)	Essential oil	0	0	0	0	0	**<1**	0	0
P. camphorata (C)	Essential oil	0	0	0	0	0	**<1**	0	0
P. elongata (A)	Acetone	0	**2**	**3**	0	0	0	0	0
P. elongata (A)	Methanol	0	0	0	0	0	0	0	0
P. elongata (B)	Essential oil	0	**2**	0	0	0	**1**	0	**2**
P. empetrifolia (B)	Acetone	0	0	0	0	0	0	0	0
P. empetrifolia (A)	Essential oil	**2**	**2**	**2**	0	0	0	0	0
P. empetrifolia (B)	Methanol	0	0	0	0	0	0	0	0
P. fasiculata	Acetone	0	**3**	**6**	0	0	0	0	**3**
P. fasiculata	Essential oil	0	0	0	0	0	0	0	0
P. fasiculata (A)	Methanol	0	**2**	**5**	0	0	0	0	**4**
P. flexicaulis (A)	Essential oil	0	0	0	0	0	0	0	0
P. flexicaulis (A)	Acetone	0	**1**	**5**	0	0	0	0	0
P. flexicaulis (A)	Methanol	0	0	**2**	**2**	0	0	0	0
P. flexicaulis (B)	Methanol	0	0	0	0	0	0	0	0
P. glomerata (A)	Acetone	0	0	0	0	0	0	0	0
P. glomerata (A)	Essential oil	0	0	0	0	0	0	0	0

P. glomerata (B)	Methanol	0	0	**5**	0	0	0	0	0
P. pallens (A)	Acetone	0	0	**3**	0	0	0	0	0
P. pallens (B)	Essential oil	0	**1**	**2**	0	0	0	0	0
P. pallens (C)	Essential oil	0	0	0	0	0	0	0	0
P. paniculata	Acetone	0	**2**	**5**	0	0	0	0	0
P. paniculata	Essential oil	0	0	0	0	0	0	0	0
P. paniculata	Methanol	0	0	**2**	0	0	0	0	0
P. viscosa (A)	Acetone	0	0	0	0	0	0	0	0
P. viscosa (A)	Essential oil	0	0	0	0	0	0	0	0
P. viscosa (B)	Methanol	0	0	0	0	0	0	0	0
Control		**5**[2]	**6**[2]	**6**[2]	**5**[2]	**4**[2]	**5**[2]	**6**[3]	**5**[3]

[1]Zone diameters measured in mm from disc edge to edge of zone
[2]Control - neomycin 50mg/ml
[3]Control – nystatin 100IU

3.3 Minimum inhibitory concentrations

Following the results determined from the disc diffusion assays, the most active species were tested further in order to determine their minimum inhibitory concentrations (MIC) at concentrations of 50 mg/ml initially. Three of the extracts displayed MIC's of <0.39 mg/ml against *S. aureus* and were tested further at 5 mg/ml in order to determine and confirm their MIC's. The same samples also displayed this antimicrobial activity against *B. cereus*. These samples *Pteronia elongata*, *P. fasiculata* and *P. flexicaulis* (the methanol and/or acetone extracts) were the same samples that exhibited various sizes of zones of inhibition in the disc diffusion assays. The results of each organism's MIC's are depicted in Table 3.

Table 3: MIC results for selected extracts and essential oils tested against *S. aureus, B. cereus and B. subtilis*.

Species	**Extract**	**MIC (mg/ml)**		
		S. aureus	*B. cereus*	*B. subtilis*
P. adenocarpa (A)	Acetone	0.15	0.08	1.56
P. adenocarpa (B)	Essential oil	32	*	2
P. elongata (A)	Acetone	6.25	**	**
P. elongata (A)	Methanol	0.33	0.26	2.08
P. elongata (B)	Essential oil	<0.25	21.33	*
P. empetrifolia (A)	Essential oil	5.33	*	10.67
P. fasiculata (A)	Acetone	0.08	0.39	2.08
P. flexicaulis (A)	Acetone	0.22	1.04	1.30
P. flexicaulis (A)	Essential oil	*	1	*
P. flexicaulis (A)	Methanol	0.15	0.39	1.56
P. pallens (A)	Essential oil	13.33	16	4
P. paniculata (A)	Acetone	0.19	1.30	*
P. paniculata (A)	Essential oil	16	*	0.5
Control Ciprofloxacin		1.0×10^3	0.5×10^3	0.3×10^3

*Not selected for testing, did not display antimicrobial activity in disc diffusion assays

** Not sufficient samples for further tests

◄

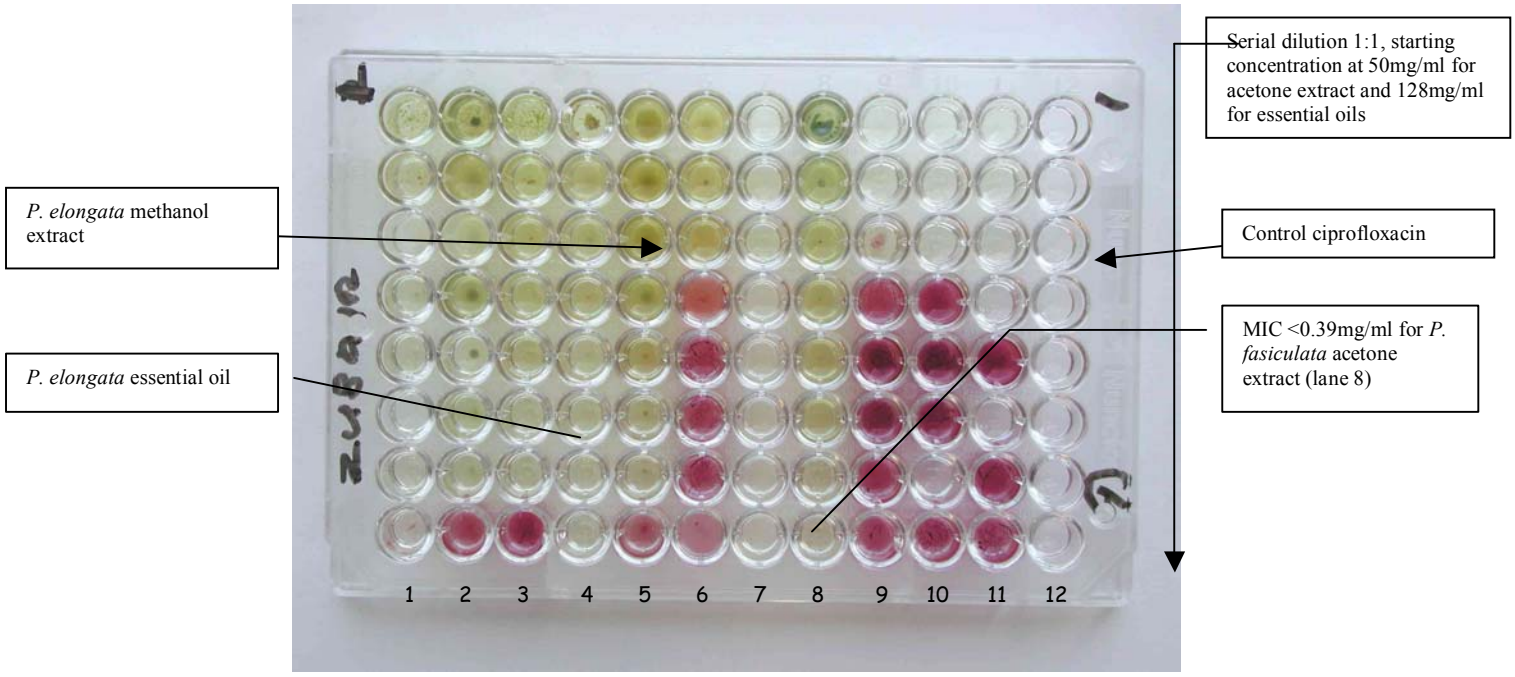


Figure 7: MIC results for *S. aureus*.

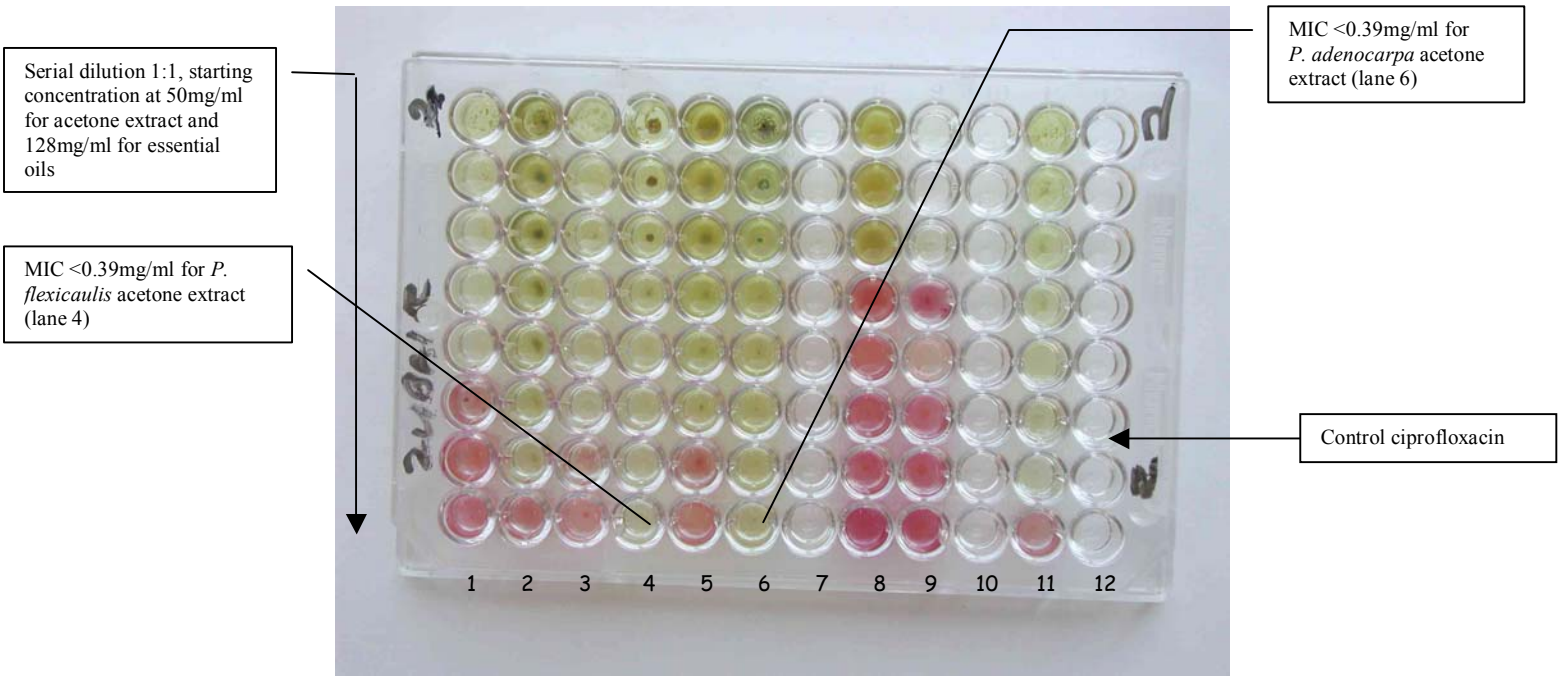


Figure 8: MIC results for *B. cereus*.

For *S. aureus* the following extracts displayed MIC's at <0.39 mg/ml, *Pteronia flexicaulis* (acetone and methanol extracts), *Pteronia fasiculata* (acetone extract) and *Pteronia adenocarpa* (acetone extract). These samples were then diluted to a concentration of 5 mg/ml in order to determine the exact MIC's for the species in question. Results are recorded in the aforementioned tables. Unfortunately the yields of the sample from *Pteronia elongata*

(essential oils and acetone extracts) were extremely low, and no further tests could be performed due to this lack of sample.

Zones of inhibition and the MIC's do not complement each other as a quantitative measure of antimicrobial activity. This shows variation in the microbiological techniques employed, and hereby just confirms what the MIC of the sample being tested is; or if the sample has any antimicrobial properties against the bacteria in the test e.g. *Pteronia adenocarpa* at a concentration of 50 mg/ml has a zone of inhibition of just 2 mm in diameter against *S. aureus* whereas the MIC at the same concentration was 0.15 mg/ml against *S. aureus*.

Therefore the disc diffusion method and the test for MIC's cannot be compared to each other, however they do compliment each other making them valuable techniques, in determining how much of an extract is required for any antimicrobial activity; or if the test sample has any antimicrobial properties against the other organisms (Njenga *et al.* 2005).

3.4 Essential oils analysis

The essential oil composition is given in Table 4, whilst the GC/MS chromatograms are included in chapter 4. These include the compositional data for each of the oils.

Table 4: Essential oil composition of the hydro-distilled essential oils as determined by GC/MS .

Rt	RRI	Compound	*P. adenocarpa*	*P. camphorata* A	*P. camphorata* B	*P. camphorata* C	*P. elongata*	*P. empetrifolia*	*P. fasiculata*	*P. flexicaulis*	*P. glomerata*	*P. pallens* B Volmoed	*P. pallens* A Lategansvlei	*P. pallens* B	*P. paniculata*	*P. viscosa*
6.4	952	1-nonene					0.5			0.04						
7.7	1035	α -thujene		0.3		0.1					2.1					
8.6	1032	α-pinene	4.8	0.5	0.2	0.7	4.5	10.3	8.7	5.1	10.2	4.2	4.7	5	12	2.4
8.9	1048	2-methyl-3-buten-2-ol					2.2									
10.3	1076	camphene						0.03								
12.3	1118	β-pinene	25.4	1.1	0.7	0.9	28.6	14.5	15.8	32.1	14.3	21.6	26.9	22.5	19	14.9
12.8	1132	sabinene	3.3	9.1	7.1	12.7	7.8	2.5	2	2.8	13.3	32.2	33.6	30.8	22	9
13.8	1142	undecene						0.4								
14.9	1176	α -phellandrene		1.6	1.7	5.5										
14.9	1174	myrcene	2.9				12.4	4.7	13.8	17.6	2.8	1.3	1.1	0.9	2.1	10.4
15.6	1188	α -terpinene			0.1	0.3	0.7			0.5	1.3	0.3	0.7	0.6	0.7	0.7
16.1	1195	dihydro 1,8-cineole	0.03		0.1	0.1										
16.6	1203	limonene	2.8	5	3.8	7.7	3	11.2	2.7	6.1	3.2	4.6	3.3	4.3	13	2.7
17.0	1213	1,8-cineole	23.5	42.7	40.4	42.6		2.5			7.7					37
17.0	1218	β-phellandrene					11.2		0.2	2.2		1.2	1.2	0.8	1.9	0.2
18.2	1246	Z-(β)-ocimene	0.6	1	0.8	1.5	3.8	0.1			0.3	0.4				
18.7	1255	δ-terpinene	0.3	1.2	0.4	0.7							0.9	0.1		1.7
18.7	1255	γ -terpinene					1.5			0.3	2.5				1.1	
19.0	1266	(E)-β-ocimene	0.2	0.2	0.03	0.1	1.4	0.3	0.9		1.1	1	0.9	1.4	0.5	0.9
19.9	1280	ρ-cymene	14.3	17.1	21.1	10	3.6	9.8	9.2	1.3	9.9	0.5	0.7	1.1	2.9	1.7
20.4	1290	terpinolene	0.2	0.3		0.1	0.5	0.1		0.3	0.6	0.1	0.2	0.03	0.3	0.4
21.9	1327	3-methyl-2-butenol					0.6				0.1					
22.1	1386	1-hexanol	0.03													
24.1	1382	*cis*-allo ocimene					0.1									
24.5	1384	α-pinene oxide												0.2		

Rt	RRI	Compound	*P. adenocarpa*	*P. camphorata* A	*P. camphorata* B	*P. camphorata* C	*P. elongata*	*P. empetrifolia*	*P. fasiculata*	*P. flexicaulis*	*P. glomerata*	*P. pallens* B Volmoed	*P. pallens* A Lategansvlei	*P. pallens* B	*P. paniculata*	*P. viscosa*
24.7	1399	methyl-octanoate					0.1	0.1			0.2					0.7
25.6	1406	presilphiperfol-7-ene								0.4		0.4	0.8	0.3		
25.9	1429	perillene	0.1				0.1	0.1								
26.2	1424	7-α-(H)-silphiperfol-5-ene											0.04	0.1		
26.5	1432	ethyl-octanoate	0.1				0.04				0.1					0.1
26.7	1450	*trans*-linalool oxide (furanoid)				0.1										
26.7	1452	α- ρ -dimethylesterene	0.02													
26.8	1451	β-thujone												0.01		
26.9	1458	*cis*-1,2-limonene epoxide												0.04		
27.2	1452	7 β -(H)-silphiperfol-5-ene								0.8			0.1	0.07		
27.4	1466	α-cubebene								0.5						
27.5	1474	*trans*-sabinene hydrate	0.1	0.4	0.8	0.6		0.1			0.4	1.1	1	1.4	1.2	0.2
27.7	1450+	*cis*-linalool oxide (furanoid)			0.1	0.1										
28.2	1479	bicycloelemene						0.1			0.1	1.1		0.01	0.1	
28.3	1482	cyclosativene								0.1						
28.7	1499	α-campholene aldehyde	0.04					0.4						0.01		
28.8	1495	silphiperfol-5-ene								1.7		0.9	1.7	0.9		
29.4	1512	dillethether			0.04									0.02		
29.6	1435	β-bourbonene								0.5						
30.3	1553	linalool	0.1	0.4	2.8	3.1	0.2				0.1					0.1
30.3	1556	*cis*-sabinene hydrate	0.04								0.2	0.8	0.7	0.8	1	
30.6	1562	isopinocamphene						0.1		0.2						0.1
30.9	1571	*trans*-ρ-menth-2-en-1-ol	0.3	0.3	0.1	0.2	0.3	0.1		0.04	0.4	0.1	0.1	0.1	0.5	0.3
31.3	1586	pinocarvone	0.7	0.1			0.1	0.6		0.1		0.1	0.2	0.4		
31.5	1577	cascarilladiene								0.1						
31.7	1583	nopinone	0.5				0.1	0.3			0.1	0.1	0.1	0.2		

Rt	RRI	Compound	*P. adenocarpa*	*P. camphorata* A	*P. camphorata* B	*P. camphorata* C	*P. elongata*	*P. empetrifolia*	*P. fasiculata*	*P. flexicaulis*	*P. glomerata*	*P. pallens* B Volmoed	*P. pallens* A Lategansvlei	*P. pallens* B	*P. paniculata*	*P. viscosa*
31.9	1588	methyl decanoate	0.03													
32.0	1604	thymol methyl ether						0.1								
32.2	1600	terpinen-4-ol	3.6	5.8	3.1	2.4	7	0.8	6.3	2.3	9.5	2.2	3.7	2.4	9.2	8.4
32.6	1661	aromadendrene									0.1				0.2	
32.9	1632	*cis*-ρ-menth-2-en-1-ol				0.2										
32.9	1639	*trans*-ρ-menth-2,8-dien-1-ol		0.4	0.4											
32.9	1632	*cis*- ρ -menth-2-en-1-ol	0.2				0.2	0.2		0.04	0.3				0.3	0.2
33.3	1648	myrtenal		0.1	0.2	0.1	0.3	0.8		0.1	0.04	0.3	0.3	0.6		
33.3	1651	sabinaketone		0.3	0.2	0.2							0.3	0.6		
33.9	1661	*trans*-pinocarveol	2.3	0.2	0.2	0.1	0.4	1.5			0.1	0.5	0.8	1		0.1
33.9	1661	(Z)-3-hexenyl hexenoate + alloaroma dendrene								0.6						
34.0	1659	γ -gurjunene									0.4					
34.2	1681	(Z)-3-hexenyl tiglate								0.04						
34.3	1682	δ-terpineol	0.3													
34.3	1678	*cis*-ρ-menth-2,8-dien-1-ol												0.04		
34.4	1671	methyl chavicol (estragol)		4.5	6.8	2.9										
34.5	1689	*trans*-piperitol									0.2		0.3			0.1
34.5	1690	cryptone	0.4				0.2									
34.5	1683	*trans*-verbenol						0.5								
34.5	1690	cryptone								0.03						
34.5	1687	α-humulene										0.3		0.5		
34.7	1700	limonen-4-ol		0.05	0.4			0.04								
35.1	1682	α -terpineol	1.7	2.3	4.8	3.9	1.9	0.8	1.3	1.1	1.4	0.1	0.2	0.2	1.6	1.5
35.3	1708	ledene									0.1					
35.4	1729	thujol	0.03													
35.7	1726	verbenone	0.3					0.7								

Rt	RRI	Compound	*P. adenocarpa*	*P. camphorata* A	*P. camphorata* B	*P. camphorata* C	*P. elongata*	*P. empetrifolia*	*P. fasiculata*	*P. flexicaulis*	*P. glomerata*	*P. pallens* B Volmoed	*P. pallens* A Lategansvlei	*P. pallens* B	*P. paniculata*	*P. viscosa*
35.7	1726	germacrene D								0.1		0.8	0.6	0.9	1.1	
36.0	1743	α-cadinene	0.3							0.4						
36.1	1740	α-muurolene						1.2			0.1					
36.1	1740	valencene												0.1		
36.1	1743	eremophilene													1	
36.4	1751	carvone	0.5	1.2	1	0.7										
36.4	1755	bicyclogermacrene					0.8	1.5		0.2	2.8	1.3	0.8	0.7	2.4	
36.8	1765	geranyl acetone			0.1	0.1										
36.8	1758	*cis*-piperitol						0.2								
36.8	1765	geranyl acetone										0.2	0.04	0.1		
37.0	1773	δ-cadinene	0.4	0.1		0.1		2.1		0.1	0.3	0.03	0.1	0.1	0.8	0.3
37.1	1776	γ-cadinene			0.1						0.1	0.1	0.05	0.2		
37.4	1786	kessane	0.1				0.1	1.5		0.1	0.5	0.6	0.7	0.7		
37.6	1797	ρ-methyl-acetophenone	0.1	0.5	0.5											
37.6	1783	*cis*-sabinol										0.1				
37.8	1802	cumin aldehyde		0.4	0.4	0.2										
37.8	1804	myrtenol	1.4		0.2	0.1	0.3	0.8		0.2	0.2	0.4	0.5	0.7		
38.0	1814	ρ-mentha-1,5-dien-7-ol		0.2												
38.2	1804	liguloxide	0.3				0.1	1.4			0.2	0.5	0.5	0.6		
39.0	1834	*trans*-carveol	0.2	0.4	0.3	0.2		0.5				0.3		0.1		
39.2	1841	calamenene						0.1		0.1					0	
39.3	1864	ρ-cymen-8-ol	0.8	0.2	0.4	0.2	0.1	0.2		0.1	0.1		0.03	0.1	0.1	0.1
39.6	1868	(E)-geranyl acetone	0.02				0.1					0.2	0.1	0.1		
39.6	1867	thymol acetate						11								
39.8	1882	*cis*-carveol		0.1		0.1										
40.1	1890	carvacryl acetate														

Rt	RRI	Compound	*P. adenocarpa*	*P. camphorata* A	*P. camphorata* B	*P. camphorata* C	*P. elongata*	*P. empetrifolia*	*P. fasiculata*	*P. flexicaulis*	*P. glomerata*	*P. pallens* B Volmoed	*P. pallens* A Lategansvlei	*P. pallens* B	*P. paniculata*	*P. viscosa*
40.4	1896	*cis*- ρ -menth-1(7),8-dien-2-ol						0.03								
40.6	1900	epicubebol						0.1							0	
40.9	1916	α-agarofuran						0.2		0.3		0.3	0.2	0.6		
41.4	1924	silphiperfolan-7 β -ol								0.1		0.1	0.1	0.1		
41.5	1941	α-calacorene						0.1								
41.8	1953	palustrol									0.1					
41.9	1957	cubebol						0.1							0.1	
41.9	1957	cubebene								0.6						
42.7	1976	cameroonan-7 α -ol								0.6		0.4	0.4			
43.1	2001	isocaryophyllene oxide												0.05		
43.4	2008	caryophyllene oxide	0.02	0.3	0.3	0.4	0.3	0.2	0.3	1.3		0.4	0.2	0.7		
43.5	2029	perilla alcohol	0.1													
43.7	2030	methyl eugenol			0.4	0.2										
43.7	2012	1-allyl-2,4-dimethoxybenzene		0.3												
43.7	2030	methyl eugenol	0.3													
43.9	2024	presilphiperfolan-9 α -ol								0.9						
44.2	2037	(E)-Nerolidol							4	0.5				0.02	0.2	
44.3	2041	tridecenyl acetate														0.1
44.4	2045	ledol									0.4					
44.5	2049	prenopsan-8-ol								0.1			0.04	0.1		
44.7	2202	germacrene D-4-ol				0.1									0.1	
44.7	2057	ρ-mentha-1,4-dien-7-ol	0.1													
44.8	2081	humulene-epoxide III	0.4		0.04											
44.9	2067	presilphiperfolan-8-ol						0.9		0.9		7.5	5.1	7.9		
45.1	2080	cubenol								0.1						
45.2	2088	1-epi-cubenol					0.1	0.1		0.2						

Rt	RRI	Compound	*P. adenocarpa*	*P. camphorata* A	*P. camphorata* B	*P. camphorata* C	*P. elongata*	*P. empetrifolia*	*P. fasiculata*	*P. flexicaulis*	*P. glomerata*	*P. pallens* B Volmoed	*P. pallens* A Lategansvlei	*P. pallens* B	*P. paniculata*	*P. viscosa*
45.4	2098	globulol					0.1				0.4			0.01	0.1	
45.7	2104	viridiflorol	0.1				0.1	1.9	31.1	2.3	0.3				0.2	
45.8	2113	cumin alcohol	0.3	0.2	0.1	0.1	0.1					0.1	0.1	0.3		
46.2	2127	10-epi- γ -eudesmol	0.03					0.7		5.4		8.8	5	6.4		
46.6	2144	spathulenol	2.4	0.1		0.1	3.6	4.7	2.1	1.3	8.6				2.3	2.2
47.4	2170	*cis*- ρ -meth-3-en-1,2-diol									0.1					
47.6	2187	T-cadinol	0.1			0.04					0.05				0.3	0.1
47.7	2183	thymol						1.7								
47.8	2187	copaborneol								0.3						
48.0	2196	T-muurolol	0.3					0.9		0.4	0.2				0.2	0.1
48.3	2209	torreyol						0.1			0.1					
48.4	2214	carvacrol					0.03	0.1							0.1	0.1
48.5	2218	isospathulenol									0.1					
48.6	2232	α-bisabolol						1.3								
48.7	2228	himachalol		0.1	0.1	0.2										
48.9	2247	*trans*-α-bergamotol						0.1			0.6			0.12		
48.9	2247	*trans*-α-bergamotol														
49.1	2255	α-cadinol	0.4	0.2				1.5	0.2		0.3	0.9		0.3		0.9
49.1	2257	β-eudesmol											0.2			
49.1	2255	α-cadinol													0.3	
49.2	2250	4- α -hydroxy-dihydro-agarofuran								0.3						
49.2	2264	intermedeol									0.8					
49.9	2282	8,13-epoxy-15,16-dinorlabol-12-ene								0.1				0.1		
50.4	2304	(2E,6Z) farnesol									0.2					
50.5	2308	(Z)-9-tricosene								0.2						
Total			99.8	99.4	99.3	99.7	96.3	99.2	98.6	96.6	99.7	99.6	99.9	95.2	98.3	98.5

The following monoterpenes were found to be present in the oil analysed; α-pinene, β-pinene, sabinene, limonene, ρ-cymene, α-terpineol and terpinen-4-ol.

However some major and minor compounds in the essential oils of the species being investigated, indicate that the composition of these essential oils differ quite extensively within the genus *Pteronia* as well. This confirms findings of Zedro *et al*. (1990) that the diterpenes and other compounds found in the *Pteronia* species are not uniform although there are some similarities.

Two distinct groups are defined. In *Pteronia pallens*, *P. empetrifolia* and *P. flexicaulis*, some rare compounds, characterize this group. These are; presilphiperfol-7-ene, 7-α-(H)-silphiperfol-5-ene, 7-α-(H)- silphiperfol-5-ene, 7-β-(H)-silphiperfol-5-ene, α-campholene aldehyde, silphiperfol-5-ene, camaroonan-7- α -ol, silphiperfolan-7- β -ol, presilphiperfolan-9- α -ol and presilphiperfolan-8-ol (a major compound in *Pteronia pallens*). These compounds have previously only been recorded once in the essential oil literature, and have been isolated from the essential oils of *Eryophyllen staechadifolium* (Kőnig *et al*. 1997).

The second group is distinctly characterised by the absence of the above compounds and all contain the following monoterpines, α-pinene, β-pinene, sabinene, limonene, ρ-cymene, α-terpineol and terpinen-4-ol, with α-pinene, β-pinene and terpinen-4-ol being the dominant compounds.

3.5 Cluster Analysis

For this cluster analysis, the percentage composition of the essential oil samples was used to determine the similarity between the different samples of *Pteronia* species investigated. Therefore, if a sample presented with a set of essential oil compares exactly like another in the dataset, the correlation co-efficient will approach 1; making them alike as far as the amount and presence of essential oil compounds. The converse would also be true, i.e. if the co-efficient is approaching 0, there are fewer similarities between the samples.

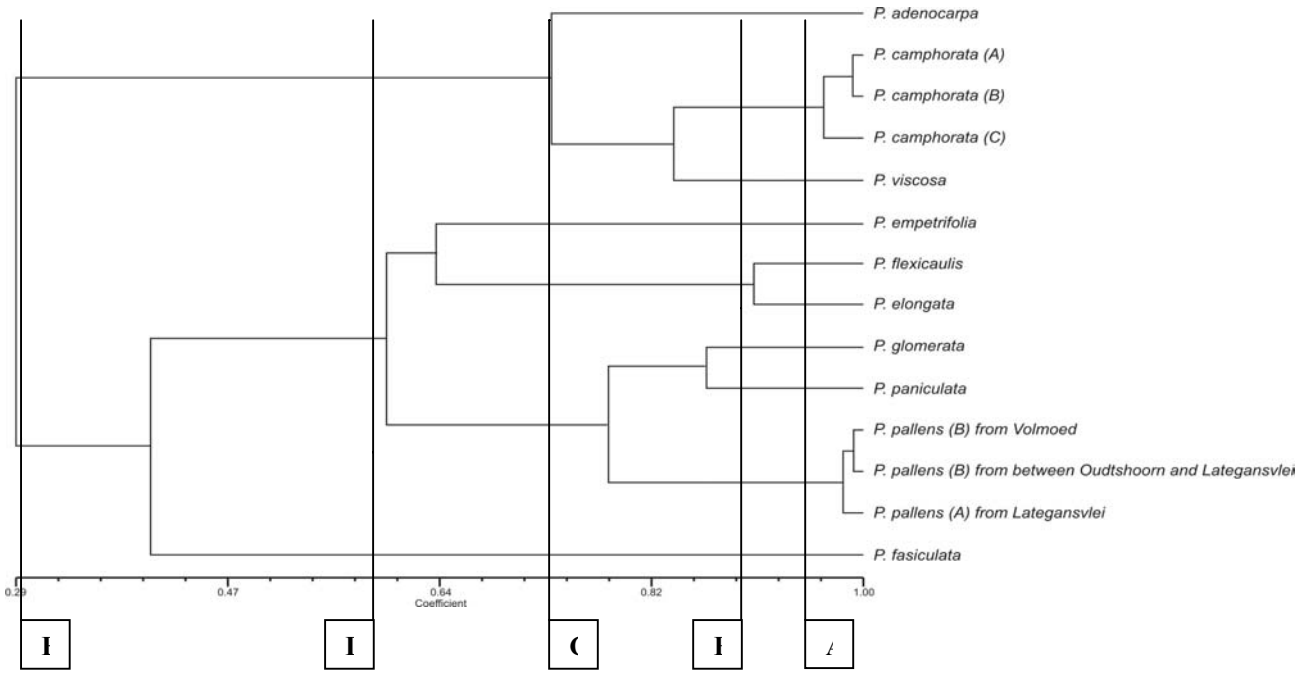


Figure 9: Cluster analysis of essential oil composition.

In order to investigate if there are any inter-plant variations of individual plants from within the same population, three samples of *P. camphorata* were collected and essential oil composition determined using the GC-MS. If an arbitrary line A is drawn in figure 9, the cluster analysis indicates that all the collections of *P. camphorata* are grouped together, with a co-efficient close to 1, thus concluding that there are no obvious differences within the species, whether it be different individual plants from the same population as is the case with *P. camphorata*. Coupled with the antimicrobial data from the disc diffusion assays, and that their antimicrobial activity against the three gram positive organisms is the similar (table 2), as well as the fairly uniform TLC results (figures 4 and 5), this concludes that there are no obvious difference in the *P. camphorata*, obtained from the same population. From this result, we can assume that this factor may be uniform in the species, and that there is no individual plant variation from the same population in essential oil composition as a genus.

In order to investigate if there are any inter-plant variations from samples from different populations, three samples of *P. pallens* were collected and essential oil composition determined using the GC-MS. Using arbitrary line A in figure 9, the cluster analysis indicates that at species level the species are grouped together, with a co-efficient close to 1, thus concluding that the species are similar to each other in essential oils composition and quantity, whether the individual plants are from different populations, as is the case with *P.*

pallens. This is further confirmed by the TLC results (figures 4 and 5) which shows no variation in the separation of the essential oils or their phenolic extracts. From this result, we can assume that this factor may be uniform in the species, and that there is no inter-plant variation from individuals from different populations.

If an arbitrary line B is drawn in figure 9, this would give us the species which have the most essential oils in common with each other. These 2 species are *P. flexicaulis* and *P. elongata*, with a co-efficient of approximately 0.91. The disc diffusion assays and MIC's also indicate a similar trend in that, the 2 species phenolic extracts display anti-microbial activity against *S. aureus*, *B. cereus* and *B. subtilis*. The concentrations during the MIC's at which these two compounds are active are also similar (table 3) to one another.

If arbitrary lines C and D are drawn in figure 9, this distinctly gives us two groupings of species. The first group consists of *Pteronia adenocarpa*, *P. camphorata* and *P. viscosa* with a co-efficient of approximately 0.72. Unique to this group are two essential oils, namely. δ-terpinene and linalool. From this point forward, there is also no correlation between the essential oils composition and the antimicrobial activity of the compounds. The second group of species is *Pteronia elongata*, *P. empetrifolia*, *P. fasiculata*, *P. flexicaulis*, *P. glomerata*, *P. pallens* and *P. paniculata* with a co-efficient of approximately 0.4. Unique to these species are also two essential oils, namely myrcene and bicyclogermacrene. There is also no correlation between the essential oils and antimicrobial activity. Arbitrary line E, gives a co-efficient of approximately 0.29. At this point the species are united with each other into the genus *Pteronia*. The essential oils that are present in all the species displayed in figure 9 are α-pinene, β-pinene, limonene, sabinene , ρ-cymene, α –terpineol and terpinen-4-ol.

CHAPTER 4: Monographs of species investigated

4.1 *Pteronia adenocarpa* Harv.

Botanical description

The plant is a rigid shrub with twigs (figure 10). Flower heads are solitary at branch tips, sticky with leaves also opposite, ovate, recurving at the tips and scabrid on the margins. Bright pink buds (figure 11) open to waxy white flowers *Pteronia adenocarpa* flowers between August and December. The plant is a very old Khoi-San 'buchu remedy'. Plants are locally abundant.

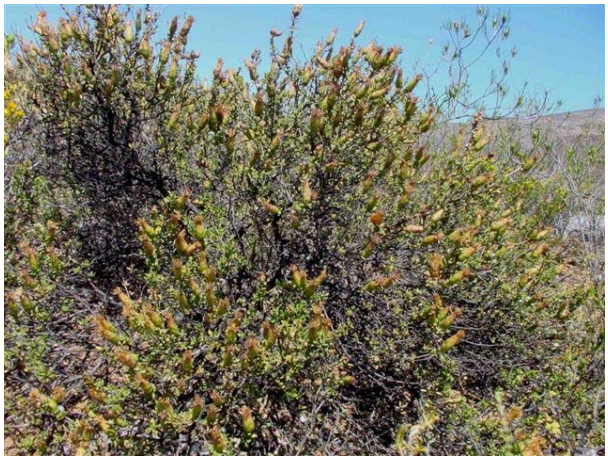


Figure 10: *P. adenocarpa.* in habit

Figure 11: Pink buds opening to flowers

Geographic distribution

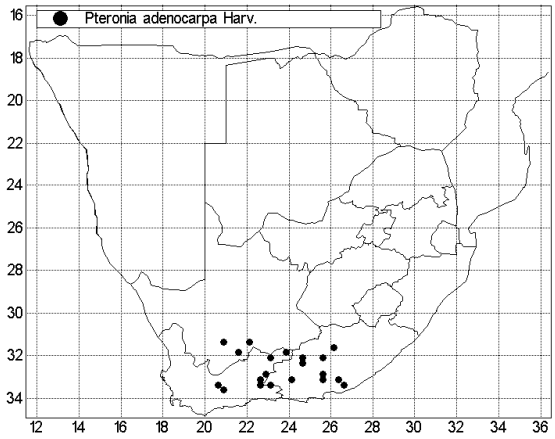


Figure 12: Geographical distribution of *P. adenocarpa*. (SANBI)

(All the distribution maps in the monographs were obtained from the South African National Botanical Institute (SANBI), Private Bag X101, Pretoria, South Africa)

The plant is distributed from the Eastern Cape, Western Cape and inland to the Karoo (figure 12). These specimens for this study were collected approximately 10km west of Klaarstroom, near the Frisgewaagd farm, South Africa.

Essential oil analysis

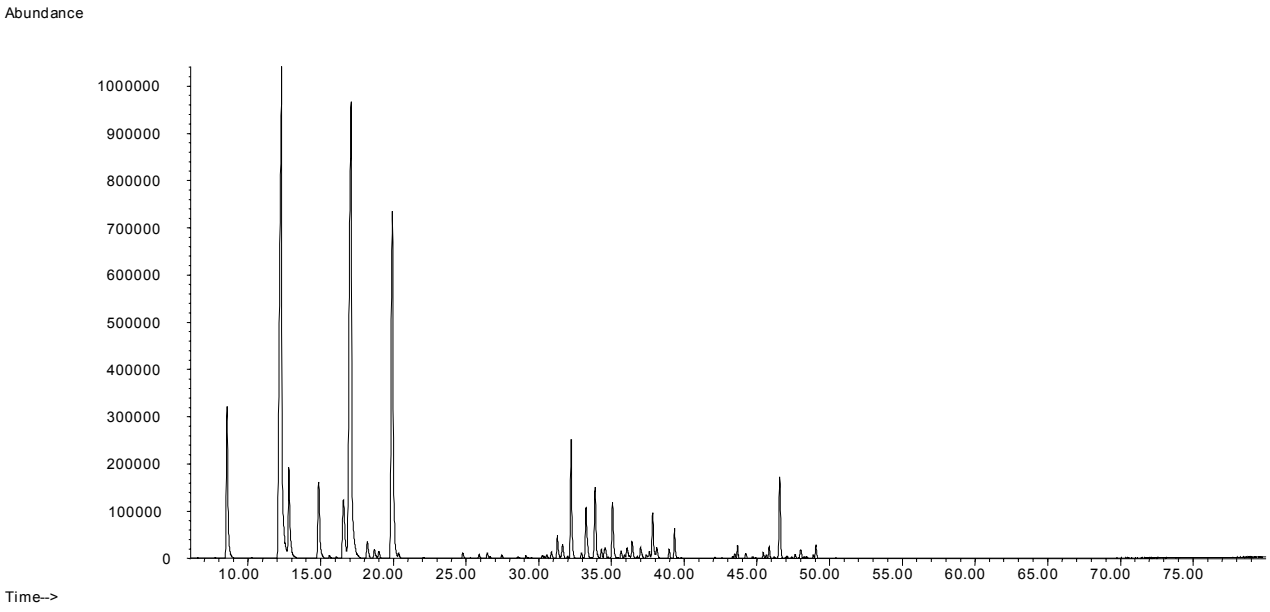


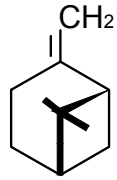
Figure 13: Total ion chromatogram of the hydrodistilled essential oil from *P. adenocarpa.*

Table 5: Compounds identified in the essential oil of *Pteronia adenocarpa.*

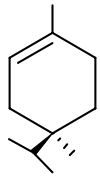
Rt	RRI	Compound	%
8.6	1032	α-pinene	4.8
12.3	1118	β-pinene	25.4
12.8	1132	sabinene	3.3
14.9	1174	myrcene	2.9
16.1	1195	dihydro 1,8-cineole	0.03
16.6	1203	limonene	2.8
17	1213	1,8-cineole	23.5
18.2	1246	Z-(β)-ocimene	0.6
18.7	1255	γ -terpinene	0.3
19	1266	(E)- β -ocimene	0.2
19.9	1280	ρ-cymene	14.3
20.4	1290	terpinolene	0.2
22.1	1386	1-hexanol	0.03
25.9	1429	perillene	0.1
26.5	1432	ethyl-octanoate	0.1

26.7	1452	α- *ρ* -dimethylesterene	0.02
27.5	1474	*trans*-sabinene hydrate	0.1
28.7	1499	α-campholene aldehyde	0.04
30.3	1556	*cis*-sabinene hydrate	0.04
30.3	1553	linalool	0.1
30.6	1562	isopinocamphene	0.1
30.9	1571	*trans*- ρ -menth-2-en-1-ol	0.2
31.3	1586	pinocarvone	0.7
31.7	1583	nopinone	0.5
31.9	1588	methyl decanoate	0.03
32.2	1600	terpinen-4-ol	3.6
32.9	1632	*cis*- ρ -menth-2-en-1-ol	0.2
33.9	1661	*trans*-pinocarveol	2.3
34.3	1682	δ-terpineol	0.3
34.5	1690	cryptone	0.4
35.1	1682	α-terpineol	1.7
35.4	1729	thujol	0.03
35.7	1726	verbenone	0.3
36	1743	α-cadinene	0.3
36.4	1751	carvone	0.5
37	1773	δ-cadinene	0.4
37.4	1786	kessane	0.1
37.6	1797	ρ-methyl-acetophenone	0.1
37.8	1804	myrtenol	1.4
38.2	1804	liguloxide	0.3
39	1834	*trans*-carveol	0.2
39.3	1864	ρ-cymen-8-ol	0.8
39.6	1868	(E)-geranyl acetone	0.02
43.4	2008	caryophyllene oxide	0.02
43.5	2029	perilla alcohol	0.1
43.7	2030	methyl eugenol	0.3
44.7	2057	ρ-mentha-1,4-dien-7-ol	0.1
44.8	2081	humulene-epoxide III	0.4
45.7	2104	viridiflorol	0.1
45.8	2113	cumin alcohol	0.3
46.2	2127	10-epi- γ -eudesmol	0.03
46.6	2144	spathulenol	2.4
47.6	2187	T-cadinol	0.1
48	2196	T-muurolol	0.3
48.4	2214	carvacrol	0.1
49.1	2255	α-cadinol	0.4
		Total	99.89

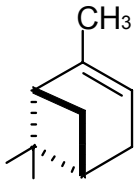
Major compounds identified in *Pteronia adenocarpa* essential oil



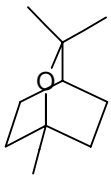
β-pinene (26%)



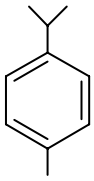
terpinen-4-ol (4%)



α-pinene (5%)



1,8-cineole (24%)



ρ-cymene (14%)

4.2 *Pteronia camphorata* (L.) L var. *camphorata*

Botanical description

Pteronia camphorata is a slender aromatic shrub (figure 14). The leaves are linear to filiform and ciliate. Florets are yellow, with the flower heads being discoid with 1-3 occurring at the branch tips (figure 15). The bracts are short and closely ciliate. Plants are locally abundant, but are localized to the area. Plant height is approximately 2.0 meters. Flowering occurs between August and November.

Figure 14: Slender shrubs of *P. camphorata* var. *camphorata*

Figure 15: Yellow flowers of *P. camphorata* var. *camphorata*

Geographical distribution

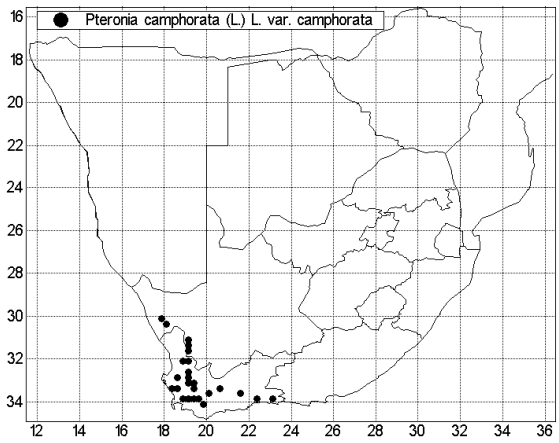


Figure 16: Geographical distribution of *P. camphorata* (L.) L. var. *camphorata.* (SANBI)

Three varieties of *Pteronia camphorata* grow in Southern Africa. These are *Pteronia camphorata* (L.) L. var. *armata* Harv., *P. camphorata* (L.) L. var. *camphorata* and *P. camphorata* (L.) L. var. *laevigata*. *P. camphorata* var. *camphorata* was selected for this report and was collected near Montagu Pass near Herold.

Essential Oil analysis

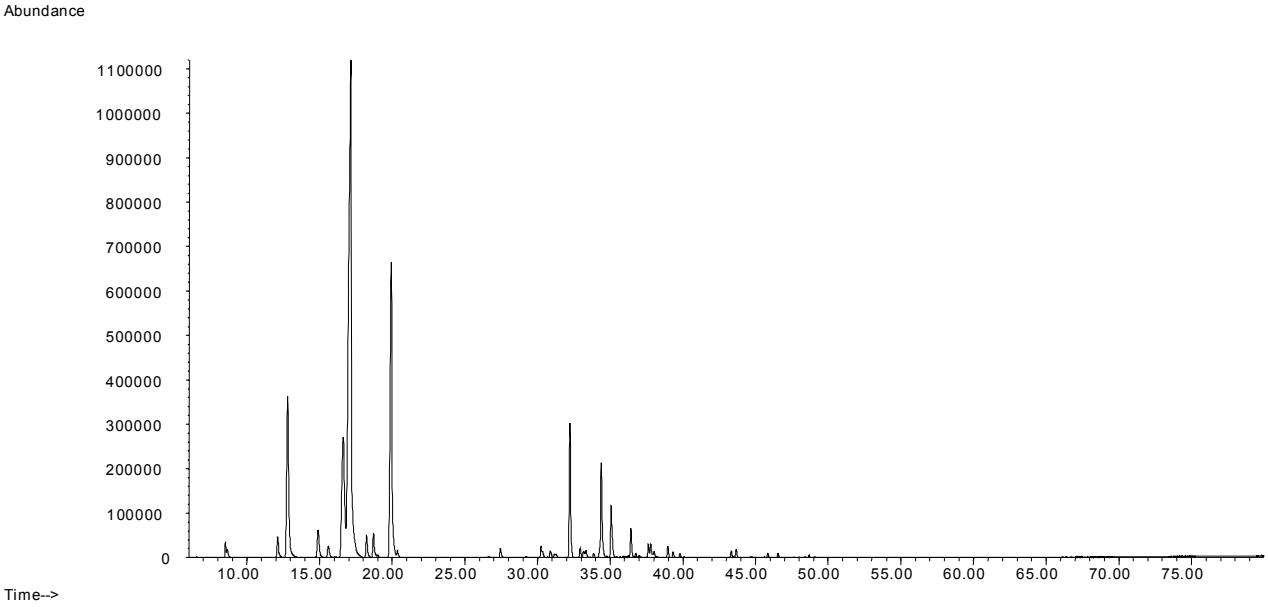


Figure 17: Total ion chromatogram of the hydrodistilled essential oil from *P. camphorata* var. *camphorata* (individual A).

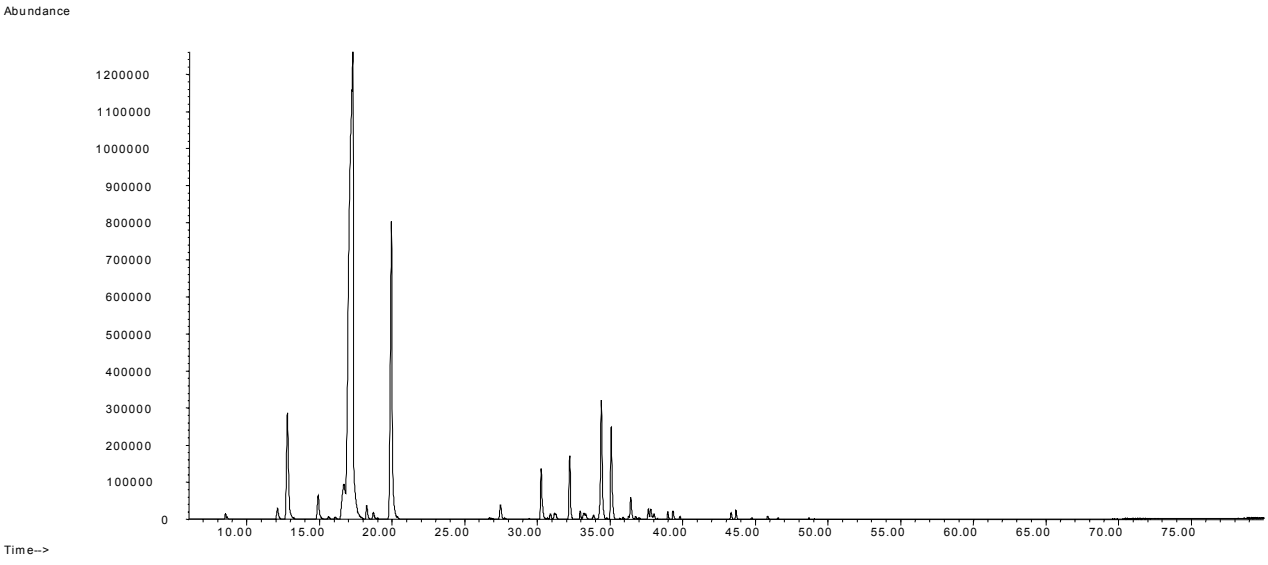


Figure 18: Total ion chromatogram of the hydrodistilled essential oil from *P. camphorata* var. *camphorata* (individual B).

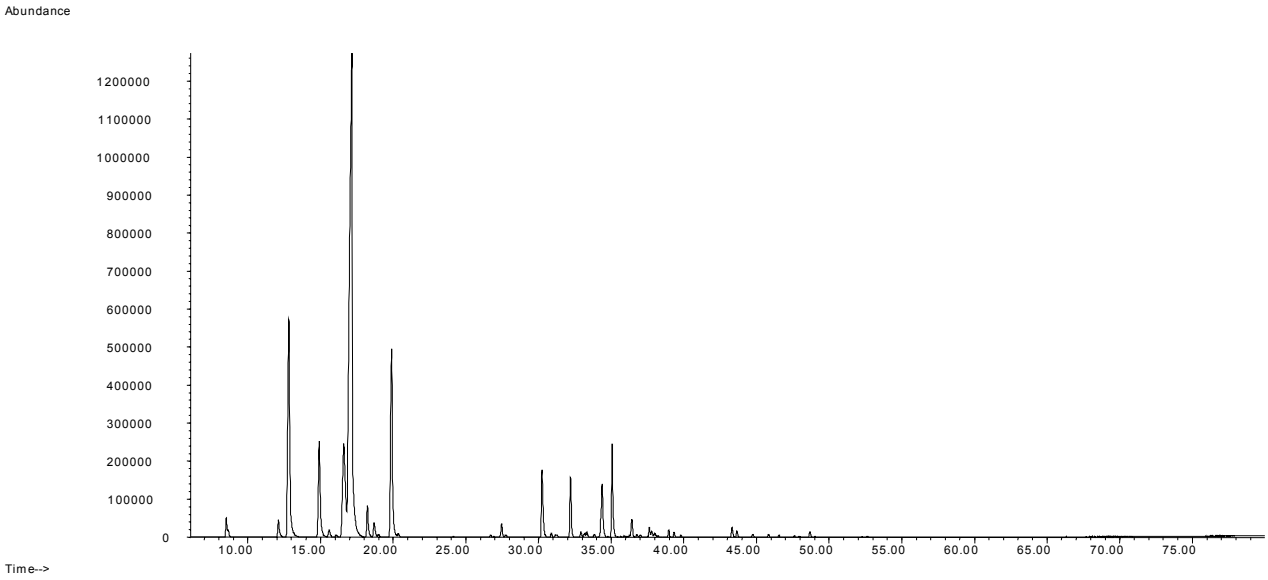


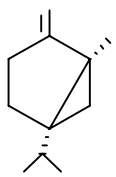
Figure 19 Total ion chromatogram of the hydrodistilled essential oil from *P. camphorata* var. c*amphorata* (individual C).

Table 6: Compounds identified in the essential oil of *P. camphorata* (three individual plants in a single population).

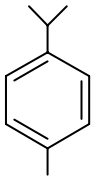
Rt	**RRI**	**Compound**	**Percentage composition**		
			***P. camphorata* (A)**	***P. camphorata* (B)**	***P. camphorata* (C)**
8.6	1032	α-pinene	0.5	0.2	0.7
7.7	1035	α -thujene	0.3		0.1
12.3	1118	β-pinene	1.1	0.7	0.9
12.8	1132	sabinene	9.1	7.1	12.7
14.9	1176	α -phelladrene	1.6	1.7	5.5
15.6	1188	α -terpinene		0.1	0.3
16.1	1195	dihydro 1,8-cineole		0.1	0.1
16.6	1203	limonene	5.0	3.8	7.7
17.0	1213	1,8-cineole	42.7	40.4	42.6
18.2	1246	Z-(β)-ocimene	1.0	0.8	1.5
18.7	1255	γ-terpinene	1.2	0.4	0.7
19.0	1266	(E)-β-ocimene	0.2	0.03	0.1
19.9	1280	ρ-cymene	17.1	21.1	10.0
20.4	1290	terpinolene	0.3		0.1
26.7	1450	*trans*-linalool oxide (furanoid)			0.1
27.5	1474	*trans*-sabinene hydrate	0.4	0.8	0.6

27.7	1450+	*cis*-linalool oxide (furanoid)		0.1	0.1
29.4	1512	dillethether		0.04	
30.3	1553	linalool	0.4	2.8	3.1
30.9	1571	*trans*-ρ-menth-2-en-1-ol	0.3	0.1	0.2
31.3	1586	pinocarvone	0.1		
32.2	1600	terpinen-4-ol	5.8	3.1	2.4
32.9	1632	*cis*-ρ-menth-2-en-1-ol			0.2
32.9	1639	*trans*-ρ-menth-2,8-dien-1-ol	0.4	0.4	
33.3	1648	myrtenal	0.1	0.2	0.1
33.3	1651	sabinaketone	0.3	0.2	0.2
33.9	1661	*trans*-pinocarveol	0.2	0.2	0.1
34.4	1671	methyl chavicol (estragol)	4.5	6.8	2.9
34.7	1700	limonen-4-ol	0.05	0.4	
35.1	1682	α -terpineol	2.3	4.8	3.9
36.4	1751	carvone	1.2	1.0	0.7
36.8	1765	geranyl acetone		0.1	0.1
37.0	1776	δ-cadinene	0.1		0.1
37.1	1797	γ-cadinene		0.1	
37.6	1804	ρ-methyl-acetophenone	0.5	0.5	
37.8	1802	myrtenol		0.2	0.1
37.8	1814	cumin aldehyde	0.4	0.4	0.2
38.0	1834	ρ-mentha-1,5-dien-7-ol	0.2		
39.0	1864	*trans*-carveol	0.4	0.3	0.2
39.3	1882	ρ-cymen-8-ol	0.2	0.4	0.2
39.8	2008	*cis*-carveol	0.1		0.1
43.4	2030	caryophyllene oxide	0.3	0.3	0.4
43.7	2012	methyl eugenol		0.4	0.2
43.7	2202	1-allyl-2,4-dimethoxybenzene	0.3		
44.7	2081	germacrene D-4-ol			0.1
44.8	2113	humulene-epoxide III		0.04	
45.8	2144	cumin alcohol	0.2	0.1	0.1
46.6	2187	spathulenol	0.1		0.1
47.6	2228	T-cadinol			0.04
48.7	2255	himachalol	0.1	0.1	0.2
49.1	2255	α-cadinol	0.2		
		Total	99.45	99.31	99.74

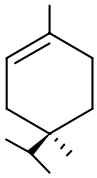
Major compounds identified in *Pteronia camphorata* essential oil



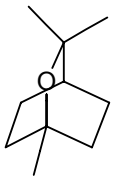
sabinene (8%)



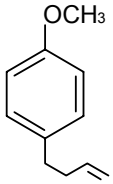
ρ-cymene (16%)



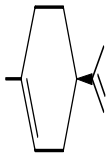
terpinen-4-ol (4%)



1, 8-cineole (41%)



methyl chavicol (5%)



limonene (6%)

4.3 *Pteronia elongata* Thunb.

Botanical description

Pteronia elongata is a rigid and much branched shrub. The leaves are oblong-lanceolate, keeled and are roughly ciliate. The flower heads are discoid and solitary at the branch tips. The florets are creamy-white. Flowering occurs between the months of August and November.

Geographic distribution

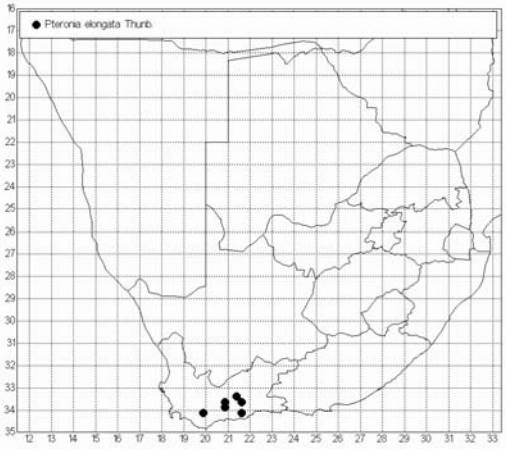


Figure 20: Geographical distribution of *P. elongate.* (SANBI)

Pteronia elongata is distributed between Oudtshoorn and Swellendam (figure 20). Plants were collected approximately 5km east of Prins Albert, next along the Klaarstroom road, South Africa.

Essential oil analysis

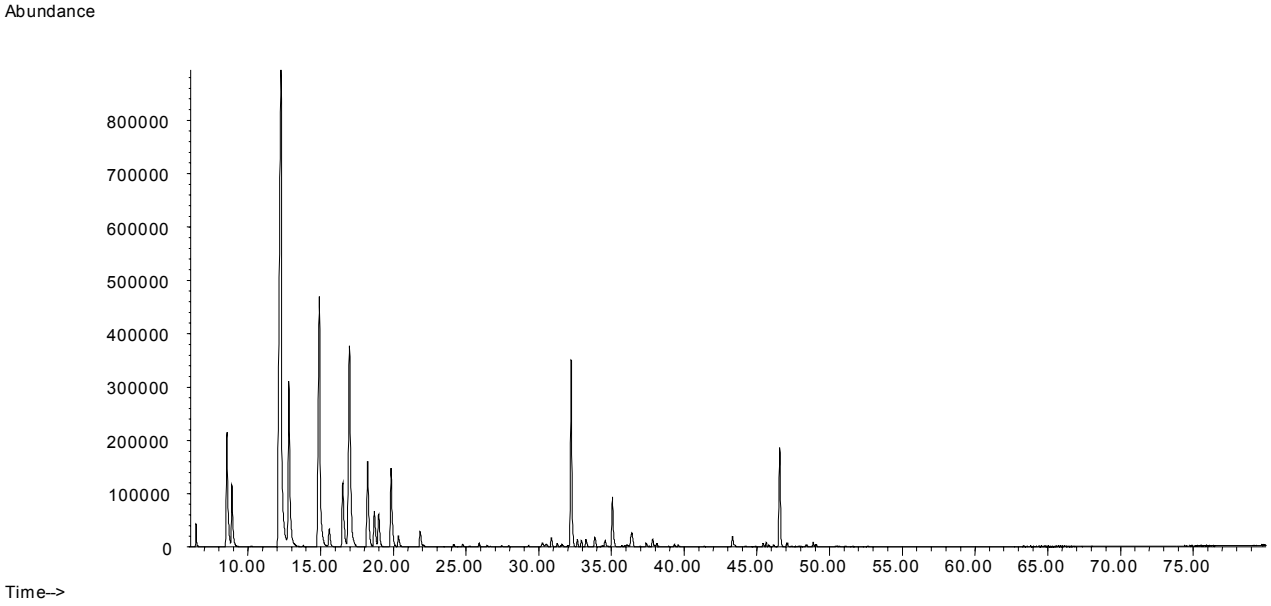


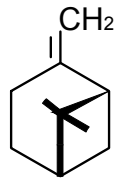
Figure 21: Total ion chromatogram of the hydrodistilled essential oil from *P. elongata.*

Table 7: Compounds identified in the essential oil of *Pteronia elongata.*

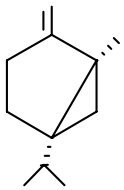
Rt	RRI	Compound	%
6.4	952	1-nonene	0.5
8.6	1032	α-pinene	4.5
8.9	1048	2-methyl-3-buten-2-ol	2.2
12.3	1118	β-pinene	28.6
12.8	1132	sabinene	7.8
14.9	1174	myrcene	12.4
15.6	1188	α-terpinene	0.7
16.6	1203	limonene	3.0
17.0	1218	β-phellandrene	11.2
18.2	1246	Z-(β)-ocimene	3.8
18.7	1255	γ -terpinene	1.5
19.0	1266	(E)- β -ocimene	1.4
19.9	1280	ρ-cymene	3.6
20.4	1290	terpinolene	0.5
21.9	1327	3-methyl-2-butenol	0.6
24.1	1382	*cis*-allo ocimene	0.1
24.7	1399	methyl-octanoate	0.1
25.9	1429	Perillene	0.1
26.5	1432	ethyl-octanoate	0.04

30.3	1553	Linalool	0.2
30.9	1571	*trans*- ρ -menth-2-en-1-ol	0.3
31.3	1586	Pinocarvone	0.1
31.7	1583	Nopinone	0.1
32.2	1600	terpinen-4-ol	7.0
32.9	1632	*cis*- ρ -menth-2-en-1-ol	0.2
33.3	1648	Myrtenal	0.3
33.9	1661	*trans*-pinocarveol	0.4
34.5	1682	cryptone	0.2
35.1	1755	α-terpineol	1.9
36.4	1786	bicyclogermacrene	0.8
37.4	1804	kessane	0.1
37.8	1804	myrtenol	0.3
38.2	1864	liguloxide	0.1
39.3	1868	ρ -cymen-8-ol	0.1
39.6	2008	(E)-geranyl acetone	0.1
43.4	2088	caryophyllene oxide	0.3
45.2	2098	1-epi-cubenol	0.1
45.4	2104	globulol	0.1
45.7	2113	viridiflorol	0.1
45.8	2144	cumin alcohol	0.1
46.6	2214	spathulenol	3.6
48.4		carvacrol	0.03
		Total	96.3

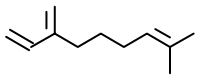
Major compounds identified in *Pteronia elongata* essential oil



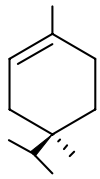
β-pinene (28%)



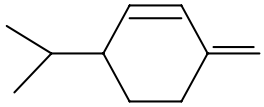
sabinene (8%)



myrcene (12%)



terpinen-4-ol (7%)



β-phellandrene (11%)

4.4 *Pteronia empetrifolia* DC.

Botanical description

Pteronia empetrifolia are rounded, erect, dense and branched shrubs (figure 22), with yellow to orange florets (figure 23), rising to approximately 0.5m off the ground. The leaves are green in colour, are connate and appear just below the flower heads. Abundance is occasional in the area.

Figure 22: *P empetrifolia* in habit (near Prins Albert).

Figure 23: Yellow flowers of *P. empetrifolia.*

Geographic distribution

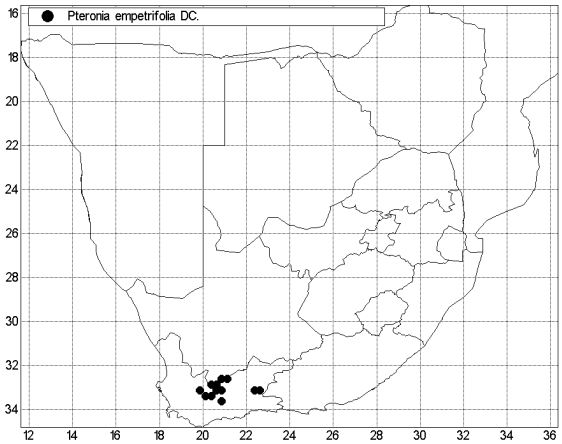


Figure 24: Geographical distribution of *P. empetrifolia.* (SANBI)

Pteronia empetrifolia is distributed in a fairly narrow range in the Southern Karoo (figure 24). The plant materials were collected approximately 5km east of Prins Albert, next to Klaarstroom road, South Africa.

Essential oil analysis

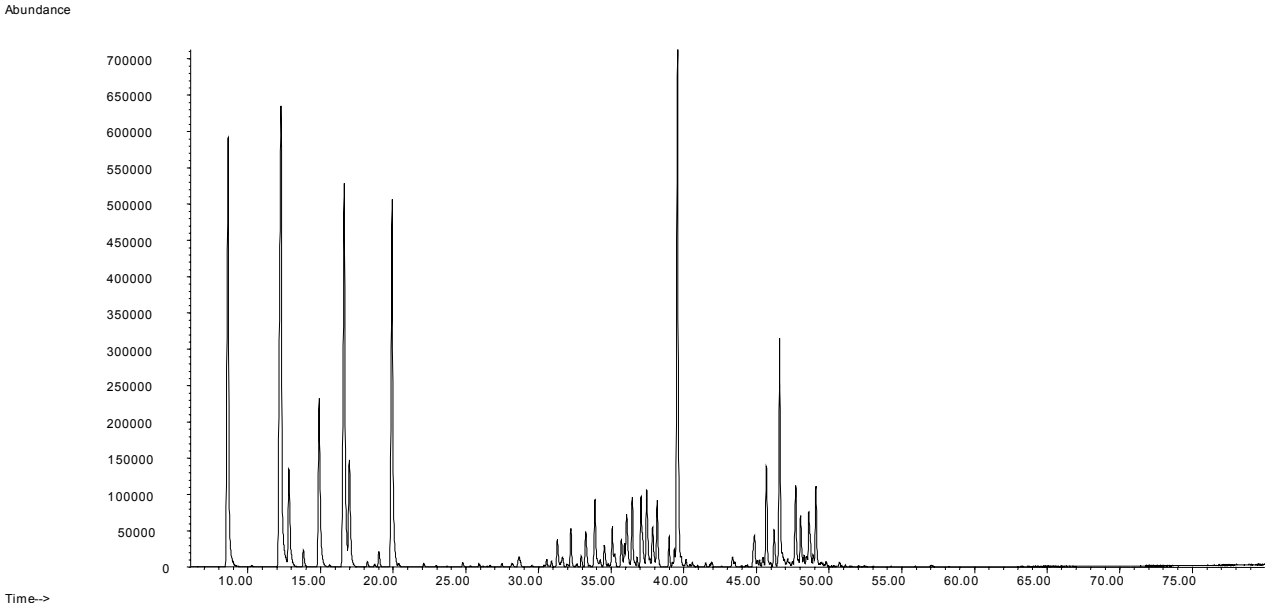


Figure 25: Total ion chromatogram of the hydrodistilled essential oil from *P. empetrifolia.*

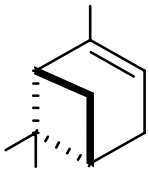
Table 8: Compounds identified in the essential oil of *Pteronia empetrifolia.*

Rt	RRI	Compound	%
8.6	1032	α-pinene	10.3
10.3	1076	camphene	0.03
12.3	1118	β-pinene	14.5
12.8	1132	sabinene	2.5
13.8	1142	undecene	0.4
14.9	1174	myrcene	4.7
16.6	1203	limonene	11.2
17.0	1213	1,8-cineole	2.5
18.2	1246	Z-(β)-ocimene	0.1
19.0	1266	(E)- β -ocimene	0.3
19.9	1280	ρ-cymene	9.8
20.4	1290	terpinolene	0.1
24.7	1399	methyl-octanoate	0.1
25.9	1429	perillene	0.1
27.5	1474	*trans*-sabinene hydrate	0.1
28.2	1479	bicycloelemene	0.1
28.7	1499	α-campholene aldehyde	0.4

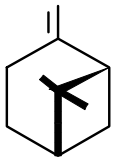
30.6	1562	isopinocamphene	0.1
30.9	1571	*trans*- ρ -menth-2-en-1-ol	0.1
31.3	1586	pinocarvone	0.6
31.7	1583	nopinone	0.3
32.0	1604	thymol methyl ether	0.1
32.2	1600	terpinen-4-ol	0.8
32.9	1632	*cis*- ρ -menth-2-en-1-ol	0.2
33.3	1648	myrtenal	0.8
33.9	1661	*trans*-pinocarveol	1.5
34.5	1683	*trans*-verbenol	0.5
34.7	1700	limonen-4-ol	0.04
35.1	1682	α-terpineol	0.8
35.7	1725	verbenone	0.7
36.1	1740	α-muurolene	1.2
36.4	1755	bicyclogermacrene	1.5
36.8	1758	*cis*-piperitol	0.2
37.0	1773	δ-cadinene	2.1
37.4	1786	kessane	1.5
37.8	1804	myrtenol	0.8
38.2	1804	liguloxide	1.4
39.0	1834	*trans*-carveol	0.5
39.2	1841	calamenene	0.1
39.3	1864	ρ-cymen-8-ol	0.2
39.6	1867	thymol acetate	11.0
40.1	1890	carvacryl acetate	0.1
40.4	1896	*cis*- ρ -menth-1(7),8-dien-2-ol	0.03
40.6	1900	epicubebol	0.1
40.9	1916	α-agarofuran	0.2
41.5	1941	α-calacorene	0.1
41.9	1957	cubebol	0.1
43.4	2008	caryophyllene oxide	0.2
44.9	2067	presilphiperfolan-8-ol	0.9
45.2	2088	1-epi-cubenol	0.1
45.7	2104	viridiflorol	1.9
46.2	2127	10-epi- γ -eudesmol	0.7
46.6	2144	spathulenol	4.7
47.7	2183	thymol	1.7

48.0	2209	T-muurolol	0.9
48.3	2214	torreyol	0.1
48.4	2232	carvacrol	0.1
48.6	2247	α-bisabolol	1.3
48.9	2255	*trans*-α-bergamotol	0.1
49.1	2255	α-cadinol	1.5
		Total	99.2

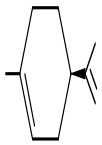
Major compounds identified in *Pteronia empetrifolia* essential oil



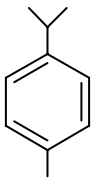
α-pinene (10%)



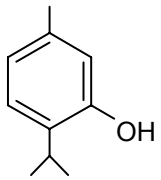
β-pinene (15%)



limonene (11%)



ρ-cymene (10%)



thymol (11%)

4.5 *Pteronia fasiculata* L *f.*

Botanical description

Pteronia fasiculata is a rigid shrub. The leaves are linear-lanceolate, rigidly coriaceous, and viscid and are crowded at the branch tips, are green in colour and are sticky to the touch. The flower heads are discoid, 1-flowered in tight round clusters and are bright yellow in colour. The stems are naked below the flower (figure 26). Plants are about 1.5m tall.

Figure 26: Yellow flowers and naked stems of *P. fasiculata.*

Geographical distribution

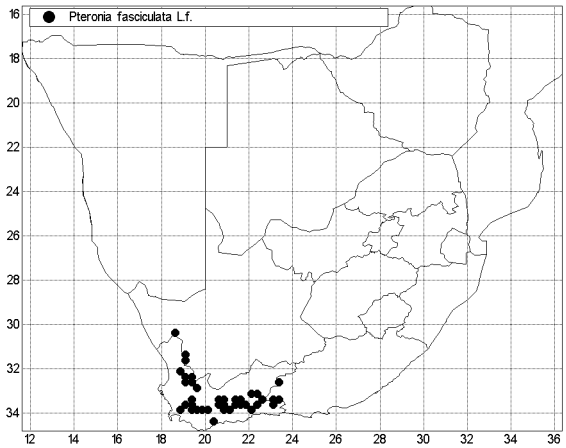


Figure 27: Geographical distribution of *P. fasiculata.* (SANBI)

Pteronia fasiculata is widely distributed in the Western Cape from the Cedarberg Mountains to Witteberg and Uniondale (figure 27). The material for the study was collected in Oudtshoorn, near Bakenskraal.

Essential oil analysis

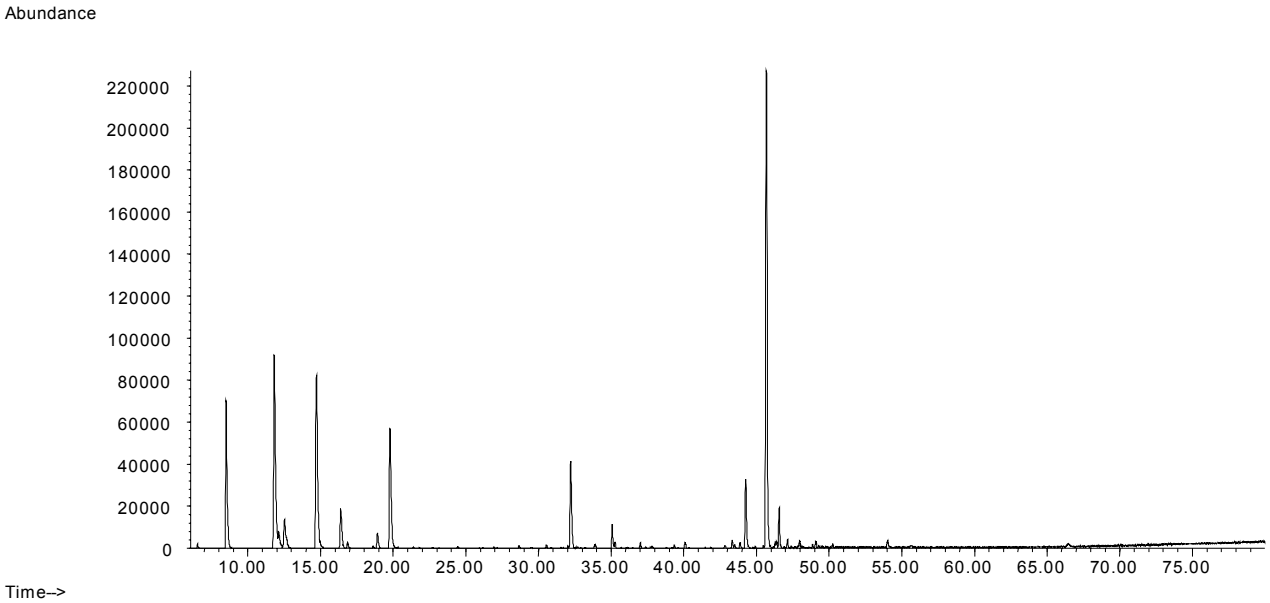
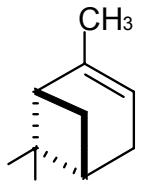


Figure 28: Total ion chromatogram of the hydrodistilled essential oil from *P. fasiculata.*

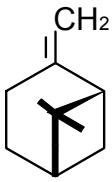
Table 9: Compounds identified in the essential oil of *Pteronia fasiculata*.

Rt	RRI	Compound	%
8.6	1032	α -pinene	8.7
12.3	1118	β-pinene	15.8
12.8	1132	sabinene	2.0
14.9	1174	myrcene	13.8
16.6	1203	limonene	2.7
17.0	1218	β-phellandrene	0.2
19.0	1266	(E)- β -ocimene	0.9
19.9	1280	ρ-cymene	9.2
32.2	1600	terpinen-4-ol	6.3
35.1	1682	α-terpineol	1.3
43.4	2008	caryophyllene oxide	0.3
44.2	2037	(E)-Nerolidol	4.0
45.7	2104	viridiflorol	31.1
46.6	2144	spathulenol	2.1
49.1	2255	α-cadinol	0.2
		Total	98.6

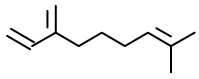
Major compounds identified in *Pteronia fasiculata* essential oil



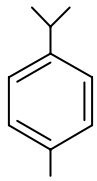
α-pinene (9%)



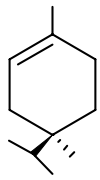
β-pinene (16%)



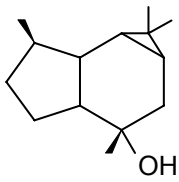
Myrcene (14%)



ρ-cymene (9%)



terpinen-4-ol (7%)



Viridiflorol (31%)

4.6 *Pteronia flexicaulis* L *f.*

Botanical description

Pteronia flexicaulis is a rigid shrub (figure 29). The leaves are subterate, viscid, connate below around the stems and clustered at the ends of branches. The flower heads occur in 1-3's around the branch tips, are also viscid, yellow in colour (figure 30) and are 20-25cm in length. The plants flower in November to December. Plants grow up to 1.5m tall. The plants are locally abundant.

Figure 29: *P. flexicaulis* in habit.

Figure 30: Flowers and leaves of *P. flexicaulis.*

Geographical distribution

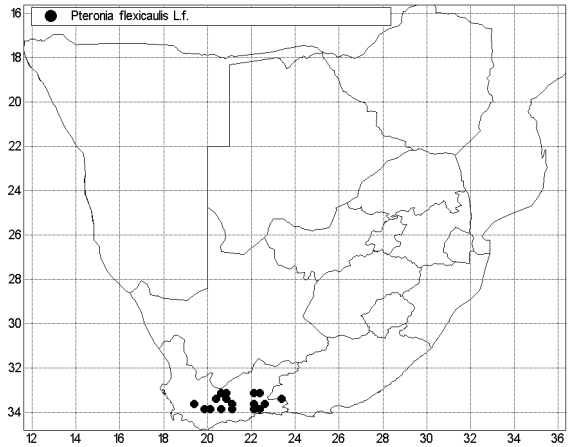


Figure 31: Geographical distribution of *P. flexicaulis.* (SANBI)

Pteronia flexicaulis is distributed from Ceres in the Western Cape to Oudtshoorn and the southern parts of the Karoo region (figure 31). Plant material was collected in Uniondale, at the southern foothills of Kamenassie Mountains near Daskop.

Essential oil analysis

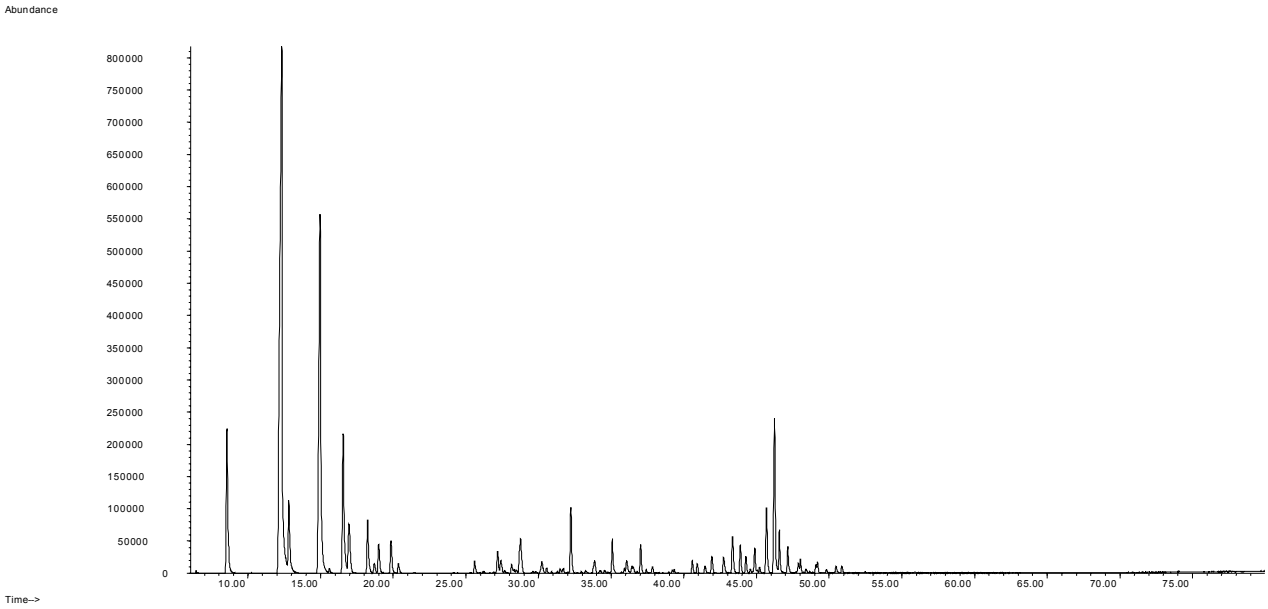


Figure 32: Total ion chromatogram of the hydrodistilled essential oil from *P. flexicaulis*.

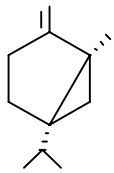
Table 10: Compounds identified in the essential oil of *Pteronia flexicaulis.*

Rt	RRI	Compound	%
6.4	952	1-nonene	0.04
8.6	1032	α-pinene	5.1
12.3	1118	β-pinene	32.1
12.8	1132	sabinene	2.8
14.9	1174	myrcene	17.6
15.6	1188	α-terpinene	0.5
16.6	1203	limonene	6.1
17.0	1218	β-phellandrene	2.2
18.7	1255	γ-terpinene	0.3
19.9	1280	ρ-cymene	1.3
20.4	1290	terpinolene	0.3
25.6	1406	presilphiperfol-7-ene	0.4
27.2	1452	7 β -(H)-silphiperfol-5-ene	0.8
27.4	1466	α-cubebene	0.5
28.3	1482	cyclosativene	0.1
28.8	1495	silphiperfol-5-ene	1.7
29.6	1435	β-bourbonene	0.5

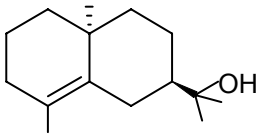
30.6	1562	isopinocamphene	0.2
30.9	1571	*trans*- ρ -menth-2-en-1-ol	0.04
31.3	1586	pinocarvone	0.1
31.5	1577	cascarilladiene	0.1
32.2	1600	terpinen-4-ol	2.3
32.9	1632	*cis*- ρ -menth-2-en-1-ol	0.04
33.3	1648	myrtenal	0.1
33.9	1661	(Z)-3-hexenyl hexenoate + alloaroma dendrene	0.6
34.2	1681	(Z)-3-hexenyl tiglate	0.04
34.5	1690	cryptone	0.03
35.1	1682	α-terpineol	1.1
35.7	1726	germacrene D	0.1
36.0	1743	α-cadinene	0.4
36.4	1755	bicyclogermacrene	0.2
37.0	1773	δ-cadinene	0.1
37.4	1786	Kessane	0.1
37.8	1804	myrtenol	0.2
39.2	1841	calamenene	0.1
39.3	1864	ρ-cymen-8-ol	0.1
40.9	1916	α-agarofuran	0.3
41.4	1924	silphiperfolan-7 β -ol	0.1
41.9	1957	cubebene	0.6
42.7	1976	cameroonan-7 α -ol	0.6
43.4	2008	caryophyllene oxide	1.3
43.9	2024	presilphiperfolan-9 α -ol	0.9
44.2	2037	(E)-Nerolidol	0.5
44.5	2049	prenopsan-8-ol	0.1
44.9	2065	presilphiperfolan-8-ol	0.9
45.1	2080	cubenol	0.1
45.2	2088	1-epi-cubenol	0.2
45.7	2104	viridiflorol	2.3
46.2	2127	10-epi- γ -eudesmol	5.4
46.6	2144	spathulenol	1.3
47.8	2187	copaborneol	0.3
48.0	2196	T-muurolol	0.4
49.2	2250	4- α -hydroxy-dihydro-agarofuran	0.3
49.9	2282	8,13-epoxy-15,16-dinorlabol-12-ene	0.1

50.5	2308	(Z)-9-tricosene	0.2
		Total	96.69

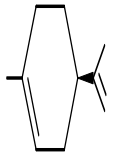
Major compounds identified in *Pteronia flexicaulis* essential oil



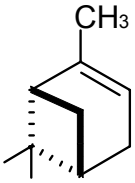
β-pinene (32%)



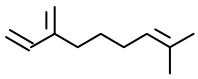
10-epi-γ-eudesmol (5%)



limonene (6%)



α-pinene (9%)



myrcene (18%)

4.7 *Pteronia glomerata* L *f.*

Botanical description

Pteronia glomerata is a small and rigid shrub usually twice as broad as high. Branches are dark, erect and thin. Buds are covered by a thin waxy layer as a protection from cold winters. Plants are approximately 0.5m tall. Plants are locally abundant.

Geographical distribution

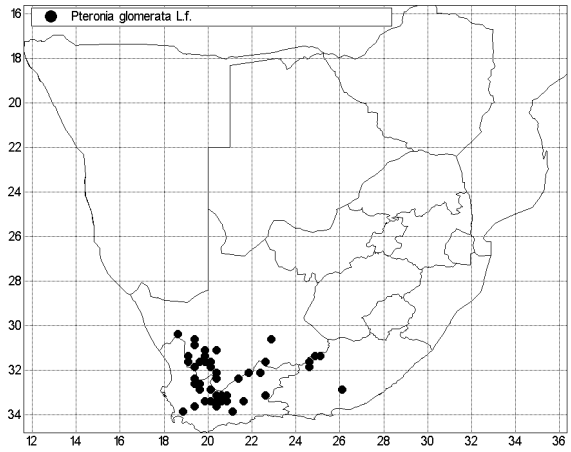


Figure 33: Geographical distribution of *P. glomerata.* (SANBI)

Pteronia glomerata is widespread in its distribution, which ranges from the northern area of the Western Cape to the Karoo region and as far east as the eastern and northern regions of the Eastern Cape (figure 33). The plants were collected approximately 10km west of Klaarstroom, near the Frisgewaagd farm.

Essential oil analysis

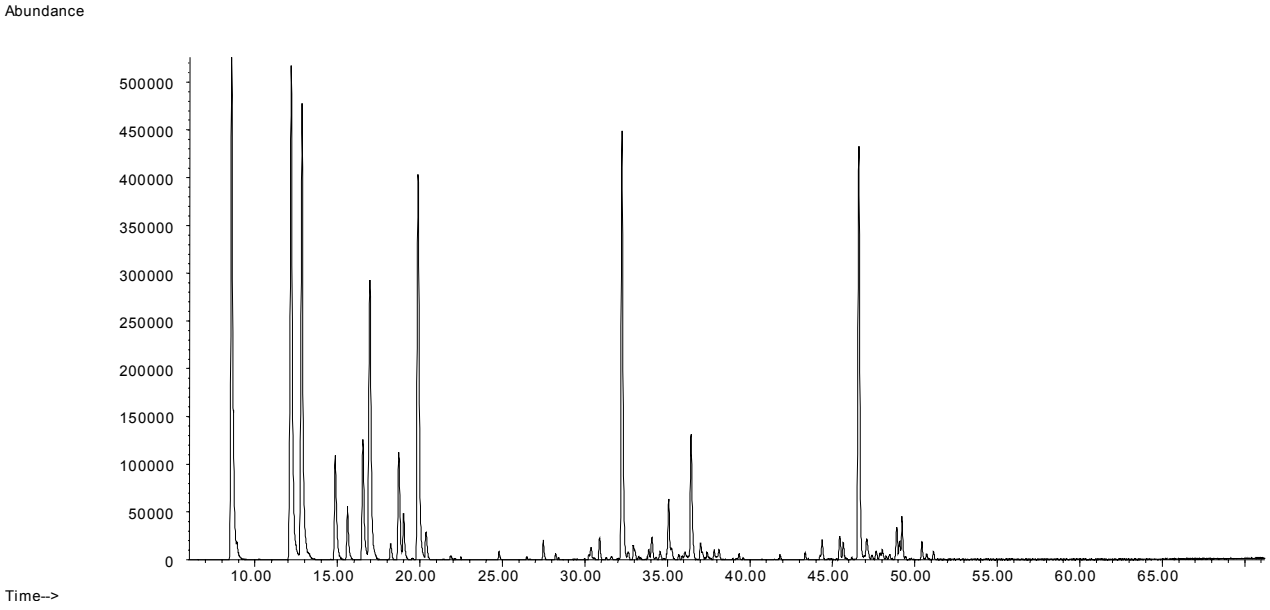


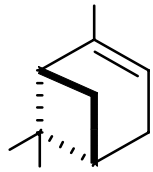
Figure 34: Total ion chromatogram of the hydrodistilled essential oil from *P. glomerata*.

Table 11: Compounds identified in the essential oil of *Pteronia glomerata.*

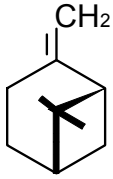
Rt	RRI	Compound	%
8.6	1032	α-pinene	10.2
7.7	1035	α-thujene	2.1
12.3	1118	β-pinene	14.3
12.8	1132	sabinene	13.3
14.9	1174	myrcene	2.8
15.6	1188	α-terpinene	1.3
16.6	1203	limonene	3.2
17.0	1213	1,8-cineole	7.7
18.2	1246	Z-(β)-ocimene	0.3
18.7	1255	γ -terpinene	2.5
19.0	1266	(E)- β -ocimene	1.1
19.9	1280	ρ-cymene	9.9
20.4	1290	terpinolene	0.6
21.9	1327	3-methyl-2-butenol	0.1
24.7	1399	methyl-octanoate	0.2
26.5	1432	ethyl-octanoate	0.1
27.5	1474	*trans*-sabinene hydrate	0.4

28.2	1479	bicycloelemene	0.1
30.3	1556	*cis*-sabinene hydrate	0.2
30.3	1553	linalool	0.1
30.9	1571	*trans*- ρ -menth-2-en-1-ol	0.4
31.7	1483	nopinone	0.1
32.2	1600	terpinen-4-ol	9.5
32.6	1661	aromadendrene	0.1
32.9	1632	*cis*- ρ -menth-2-en-1-ol	0.3
33.3	1648	myrtenal	0.04
33.9	1661	*trans*-pinocarveol	0.1
34.0	1659	γ -gurjunene	0.4
34.5	1689	*trans*-piperitol	0.2
35.1	1682	α-terpineol	1.4
35.3	1708	Ledene	0.1
36.1	1740	α-muurolene	0.1
36.4	1755	Bicyclogermacrene	2.8
37.0	1773	δ-cadinene	0.3
37.1	1776	γ-cadinene	0.1
37.4	1786	Kessane	0.5
37.8	1804	myrtenol	0.2
38.2	1804	liguloxide	0.2
39.3	1864	ρ-cymen-8-ol	0.1
41.8	1953	palustrol	0.1
44.4	2045	ledol	0.4
45.4	2098	globulol	0.4
45.7	2104	viridiflorol	0.3
46.6	2144	spathulenol	8.6
47.4	2170	*cis*- ρ -meth-3-en-1,2-diol	0.1
47.6	2187	T-cadinol	0.05
48.0	2196	T-muurolol	0.2
48.3	2209	torreyol	0.1
48.5	2218	isospathulenol	0.1
48.9	2247	*trans*-α-bergamotol	0.6
49.1	2255	α-cadinol	0.3
49.2	2264	intermedeol	0.8
50.4	2304	(2E,6Z) farnesol	0.2
		Total	99.79

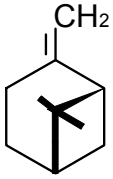
Major compounds identified in *Pteronia glomerata* essential oil



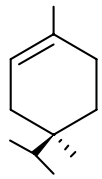
α-pinene (10%)



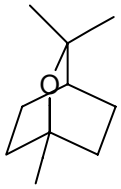
β-pinene (14%)



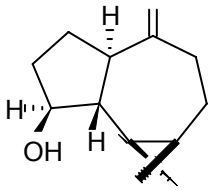
sabinene (13%)



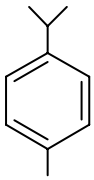
terpinen-4-ol (10%)



1,8-cineole (8%)



spathulenol (9%)



ρ-cymene (10%)

4.8 *Pteronia pallens* L *f.*

Botanical description

Pteronia pallens is a twiggy shrub. The leaves occur subterately, are viscid and connate below the stem. The flower heads are narrow with several occurring in crowded corymbs at the tip of the branches and are yellow to orange in colour (figure 35). The plant flowers from the months of September to December. The bracts are also minutely ciliate on the margins. Plants are up to 0.5m tall and are locally abundant.

Figure 35: Yellow florets and leaves of *P pallens.*

Geographical distribution

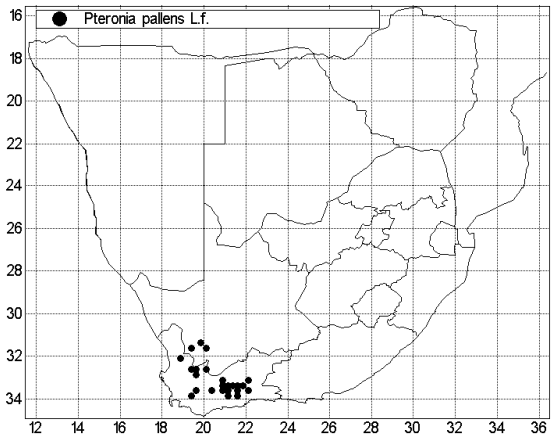


Figure 36: Geographical distribution of *P. pallens.* (SANBI)

Pteronia pallens is distributed from Calvinia to the Little Karoo (figure 36). The material was collected at Lategansvlei, Volmoed and between Oudtshoorn and Lategansvlei in the southern Cape.

Essential oil analysis

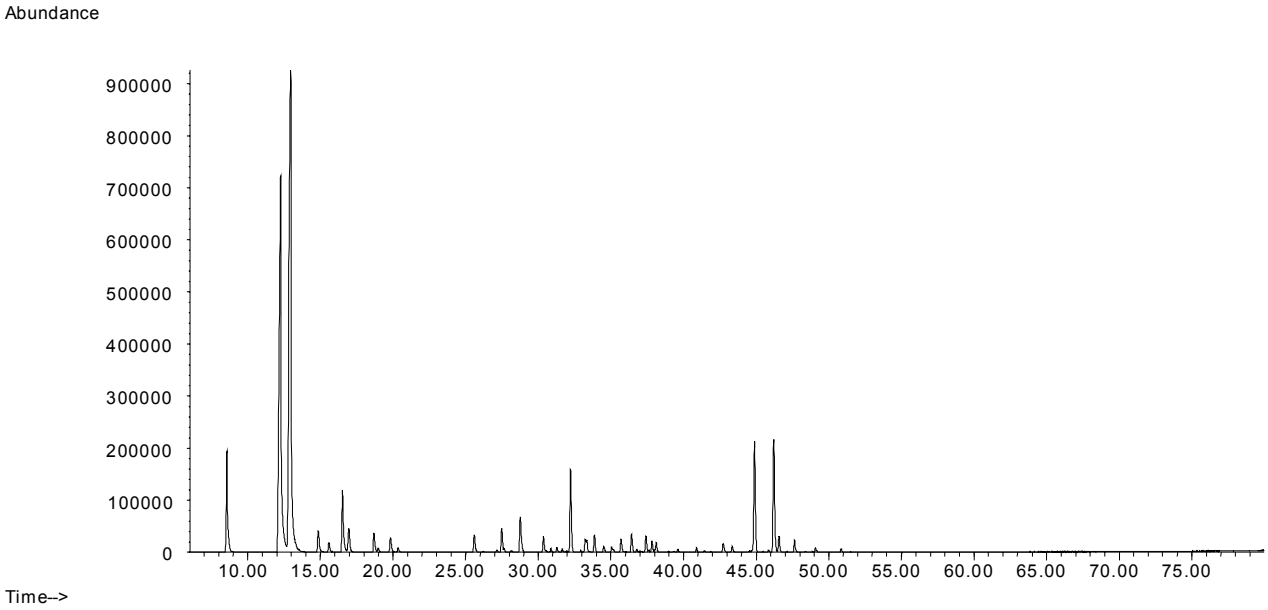


Figure 37: Total ion chromatogram of the hydrodistilled essential oil from *P. pallens* from Lategansvlei (individual A).

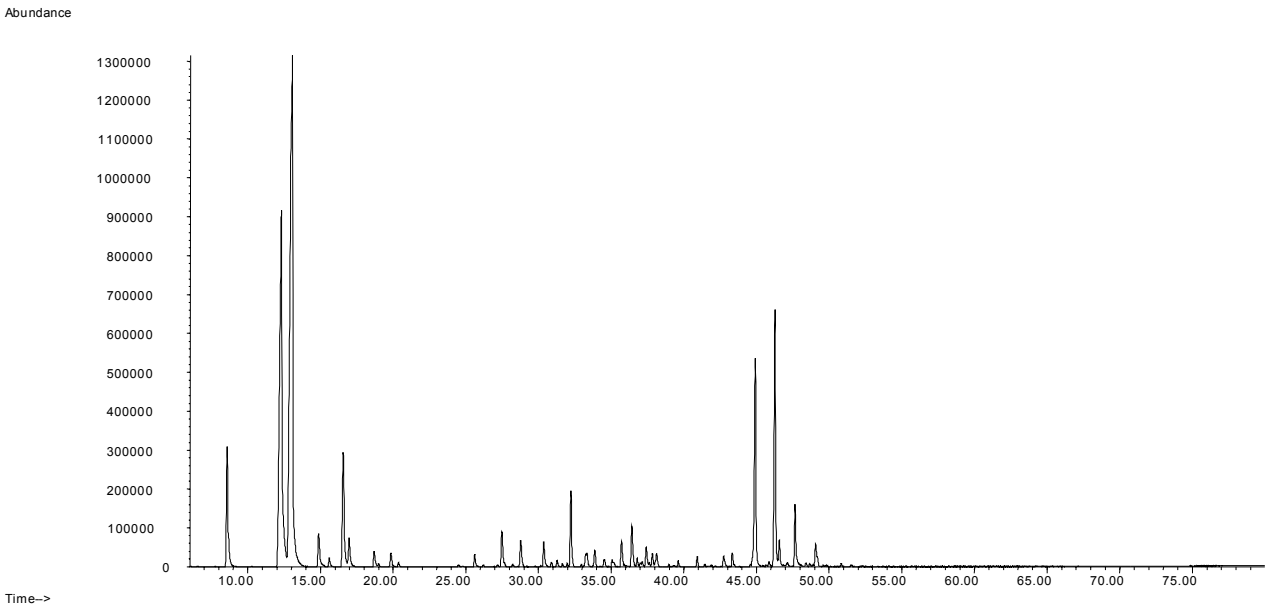


Figure 38: Total ion chromatogram of the hydrodistilled essential oil from *P. pallens* from Volmoed (individual B).

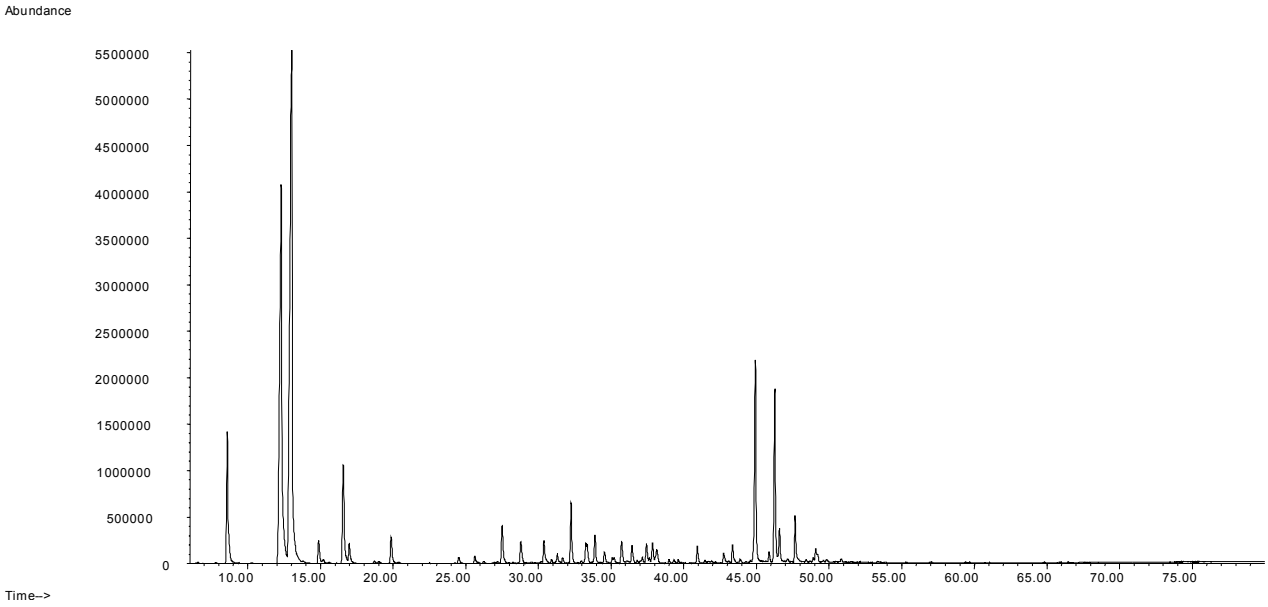


Figure 39: Total ion chromatogram of the hydrodistilled essential oil from *P. pallens* collected between Oudtshoorn and Lategansvlei (individual B).

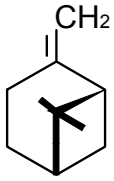
Table 12: Compounds identified in the essential oil of *P. pallens* (three individual plants in a single population).

Rt	RRI	Compound	Percentage composition		
			***P. pallens* (A) (Lategansvlei)**	***P. pallens* (B) (Volmoed)**	***P. pallens* (B) between Oudtshoorn and Lategansvlei**
8.6	1032	α-pinene	4.7	4.2	5.0
12.3	1118	β-pinene	26.9	21.6	22.5
12.8	1132	sabinene	33.6	32.2	30.8
14.9	1174	myrcene	1.1	1.3	0.9
15.6	1188	α-terpinene	0.7	0.3	0.6
16.6	1203	limonene	3.3	4.6	4.3
17.0	1218	β-phellandrene	1.2	1.2	0.8
18.2	1246	Z-(β)-ocimene		0.4	
18.7	1255	γ-terpinene	0.9		0.1
19.0	1266	(E)-β-ocimene	0.9	1.0	1.4
19.9	1280	ρ-cymene	0.7	0.5	1.1
20.4	1290	terpinolene	0.2	0.1	0.03
24.5	1384	α-pinene oxide			0.2

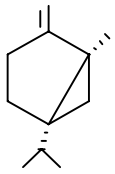
25.6	1406	presilphiperfol-7-ene	0.8	0.4	0.3
26.2	1424	7-α-(H)-silphiperfol-5-ene	0.04		0.1
26.8	1451	β-thujone			0.01
26.9	1458	*cis*-1,2-limonene epoxide			0.04
27.2	1452	7-β-(H)-silphiperfol-5-ene	0.1		0.07
27.5	1474	*trans*-sabinene hydrate	1.0	1.1	1.4
28.2	1479	bicycloelemene		1.1	0.01
28.7	1499	α-campholene aldehyde			0.01
28.8	1495	silphiperfol-5-ene	1.7	0.9	0.9
29.4	1512	dillethether			0.02
30.3	1556	*cis*-sabinene hydrate	0.7	0.8	0.8
30.9	1571	*trans*- ρ -menth-2-en-1-ol	0.1	0.1	0.1
31.3	1586	pinocarvone	0.2	0.1	0.4
31.7	1583	nopinone	0.1	0.1	0.2
32.2	1600	terpinen-4-ol	2.2	3.7	2.4
33.3	1648	myrtenal	0.3	0.3	0.6
33.3	1651	sabinaketone	0.3		0.6
33.9	1661	*trans*-pinocarveol	0.8	0.5	1.0
34.3	1678	*cis*-ρ-menth-2,8-dien-1-ol			0.04
34.5	1683	*trans*-verbenol	0.3		
34.5	1687	α-humulene		0.3	0.5
35.1	1682	α-terpineol	0.2	0.1	0.2
35.7	1726	germacrene D	0.6	0.8	0.9
36.1	1740	valencene			0.1
36.4	1755	bicyclogermacrene	0.8	1.3	0.7
36.8	1765	geranyl acetone	0.04	0.2	0.1
37.0	1773	δ-cadinene	0.1	0.03	0.1
37.1	1776	γ-cadinene	0.05	0.1	0.2
37.4	1786	kessane	0.7	0.6	0.7
37.6	1783	*cis*-sabinol		0.1	
37.8	1804	myrtenol	0.5	0.4	0.7
38.2	1804	liguloxide	0.5	0.5	0.6
39.0	1834	*trans*-carveol		0.3	0.1

39.3	1864	ρ-cymen-8-ol	0.03		0.1
39.6	1868	(E)-geranyl acetone	0.1	0.2	0.1
40.9	1916	α-agarofuran	0.2	0.3	0.6
41.4	1924	silphiperfolan-7-β-ol	0.1	0.1	0.1
42.7	1976	cameroonan-7-α-ol	0.4	0.4	
43.1	2001	isocaryophyllene oxide			0.05
43.4	2008	caryophyllene oxide	0.2	0.4	0.7
44.2	2037	(E)-Nerolidol			0.02
44.5	2049	prenopsan-8-ol	0.04		0.1
44.9	2065	presilphiperfolan-8-ol	5.1	7.5	7.9
45.4	2098	globulol			0.01
45.8	2113	cumin alcohol	0.1	0.1	0.3
46.2	2127	10-epi-γ-eudesmol	5	8.8	6.4
48.9	2247	*trans*-α-bergamotol			0.12
49.1	2255	α-cadinol		0.9	0.3
49.1	2257	β-eudesmol	0.2		
49.9	2282	8,13-epoxy-15,16-dinorlabol-12-ene			0.1
		Total	99.9	99.6	95.24

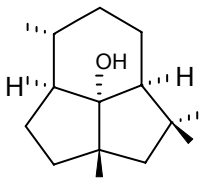
Major compounds identified in *Pteronia pallens* essential oil



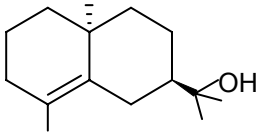
β-pinene (24%)



sabinene (32%)



presilphiperfolan-8-ol (7%)



10-epi-γ-eudesmol (7%)

Research note

Three samples of *Pteronia pallens* from different localities were analysed. There were negligible differences in the essential oil composition.

4.9 *Pteronia paniculata* Thunb.

Botanical description

Pteronia paniculata is an aromatic, much branched bushy shrub, which grows up to one meter tall (figure 40). Flowers are bright golden yellow and leaves are dark green. The flower heads are narrow, and are in several crowded corymbs at branch tips (figure 41). Flowering occurs between November to January.

Figure 40: Bushy shrub of *P. paniculata.*

Figure 41: Crowded corymbs of *P. paniculata.*

Geographical distribution

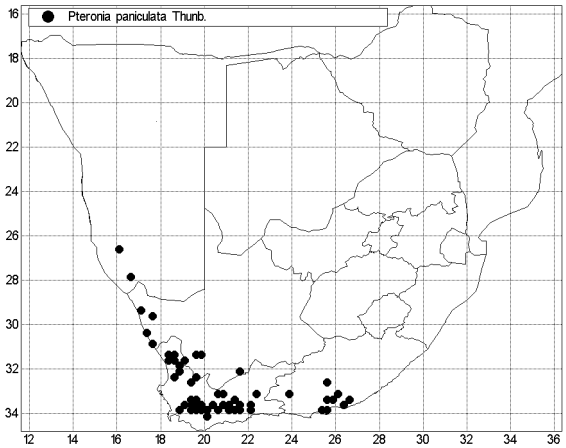


Figure 42: Geographical distribution of *P. paniculata.* (SANBI)

Pteronia paniculata is widely distributed and is found from Namibia to Port Elizabeth (figure 42). Plant material was collected from Oudtshoorn, near Bakenhoogte.

Essential oil analysis

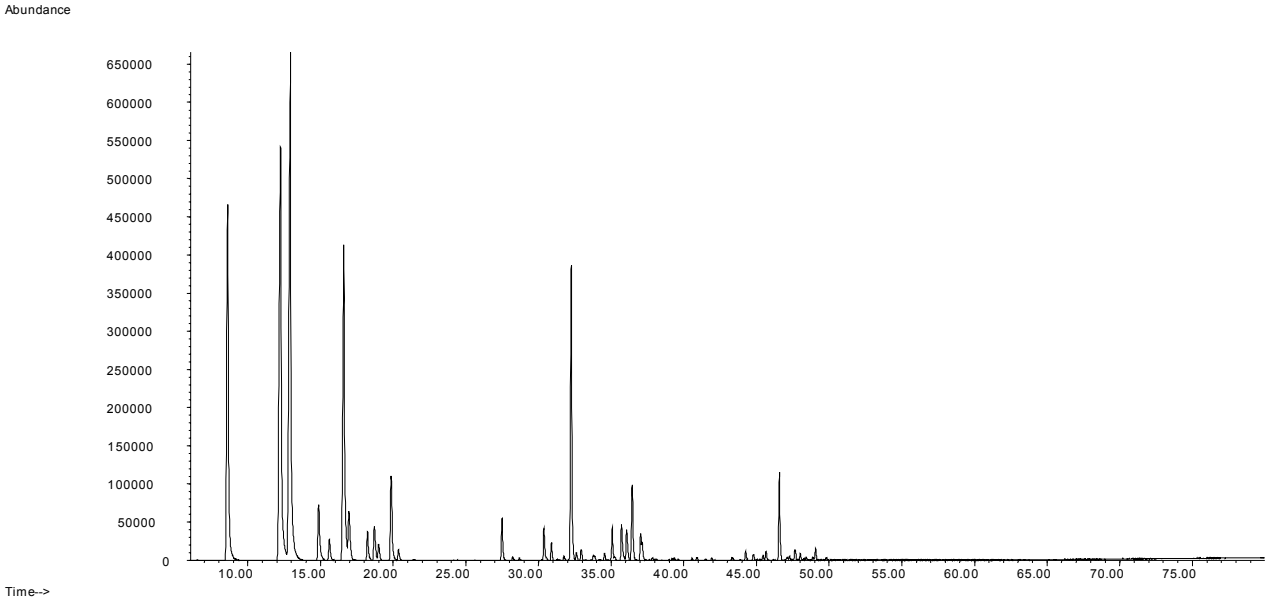


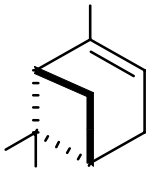
Figure 43: Total ion chromatogram of the hydrodistilled essential oil from *P. paniculata* (B).

Table 13: Compounds identified in the essential oil of *Pteronia paniculata.*

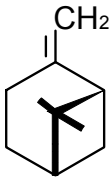
Rt	RRI	Compound	%
8.6	1032	α-pinene	12.3
12.3	1118	β-pinene	19.1
12.8	1132	sabinene	22.2
14.9	1174	myrcene	2.1
15.6	1188	α-terpinene	0.7
16.6	1203	limonene	12.8
17.0	1218	β-phellandrene	1.9
18.7	1255	γ -terpinene	1.1
19.0	1266	(E)- β -ocimene	0.5
19.9	1280	ρ-cymene	2.9
20.4	1290	terpinolene	0.3
27.5	1474	*trans*-sabinene hydrate	1.2
28.2	1479	bicycloelemene	0.1
30.3	1556	*cis*-sabinene hydrate	1.0
30.9	1571	*trans*- ρ -menth-2-en-1-ol	0.5
32.2	1600	terpinen-4-ol	9.2
32.6	1661	aromadendrene	0.2
32.9	1632	*cis*- ρ -menth-2-en-1-ol	0.3

35.1	1682	α-terpineol	1.6
35.7	1726	germacrene D	1.1
36.1	1743	eremophilene	1.0
36.4	1755	bicyclogermacrene	2.4
37.0	1773	δ-cadinene	0.8
39.2	1841	calamenene	0.04
39.3	1864	ρ-cymen-8-ol	0.1
40.6	1900	epicubebol	0.04
41.9	1957	cubebol	0.1
44.2	2037	(E)-Nerolidol	0.2
44.7	2202	germacrene D-4-ol	0.1
45.4	2098	globulol	0.1
45.7	2104	viridiflorol	0.2
46.6	2144	spathulenol	2.3
47.6	2187	T-cadinol	0.3
48.0	2196	T-muurolol	0.2
48.4	2214	carvacrol	0.1
49.1	2255	α-cadinol	0.3
		Total	98.38

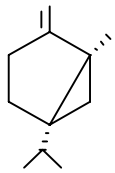
Major compounds identified in *Pteronia paniculata* essential oil



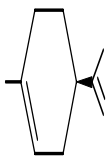
α-pinene (12%)



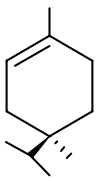
β -pinene (19%)



sabinene (22%)



limonene (13%)



terpinen-4-ol (9%)

4.10 *Pteronia viscosa* Thunb.

Botanical description

Pteronia viscosa is a rigid, round and erect shrub (figure 44). The flowers occur singly at the tip of the branch, are yellow in colour and sticky. The leaves are green and connate and are just below the flower heads (figure 45). The stems below the leaves are naked. Plants grow up to 1.0m tall and are locally abundant.

Figure 44: Rigid, erect shrub of *P. viscosa.*

Figure 45: Flowers and leaves of *P. viscosa.*

Geographical distribution

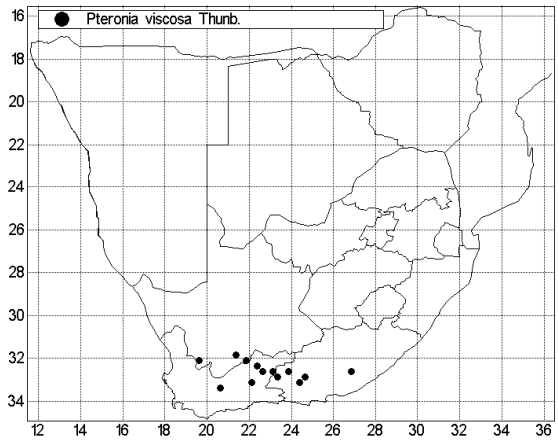


Figure 46: Geographical distribution of *P. viscosa.* (SANBI)

Pteronia viscosa although abundant in its habitat, has a fairly narrow distribution range in the upper, Little Karoo (figure 46). Plants were collected approximately 8km north of Prins Albert next along the Merweville road.

Essential oil analysis

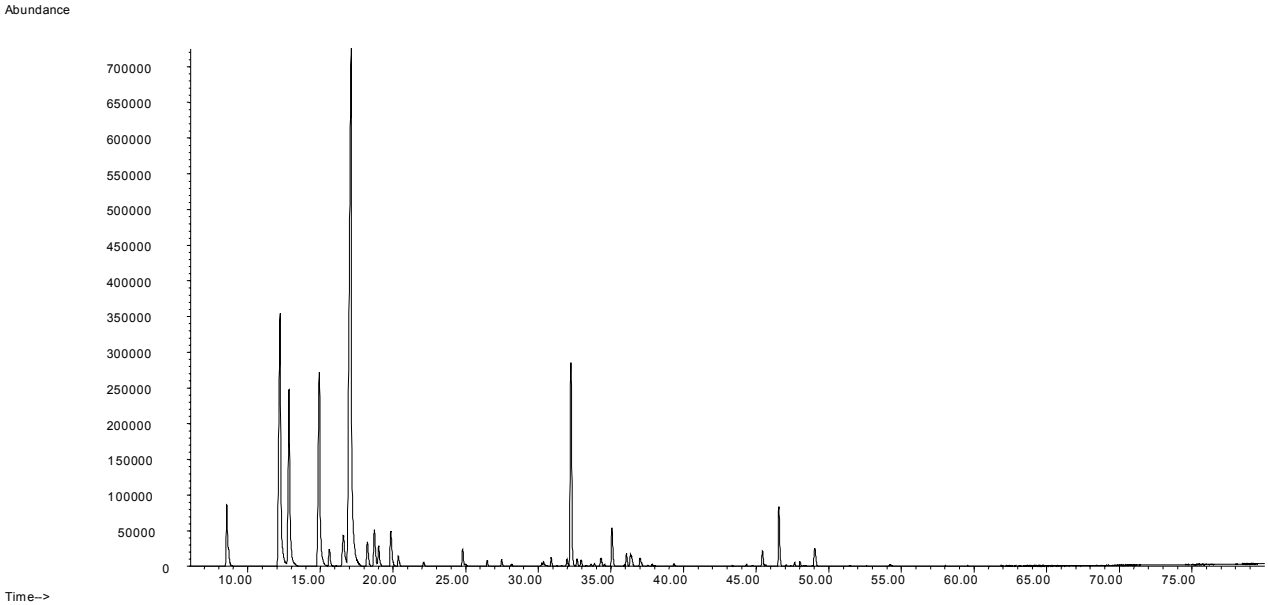


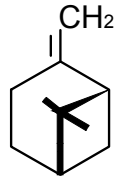
Figure 47: Total ion chromatogram of the hydrodistilled essential oil from *P. viscosa*.

Table 14: Compounds identified in the essential oil of *Pteronia viscosa*.

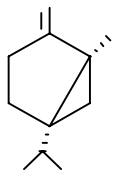
Rt	RRI	Compound	%
8.6	1032	α-pinene	2.4
12.3	1118	β-pinene	14.9
12.8	1132	sabinene	9.0
14.9	1174	myrcene	10.4
15.6	1188	α-terpinene	0.7
16.6	1203	limonene	2.7
17.0	1218	β-phellandrene	0.2
17.0	1213	1,8-cineole	37.0
18.7	1255	γ-terpinene	1.7
19.0	1266	(E)- β -ocimene	0.9
19.9	1280	ρ-cymene	1.7
20.4	1290	terpinolene	0.4
24.7	1399	methyl-octanoate	0.7
26.5	1432	ethyl-octanoate	0.1
27.5	1474	*trans*-sabinene hydrate	0.2
30.3	1553	linalool	0.1
30.9	1571	*trans*- ρ -menth-2-en-1-ol	0.3
32.2	1600	terpinen-4-ol	8.4
32.9	1632	*cis*- ρ -menth-2-en-1-ol	0.2

33.9	1661	*trans*-pinocarveol	0.1
34.5	1689	*trans*-piperitol	0.1
35.1	1682	α-terpineol	1.5
37.0	1773	δ-cadinene	0.3
39.3	1864	ρ-cymen-8-ol	0.1
44.3	2041	tridecenyl acetate	0.1
46.6	2144	spathulenol	2.2
47.6	2187	T-cadinol	0.1
48.0	2196	T-muurolol	0.1
49.1	2255	α-cadinol	0.9
		Total	98.5

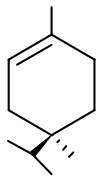
Major compounds identified in *Pteronia viscosa* essential oil



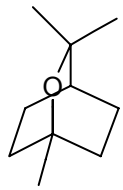
β-pinene (15%)



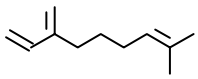
sabinene (9%)



terpinen-4-ol (8%)



1,8-cineole (37%)



myrcene (10%)

CHAPTER 5: Conclusion

This report has for the first time chromatographically recorded the essential oil composition of 10 species of *Pteronia*. Essential oils analysis, with the use of cluster analysis has revealed 2 distinct sub-groupings in the genus *Pteronia*. The results also show little chemical variation within species, regardless if they were collected from geographically different area or different specimens from within the same locality. Furthermore, rare compounds such as presilphiperfolol-7-ene, 7-α-(H)-silphiperfol-5-ene, 7-α-(H)- silphiperfol-5-ene, 7-β-(H)-silphiperfol-5-ene, α-campholene aldehyde, silphiperfol-5-ene, camaroonan-7-α-ol, silphiperfol-7- β -ol, presilphiperfolan-9- α -ol and presilphiperfolan-8-ol (a major compound in *Pteronia pallens*) were recorded. These compounds have only been recorded once before in the essential oils of *Eryophyllen staechadifolium* (Kőnig *et al*. 1997). The following monoterpenes were found to be present in the oil of all species analysed; α-pinene, β-pinene, sabinene, limonene, ρ-cymene, α-terpineol and terpinen-4-ol.

Many species of *Pteronia* display antimicrobial properties, as reflected in the disc diffusion results. The most potent species were *Pteronia fasiculata, P. adenocarpa, P. flexicaulis* and *P. elongata,* showing potential for therapeutic doses at the concentrations they inhibit growth of especially the Gram-positive organisms (*S. aureus, B. cereus and B. subtilis*).

The antimicrobial results obtained from the various tests confirm the antimicrobial activity and forms a scientific basis for the ethnobotanical use of *Pteronia* species.

Recommendations

With the antimicrobial results obtained from this study, further investigations of the exudates secreted from *P. fasiculata, P. adenocarpa, P. flexicaulis* and *P. elongata* would be warranted. There may be room on the market for these compounds, as they are specifically more active against the Gram-positive bacteria, which have shown to becoming more resistant to antibiotics available on the market today. There is also much to learn about essential oils before they can be used confidently to treat infectious borne diseases. This includes obtaining the knowledge of absorption rates, their pharmaco-kinetics and pharmaco-dynamics as well as their toxicity in humans. The toxicity in the ovine animal populations has

been well documented in the literature; especially that *Pteronia pallens* is hepatotoxic (Milton, 1995) and also extremely unpalatable to them (Watt and Breyer-Brandwijk, 1997). This makes it particularly interesting to the agricultural sector to use in the protection of there crops i.e. a possible natural protection against grazing and a possible insecticide.

In this process safety remains the most important starting point and efficacy would just now be a matter of validation. It will be in the interest of all concerned whether it be the researches, or possibly down the line, the pharmaceutical giants of industry or the agricultural community, if these compounds are further investigated.

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