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Pteronia divaricata (Asteraceae): A newly recorded Cape herbal medicine

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Abstract

The first ethnobotanical data on *Pteronia divaricata* is presented, which shows that the plant is an important traditional Cape medicine, especially in the Cederberg region (Western Cape Province) and in the Middelpos and Nieuwoudtville districts of the Northern Cape Province. Surprisingly, not a single publication could be found that records any uses for the species. We accurately documented nine separate anecdotes, which include various medicinal uses (for the treatment of colds, fever, influenza, stomach pain, diarrhoea, back pain, chest ailments, high blood pressure and tuberculosis). The novel data also includes seven previously unrecorded vernacular names, namely *flip-se-bos*, *inflammasiebos*, *pylbos*, *dassiebos*, *dassiepisbos*, *perdebos* and *boegabos*. Since the species is poorly known, its general morphology, leaf anatomy and essential oil composition were studied. Oil is produced in secretory ducts along the midribs below the main vascular bundle; secretory trichomes are also present on the leaf surface. The oil is relatively complex and contains a combination of sabinene, myrcene, β-caryophyllene and bicyclogermacrene as main compounds with smaller amounts of limonene, *p*-cymene, tetradecane, pentadecane, terpinen-4-ol and δ-cadinene. Dichloromethane extracts exhibited antibacterial activity (especially against *Bacillus cereus*) at MIC values as low as 1.0 mg/ml. Other solvent extracts and the essential oils were less active.

Keywords: Asteraceae; Essential oil; Ethnobotany; Leaf anatomy; MIC values; *Pteronia divaricata*

1. Introduction

The genus *Pteronia* L. comprises some 70 species of woody perennials sub-endemic to southern Africa (Harvey and Sonder, 1865; Hutchinson and Phillips, 1917; Leistner, 2000). Most of the species are aromatic and seven of them have published records of medicinal uses. These are *P. adenocarpa* Harv., *P. camphorata* (L.) L., *P. incana* (Burm.) DC., *P. lucilioides* DC., *P. onobromoides* DC., *P. succulenta* Thunb. and *P. stricta* Aiton.

Recent publications (Hulley et al., 2010; Viljoen et al., 2010) have shown that *Pteronia* species are poorly known and in need of further study, especially since several of them are of ethnobotanical and toxicological interest.

During recent ethnobotanical field surveys, several medicinal anecdotes were recorded for *Pteronia divaricata* (P.J. Bergius) Less. (Table 1). Surprisingly, this species has not yet been recorded as a medicinal plant in the literature (Arnold et al., 2002; Watt and Breyer-Brandwijk, 1962) despite its local importance in the Western and Northern Cape Provinces of South Africa. It is one of the most common shrubs in the dry parts of the winter rainfall region. The known geographical distribution of the species in South Africa (based on the herbarium collections of BOL, NBG and PRE) is shown in Fig. 1. The plants are up to 1.8 m high (Fig. 2) and summer-deciduous. The broad, trinerved and finely scabrid leaves emerge in winter (around June) and flowering occurs from September to November. Clusters of small, bright yellow flower

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Table 1
Ethnobotanical anecdotes recorded for *Pteronia divaricata*.

Date	Locality	Anecdote	Vernacular name	Source of information	Voucher specimen (all in JRAU)
19-08-1997	Oorlogskloof, Nieuwoudtville district	Used to treat colds, stomach pain and backache; an important medicine; "use in winter; too strong when in flower"	*Pennebos* (stems can puncture a bakkie tyre)	Willem "Blikkies" Steenkamp	*B.-E. Van Wyk & A.M. Viljoen 3700*
07-09-2008	Wupperthal	Used to treat inflammation, gastric fever, colds and tuberculosis	*Inflammasiebos*, *flip-se-bos*	Amelia Koopman (née Jooste)	*A. Koopman s.n. sub B.-E. Van Wyk 4325*
15-12-2008	Renosterhoek (near Eendekuil)	Stems sharpened and used as arrows	*Pylbos* ("arrow bush")	Johannes Hekter	*B.-E. & M. Van Wyk 4306*
03-07-2009	Citrusdal ("Modderfontein se dam")	Used to treat backache	*Dassiepisbos*	William Peter ex Willem Hanekom	No voucher
24-09-2009	Middelpos	Dry leaves are mixed with tobacco and smoked to treat chest ailments	*Boegabos*	Jakop Tromp	*B.-E. Van Wyk, J. De Beer & P.M. Tilney 4401*
04-10-2009	Brugkraal/Grasvlei near Wupperthal	Used to treat diarrhoea, fever, colds and influenza. Mixed with *wynruik* [*wynruit*, *Ruta graveolens*] and *wildeals* [*Artemisia afra*] to treat fever and colds	*Flip-se-bos*	Lydia Ockhuis	No voucher
04-10-2009	Agterstevlei near Wupperthal	Used medicinally for fever	*Flip-se-bos*	Piet Horing	No voucher
04-10-2009	Agterstevlei near Wupperthal	Used to treat high blood pressure and tuberculosis	*Flip-se-bos*	Johanna Horing	No voucher
04-10-2009	Kleinvlei near Wupperthal	Used for all pains, especially stomach pain, and lung ailments such as coughs and colds	*Flip-se-bos*	Johanna Zimri	*B.-E. van Wyk, I.M. Hulley & P.M. Tilney 4442*

heads are congested towards the branch ends and are followed by an abundance of fluffy seeds (ripening during December, at which time the leaves are shed). *P. divaricata* was described and illustrated in four of the Botanical Society's Wild Flower Guides (Le Roux and Schelpe, 1981, 1988; Manning and Goldblatt, 1996, 1997).

The aims of our study were (1) to document accurately, for the first time, indigenous knowledge about the uses of this species; (2) to explore the leaf anatomy, focussing on the secretory structures; (3) to determine, for the first time, the chemical composition of the essential oil; and (4) to investigate possible antimicrobial activity.

2. Material and methods

2.1. Materials studied

The material was collected from five localities, numbered 1 to 5 (from north to south) in Fig. 1: (1) Van Rhyns Pass [31° 19° AC], (2) Lamberts Bay [32° 18° AB], (3) Wupperthal near Clanwilliam [32° 19° CB], (4) Renosterhoek near Eendekuil and Piquetberg [32° 18° BD] and (5) Yzerfontein [33° 18° AD]. Voucher specimen details are presented in Table 2. The samples were carefully air-dried.

2.2. Anatomical procedures

Fresh leaf material was preserved in the field in formalin-acetic acid-alcohol (FAA). Small portions of the middle of the lamina were cut and treated using the glycol methacrylate (GMA) method of Feder and O'Brien (1968). Briefly, this procedure involves dehydration through a graded series of alcohol, infiltration in GMA and embedding in gelatin capsules, followed by polymerization in an oven (24 h at 60 °C). Transverse sections, 3–5 µm thick, were cut with an ultramicrotome and glass knives. Staining was done with Schiff's reagent and toluidine blue. After permanent mounting using Entellan, the microscope slides were observed under a light microscope.

2.3. Distillation and analysis of essential oil

A selection of 11 air-dried leaf samples from three localities (Table 2) was hydrodistilled for 180 min in a Clevenger-type apparatus. The oil samples were weighed and stored in sealed vials in the dark at 4 °C awaiting analysis. They were diluted in hexane (20% v/v) and analysed by Gas Chromatography–Mass Spectrometry (Agilent 6890N GC system coupled directly to a 5973 MS). The injection volume (by autosampler, at 24.79 psi) was 1 µL, the split ratio 200:1 and the inlet temperature 250 °C. For the GC analysis, we used a HP-Innowax polyethylene glycol column (60 m × 250 µm i.d. × 0.25 µm film thickness) and programmed the temperature as follows: 60 °C for 10 min, then increasing at a rate of 4 °C/min to 220 °C, held for 10 min and then increasing again at 1 °C/min to 240 °C. Helium was the carrier gas at a constant flow rate of 1.2 ml/min. Electron impact mass spectra were recorded at 70 eV (scanning from 35 to 550 m/z). The composition of the oil was calculated from electronic integration measurements using flame ionization detection (FID, 250 °C). For the calculation of relative retention indices (RRI), a series of *n*-alkanes was used as reference

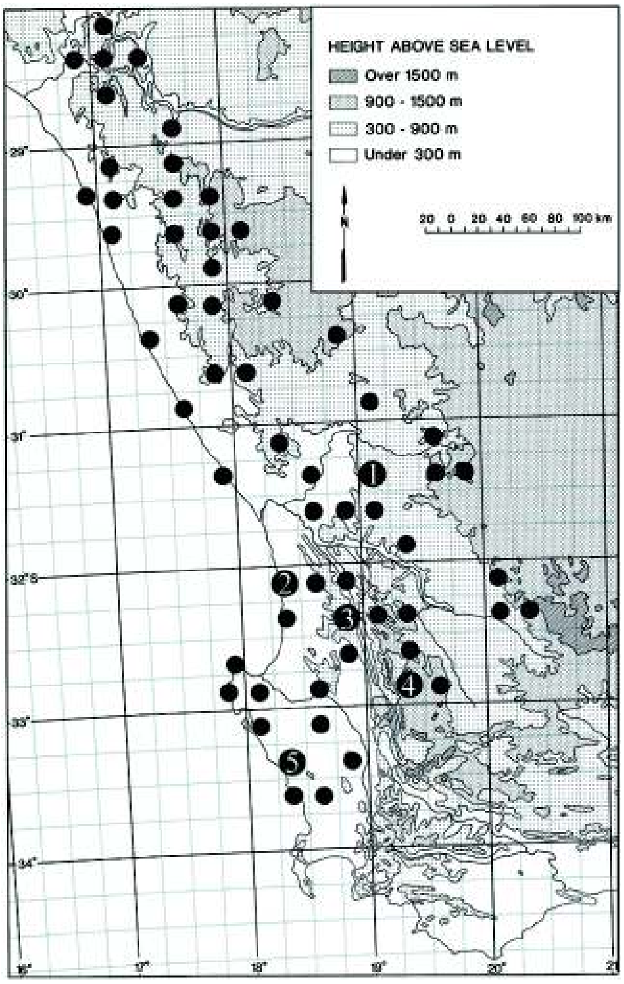

Fig. 1. The recorded geographical distribution of *Pteronia divaricata* in South Africa. The distribution extends into the extreme southern part of Namibia, south of Lüderitz. Localities where samples were collected are indicated by numbers: Van Rhyns Pass (1); Lamberts Bay (2); Wupperthal near Clanwilliam (3); Renosterhoek near Piquetberg (4) and Yzerfontein (5).

points. Compounds were identified (Table 3) by comparing their mass spectra and retention indices with library data (searches of NIST®, Mass Finder® and Flavour® libraries).

2.4. Antibacterial studies

Various extracts and oil samples (Table 3) were investigated for antimicrobial activity using the minimum inhibitory concentration (MIC) microtitre plate method described by Eloff (1998). One gram of powdered dry leaf material was mixed with 25 ml of solvent and left overnight. The solvents used were a 1:1 mixture of methanol and water, a 1:1 mixture of methanol and dichloromethane, and sterilized water. These were filtered and dried in a fume hood (organic solvents) or freeze-dried (aqueous solvents). All MIC assays were undertaken in triplicate. Two Gram-positive bacterial strains (*Bacillus cereus* ATCC 11778 and *Enterococcus faecalis* ATCC 29212) and two Gram-negative bacterial strains (*Escherichia coli* ATCC 8739 and *Klebsiella pneumoniae* ATCC 13883) were selected. *B. cereus* and *E. coli* are commonly associated with stomach and intestinal infections and *E. faecalis* and *K. pneumoniae* are associated with respiratory tract infections (see ethnobotanical uses in Table 1). Bacterial cultures were subcultured from stock agar plates and grown in Tryptone Soya broth for 18 h. Extracts diluted in acetone or water and oils diluted in acetone were applied (100 µl) to the first row of the microtitre plates at starting concentrations of 32 mg/ml (extracts) and 64 mg/ml (essential oils). Serial doubling dilutions were performed to yield concentrations varying from 16 mg/ml to 0.075 mg/ml. The cultures were diluted to an approximate inoculum size of 1×10^8 colony forming units (CFU)/ml and then introduced to all wells of the microtitre plate. Ciprofloxacin at a starting stock concentration of 0.01 mg/ml was used as a positive control against all test pathogens. Negative controls were included to determine the antimicrobial effects of the solvents used. The microtitre plates were sealed with sterile adhesive and incubated for 18 h at 37 °C. The colour reagent *p*-iodonitrotetrazolium violet (INT) was prepared (0.4 mg/ml) and 40 µl was transferred to all the inoculated wells after incubation. The microtitre plates were examined for colour changes (indicating microbial growth) after 6 h. The MIC value was determined as the lowest dilution having no evidence of bacterial growth.

3. Results and discussion

3.1. Novel ethnobotanical information

Literature records of medicinal uses exist for only seven species of *Pteronia*. With the exception of *P. onobromoides* (Hulley et al., 2010), these are all limited to a single brief anecdote or one short reference to a medicinal use: *P. adenocarpa* (Shearing, 1994), *P. camphorata* (Van Wyk and Gericke, 2000), *P. incana* (Montagu Museum, 1998), *P. lucilioides* (Archer, 1994), *P. succulenta* (Smith, 1966) and *P. stricta* (Watt and Breyer-Brandwijk, 1962). The vernacular name "*koorts-bos*" for *P. camphorata* was recorded on a herbarium specimen in 1925 [*Watermeyer 6350* (PRE), from Little Namaqualand], confirming the single published record of medicinal use for this species. In the case of *P. onobromoides*, there are several early references to its topical use as a buchu. The species was first recorded by Simon van der Stel in 1685 and later also by Harvey and Sonder (1865), Hutchinson and Phillips (1917), Laidler (1928), Marloth (1932), Watt and Breyer-Brandwijk (1962) and Smith (1966) — for a review, see Hulley et al. (2010).

We present here the first published information on the medical ethnobotany of *P. divaricata* (Table 1). The first record dates back to 1997 but recent field work has shown that the plant is well known and widely used by rural communities, especially in Wupperthal and associated small settlements in the Cederberg Mountains. The plant is known here as *flip-se-bos* or *inflammasiebos* and is considered to be one of the most important items of the local *materia medica*, along with plants such as *wildeals* (*Artemisia afra* Jacq. ex Willd.), *kouterbos* [*Athanasia trifurcata* (L.) L], *wynruit* (*Ruta graveolens* L.) and *brandnetel* (*Urtica urens* L.).

All anecdotes collected during the various ethnobotanical field surveys from different participants are presented in Table 1. According to Willem "Blikkies" Steenkamp from

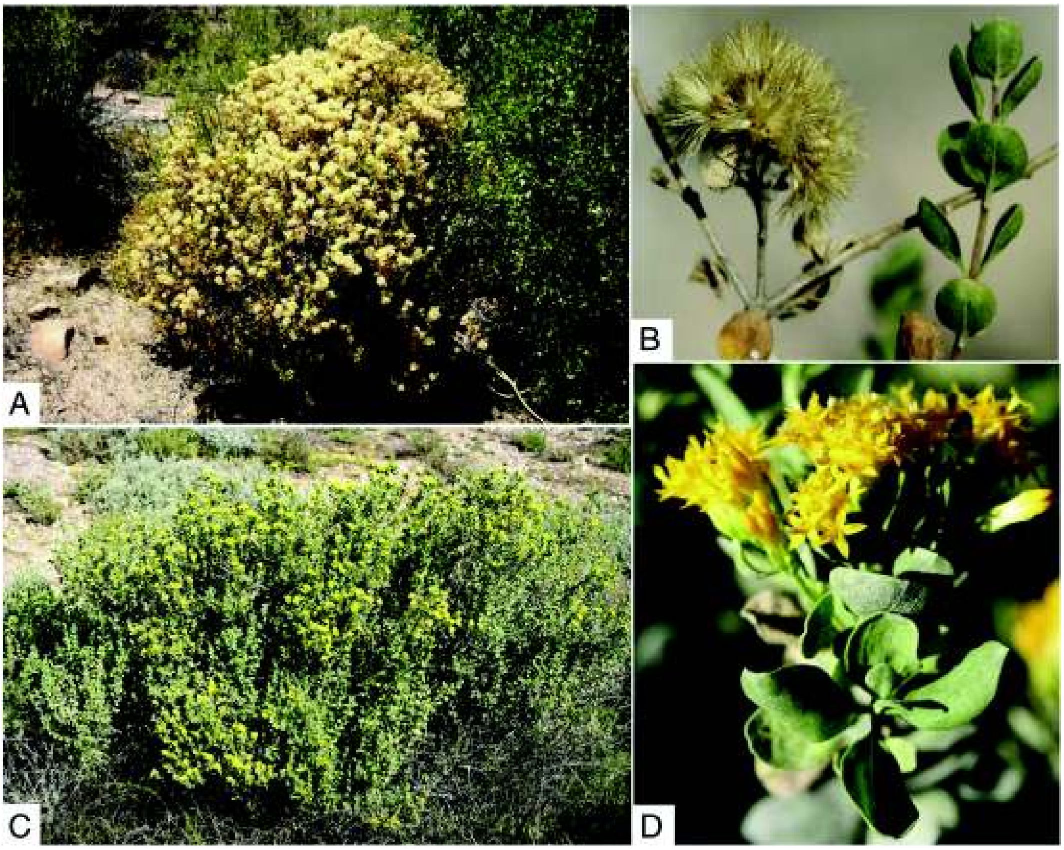

Fig. 2. *Pteronia divaricata*: habit, showing plant in fruit in December (A); leaves and fruit (B); habit, showing plant in flower in September (C); leaves and flower heads (D). Photographs taken by B.-E. Van Wyk.

Nieuwoudtville, *P. divaricata* is used to treat colds, stomach pain and backache. It is considered safe to use only during winter — it becomes "too strong" when flowering in the spring. *P. divaricata* has many uses in the Wupperthal area. Amelia Koopman (née Jooste), one of the most knowledgeable of the local herbalists, uses it to treat inflammation, gastric fever, colds and tuberculosis. According to her, the plant was used many years ago to cure a Wupperthal man by the name of Flip (surname unknown) who suffered from chronic stomach pain. Lydia Ockhuis uses *flip-se-bos* to treat diarrhoea and influenza.

Table 2
Voucher specimen details of the plant material of *Pteronia divaricata* used in the various studies (samples were collected from single plants at five localities).

Sample number	Locality	Date collected	Voucher specimens	Anatomy (A) Extracts for MIC studies (MIC) GC–MS
1	Van Rhyns Pass	12-12-2008	*B.-E. & M. Van Wyk 4323a*	A, MIC
2	Van Rhyns Pass	12-12-2008	*B.-E. & M. Van Wyk 4323b*	A, MIC
3	Van Rhyns Pass	12-12-2008	*B.-E. & M. Van Wyk 4323c*	A, MIC
4	Lamberts Bay	12-12-2008	*B.-E. & M. Van Wyk 4272a*	A, MIC
5	Lamberts Bay	12-12-2008	*B.-E. & M. Van Wyk 4272b*	A, MIC
6	Lamberts Bay	12-12-2008	*B.-E. & M. Van Wyk 4272c*	A, MIC
7	Yzerfontein	11-06-2009	*B.-E. Van Wyk 4362a1*	MIC, GC–MS
8	Yzerfontein	11-06-2009	*B.-E. Van Wyk 4362b1*	MIC, GC–MS
9	Yzerfontein	11-06-2009	*B.-E. Van Wyk 4362c1*	MIC, GC–MS
10	Renosterhoek	07-2009	*B.-E. & M. Van Wyk 4306*	GC–MS
11	Renosterhoek	07-2009	*B.-E. & M. Van Wyk 4306*	GC–MS
12	Yzerfontein (same plant as sample 7)	01-11-2009	*B.-E. Van Wyk & M.M. Le Roux 4463a2*	MIC, GC–MS
13	Yzerfontein (same plant as sample 8)	01-11-2009	*B.-E. Van Wyk & M.M. Le Roux 4463b2*	MIC, GC–MS
14	Yzerfontein (same plant as sample 9)	01-11-2009	*B.-E. Van Wyk & M.M. Le Roux 4463c2*	MIC, GC–MS
15	Wuppertal	04-10-2009	*B.-E. Van Wyk, I.M. Hulley & P.M. Tilney 4442a*	MIC, GC–MS
16	Wuppertal	04-10-2009	*B.-E. Van Wyk, I.M. Hulley & P.M. Tilney 4442b*	MIC, GC–MS
17	Wuppertal	04-10-2009	*B.-E. Van Wyk, I.M. Hulley & P.M. Tilney 4442c*	MIC, GC–MS

When mixed with *Ruta graveolens* and *Artemisia afra*, it can be used against fever and colds. Independent records from other inhabitants of the Cederberg confirm the tuberculosis and fever anecdotes but added high blood pressure (Table 1). An interesting use was recorded in the Middelpos-Gannaga Pass area. Jakop Tromp informed us that the leaves are mixed with tobacco and smoked by persons suffering from chest ailments. In the Hantam region, the plant is well known but it does not seem to have any local uses or local vernacular names.

Smith (1966) gave common names for several *Pteronia* species but *P. divaricata* was not listed among them. The only published vernacular names for *P. divaricata* are *geel knopbos* (Le Roux and Schelpe, 1981), *geelknopbos* or *spalkpenbos* (Le Roux and Schelpe, 1988), *geelgombos* (Manning and Goldblatt, 1996, 1997) and *penbos* (Powrie, 2004). The name *gombos* is often used for *Pteronia* species (Smith, 1966); *geel* means yellow. The name *penbos* (or variants thereof) refers to the hard and tough wood of the stems, making them suitable for use as pegs, splints and splices. In Nieuwoudtville, for example, the name *pennebos* is used because of the tough and hard twigs that can pierce a rubber tyre when a vehicle is driven over the shrub. The five new names recorded during this study (Table 1) are *inflammasiebos* or *flip-se-bos* (Wupperthal district), *boegabos* (a corruption of *boegoebos* or "buchu bush" — Middelpos district), *dassiepisbos* (probably named after the strong odour of the plant, reminiscent of rock rabbit urine — Citrusdal district) and *pylbos* because the straight strong stems can be sharpened and used as arrows (Piquetberg district). Two additional vernacular names have been recorded on herbarium specimens, namely *dassiebos* [*W.J. Hanekom 946* (PRE)] and *perdebos* [*P. P. Van Breda 202* (PRE)].

3.2. Leaf anatomy

The leaves are amphistomatic and have a thin cuticle. The outer periclinal walls of the epidermal cells are cutinized. The mesophyll is distinctly differentiated into palisade and compact spongy parenchyma. There are numerous small vascular bundles (Fig. 3A). In the midrib area, adjacent to the phloem (Fig. 3B), is a secretory duct. This type of secretory structure is very different from that observed in *P. onobromoides* (Hulley et al., 2010) where the oil is produced in cavities that appear as translucent dots, similar in structure and origin to those found in the Rutaceae (Beck, 2005). Secretory and non-secretory trichomes are present on the upper and lower leaf surfaces. The latter are responsible for the scabrid appearance and texture of the leaves (Fig. 3C). Surface trichomes occur in several other *Pteronia* species and have indeed been used indirectly as a taxonomic character by Hutchinson and Phillips (1917), who based their sectional classification system mainly on leaf surface texture.

3.3. Essential oil composition

Nothing appears to be known about the essential oil chemistry of *P. divaricata*. This species was not included in a survey of *Pteronia* oils by Coovadia (2007) nor in a recent publication on rare sesquiterpenoids of *Pteronia* species (Viljoen et al., 2010). The only published information on the chemistry of *P. divaricata* appears to be a report of a large number of diterpenes (mainly clerodanes) and various other phenolic compounds (Zdero et al., 1990). These include divaricatic acid, furodivaricatic acid, 3-hydroxypteronialactone and 3-oxopteronialactone.

The essential oil yields of eleven individual plants collected from three different localities (Renosterhoek, Wupperthal and Yzerfontein) are listed in Table 3. Yields were exceptionally variable, ranging from 0.068% to 0.33% of dry weight. The variation seems to be unrelated to provenance and date of collection. The Yzerfontein plants were collected in their pre-flowering (vegetative) state (11-06-2009) as well as their flowering state (01-11-2009), while the Renosterhoek plants were in full flower.

A total of 76 volatile components were identified in the eleven samples studied. The major compounds are several monoterpenes as well as sesquiterpenes (Table 3). Sabinene, myrcene, pentadecane, terpinen-4-ol, β-caryophyllene and bicyclogermacrene are main compounds in most of the samples, with lower levels of β-pinene, limonene, β-phellandrene, γ-terpinene, (*E*)-β-ocimene, *p*-cymene, tetradecane, hexadecane, heptadecane, α-muurolene, δ-cadinene, germacrene B, caryophyllene oxide, T-muurolene, 4α-hydrogermacra-1(10),5-diene and α-cadinol in several samples. Despite considerable quantitative variation, the three samples from Yzerfontein were fairly uniform in having the combination of sabinene, myrcene, β-caryophyllene and bicyclogermacrene as main constituents, regardless of the season when the plants were collected (Table 3). The two Renosterhoek samples differed from all others in the presence of valeranone as a major compound.

Sabinene and myrcene occur in several species of *Pteronia* (Coovadia, 2007; Hulley et al., 2010; Viljoen et al., 2010), and most of the other compounds listed in Table 3 are also present in other *Pteronia* species. However, none of the unusual *Pteronia* sesquiterpenoids reported recently (Viljoen et al., 2010) was found in *P. divaricata*.

3.4. Antibacterial activity

An exploratory study was conducted to see if *P. divaricata* has any antimicrobial activity against pathogens responsible for intestinal and respiratory infections (as suggested by the traditional uses). We therefore examined, for the first time in this species, possible antibacterial activity against a selection of four bacteria (Table 4).

The minimum inhibitory concentrations (MIC) were determined at initial concentrations of 32 mg/ml for the extracts and 64 mg/ml for the essential oils. The results (Table 4) showed that the methanol:dichloromethane (MeOH:CH_2Cl_2) extracts were the most active against both the Gram-positive (*B. cereus* and *E. faecalis*) and Gram-negative (*E. coli* and *K. pneumoniae*) bacteria. MIC values as low as 0.5–1.0 mg/ml were obtained when tested against *B. cereus*. All negative controls had no marked effect on the results presented.

Table 3
The main compounds (percentage area) of essential oil samples from eleven individual plants of *Pteronia divaricata* collected at three localities, as identified by GC–MS. Sample numbers of the individual plants studied are given as in Table 1. At Yzerfontein, the same three individual shrubs were sampled twice. Yield figures are in % w/w. (*T*=tentative identification).

	Locality	Renosterhoek		Wupperthal			Yzerfontein (pre-flowering, 11-06-2009)			Yzerfontein (flowering, 01-11-2009)		
	Sample number	10	11	15	16	17	7	8	9	12	13	14
RRI	Major compounds/yield	0.30	0.33	0.071	0.32	0.29	0.068	0.32	0.17	0.10	0.32	0.29
1000	Decane	–	–	0.2	3.3	1.1	–	–	–	0.2	0.3	0.9
1017	α-Pinene	0.2	1.0	0.3	0.5	0.2	0.1	0.2	0.1	0.4	0.2	–
1020	α -Thujene	0.1	–	0.2	–	0.2	0.1	0.2	0.1	0.1	0.2	–
1100	Undecane	–	–	–	1.3	–	–	–	–	–	–	–
1103	β-Pinene	0.3	0.6	0.5	0.4	0.4	0.4	0.4	0.6	2.8	1.7	0.5
1117	Sabinene	7.4	0.4	28.3	0.5	30.7	9.6	21.3	3.4	11.9	12.8	11.2
1160	Myrcene	13.9	30.5	16.6	16.4	13.7	37.5	26.3	10.1	17.5	45.0	55.6
1174	α-Terpinene	0.3	–	1.0	–	1.4	0.4	0.5	0.4	0.8	0.8	0.5
1193	Limonene	0.6	2.9	1.0	4.6	0.9	1.5	1.4	0.6	0.8	1.1	1.2
1200	Dodecane	–	–	–	1.8	–	–	–	–	–	–	–
1213	1,8-Cineole	–	–	0.9	–	0.6	–	–	–	–	–	0.9
1213	β-Phellandrene	0.3	1.5	0.6	4.1	0.6	0.7	0.9	0.3	0.5	0.9	–
1224	*trans* 2-Penthylfuran	0.1	–	–	–	–	–	–	0.1	–	–	–
1231	Thymol methyl ether	0.2	–	–	–	–	–	–	–	–	–	–
1238	(*Z*)-β-Ocimene	–	–	–	–	–	–	–	0.1	–	–	–
1241	γ-Terpinene	0.6	–	1.9	–	3.0	1.0	1.3	0.9	1.5	1.5	0.9
1260	(*E*)-β-Ocimene	0.9	0.7	0.2	4.2	2.4	–	–	0.6	3.1	1.1	0.5
1267	*p*-Cymene	1.8	2.3	1.1	2.6	1.0	1.2	0.9	0.9	1.4	0.9	0.7
1290	Terpinolene	0.2	0.1	0.5	–	0.8	0.3	0.4	0.3	0.5	0.6	–
1345	*cis* 2-Penthylfuran	–	–	–	–	–	–	–	0.1	–	–	–
1396	3-Nonanone	–	–	0.1	–	–	–	–	–	–	–	–
1400	Tetradecane	3.2	3.8	8.5	3.4	1.8	–	1.1	2.1	1.5	1.1	0.5
1429	Perillen	0.5	1.3	–	0.3	0.3	1.7	0.8	0.4	–	–	–
1430	α-Gurjunene	–	–	–	–	–	–	–	0.1	–	–	–
1466	α-Cubebene	–	–	–	–	–	–	–	0.1	–	–	–
1474	*trans*-Sabine hydrate	0.6	–	–	–	–	0.6	1.9	0.3	–	–	–
1500	Pentadecane	3.9	3.9	8.4	0.9	1.8	0.4	1.2	4.0	1.5	1.2	0.6
1544	*trans p*-Menth -2-en-1-ol	0.2	–	0.4	–	–	–	–	–	–	–	–
1553	Linalool	–	0.8	–	–	–	–	–	–	–	–	–
1556	*cis*-Sabine hydrate	–	–	–	–	–	0.6	1.9	0.3	–	–	–
1568	α-Bergamotene	–	–	–	–	–	–	–	0.1	–	–	–
1600	β-Elemene	–	–	–	–	–	–	–	0.2	–	–	–
1600	Hexadecane	–	–	7.3	3.4	1.6	–	–	–	1.4	1.2	0.7
1611	Terpinen-4-ol	4.0	4.0	6.5	0.7	12.4	4.2	4.4	–	3.5	5.5	3.8
1612	β-Caryophyllene	2.8	–	0.2	0.2	–	2.1	8.9	16.1	6.9	0.3	–
1628	Aromadendrene	–	–	0.1	0.3	–	–	–	0.2	0.2	0.1	–
1629	*cis*-α-Bisabolene	–	–	0.3	–	0.5	–	–	–	0.2	0.2	0.2
1650	γ-Elemene	–	–	–	–	–	–	0.3	0.3	–	–	–
1661	*allo* Aromadendrene	0.1	0.2	–	–	–	0.3	0.3	0.5	–	–	–
1687	α-Humulene	0.2	0.3	0.3	0.5	0.6	0.3	0.3	0.9	0.8	0.4	0.2
1689	Cryptone	–	0.5	–	0.4	–	–	–	–	–	–	–
1700	Heptadecane	–	–	2.1	1.1	1.4	–	–	–	1.4	1.3	0.6
1704	γ-Muurolene	–	–	0.3	1.3		0.3	0.2	0.2	2.7	1.2	–
1719	Germacrene D	–	–	–	–	–	0.9	0.8	2.1	–	–	–
1721	α-Farnesene	–	–	–	–	–	–	–	0.2	–	–	–
1740	α-Muurolene	–	–	0.6	2.5	1.2	1.6	0.8	2.3	1.4	–	0.5
1742	β-Selinene	–	–	–	–	–	–	–	5.1	–	–	–
1749	1,3,5,Octatrien-3-ol, 2,6-dimethyl	–	–	–	–	–	–	1.3	–	–	–	–
1755	Bicyclogermacrene	–	–	3.2	7.9	4.9	6.2	5.3	13.6	14.0	3.7	4.8
1773	δ-Cadinene	–	–	1.2	4.8	2.3	2.5	1.6	2.9	4.6	3.2	2.2
1776	γ-Cadinene	–	–	0.2	0.9	0.4	0.6	0.4	0.7	0.7	0.5	–
1782	α-Cadinene	–	–	–	–	–	–	–	2.0	–	–	–
1800	Octadecane	–	–	–	1.4	0.4	–	–	–	0.5	–	0.2
1854	Germacrene B	–	–	1.1	2.4	1.2	–	–	2.1	1.3	0.7	–
1861	3,10-Dihydro, 1,4-dimethyl azulene (T)	–	–	1.7	5.3	2.6	–	–	–	0.6	0.5	–
1891	*epi*-Cubebol	–	–	–	–	–	–	–	0.5	–	–	–

(continued on next page)

Table 3 (*continued*)

	Locality	Renosterhoek		Wupperthal			Yzerfontein (pre-flowering, 11-06-2009)			Yzerfontein (flowering, 01-11-2009)		
	Sample number	10	11	15	16	17	7	8	9	12	13	14
RRI	Major compounds/yield	0.30	0.33	0.071	0.32	0.29	0.068	0.32	0.17	0.10	0.32	0.29
1900	Nonadecane	–	–	–	–	–	–	–	0.4	–	–	–
1941	α-Calacorene	–	–	–	–	–	–	–	–	0.3	0.2	–
1948	Scapanol	–	–	–	0.3	–	–	–	–	0.2	0.1	–
1958	Cubebol	–	–	–	–	–	–	–	0.4	–	–	–
1982	10-*epi*-Junenol	–	–	–	0.4	–	–	–	1.0	0.6	0.3	–
2008	Caryophyllene oxide	–	–	–	–	–	0.3	3.7	1.1	–	–	–
2064	T-Muurolene	–	–	–	2.7	0.8	–	–	–	1.4	0.8	–
2073	4α-Hydrogermacra-1 (10), 5-diene	–	–	–	–	–	2.0	2.3	4.4	–	–	–
2080	Cubenol	–	–	–	0.4	–	–	–	–	0.2	0.2	
2098	Globulol	–	–	–	–	–	–	–	0.8	–	–	–
2102	*cis*-β-Guaiene (T)	–	–	–	1.1	–	–	–	–	–	0.3	–
2105	Phytol (T)	–	–	–	–	–	7.5	–	–	–	–	–
2185	γ-Eusdemol	9.0	–	–	–	–	–	–	–	–	–	–
2144	Spathulenol	–	–	–	–	–	0.6	0.7	1.2	–	–	–
2153	Valeranone	16.4	12.6	–	–	–	–	–	–	–	–	–
2187	T-Cadinol	–	–	–	1.7	0.7	0.9	0.4	1.1	0.2	–	0.6
2209	T-Muurolol	–	–	–	–	–	1.2	0.5	1.4	–	–	–
2246	4,10,Guaia-1-(5)-diene (T)	–	–	–	6.0	2.3	–	–	–	4.2	3.1	2.9
2255	α-Cadinol	–	–	–	–	–	2.1	0.7	3.0	–	–	–
2257	β-Eusdemol	–	–	–	–	–	–	–	1.0	–	–	–
Total		85.0	84.0	92.4	95.8	94.2	89.7	93.6	91.7	91.8	93.2	90.7

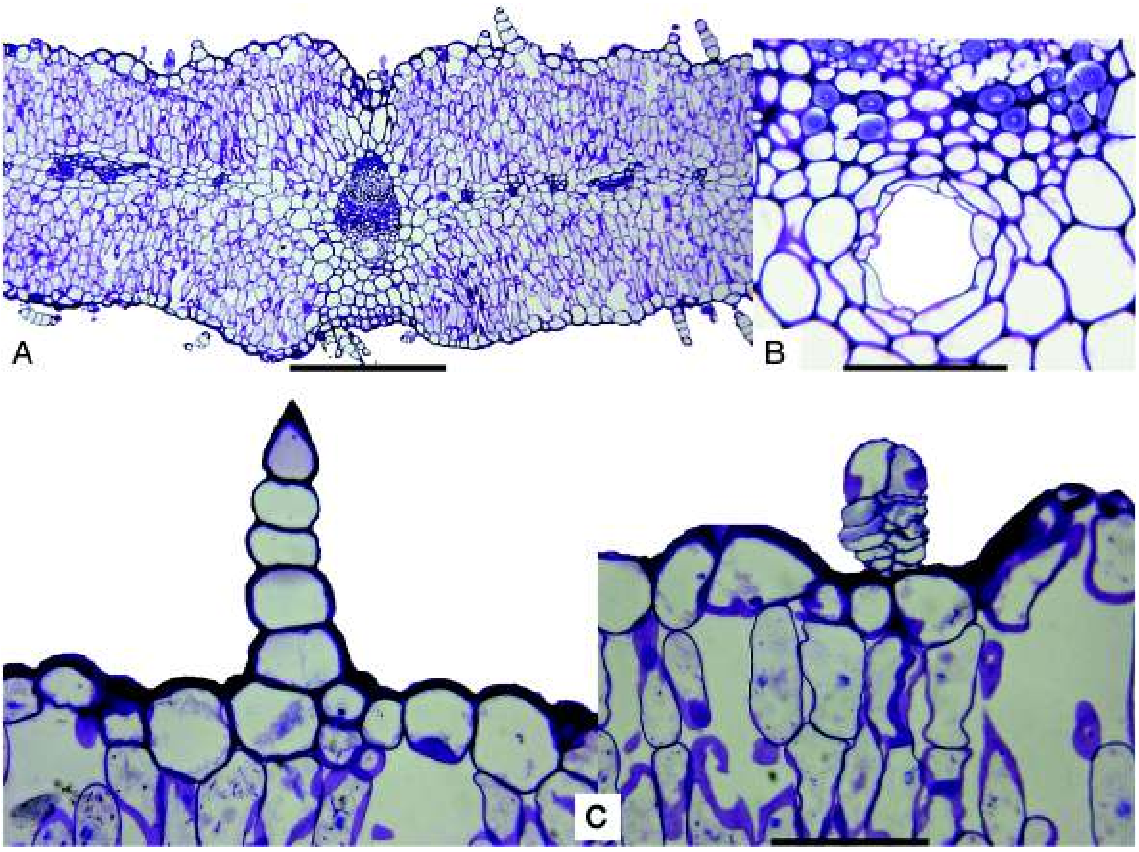

Fig. 3. *Pteronia divaricata*: transverse sections of a leaf showing a secretory duct and surface trichomes (A); single secretory duct in midrib area (B); non-secretory trichome (left) and secretory trichome (C). Scale bars: $A = 0.4$ mm; B and $C = 0.07$ mm.

Table 4
Minimum inhibitory concentrations (MIC's) for extracts and essential oils of *Pteronia divaricata*, tested on two Gram-positive (*Bacillus cereus* and *Enterococcus faecalis*) and two Gram-negative bacteria (*Escherichia coli* and *Klebsiella pneumoniae*).

Extracts or samples used	Sample number (as in Table 2)	MIC (mg/ml)			
		B. cereus ATCC 11778	*E. faecalis* ATCC 29212	*E. coli* ATCC 8739	*K. pneumoniae* ATCC 13883
H_2O extract	8	>8	>8	>8	>8
H_2O extract	9	>8	>8	6.7	>8
H_2O extract	12	>8	>8	>8	>8
H_2O extract	13	>8	>8	>8	> 8
H_2O extract	14	8.0	>8	>8	>8
H_2O extract	15	8.0	>8	>8	>8
H_2O extract	16	>8	>8	>8	>8
H_2O extract	17	>8	>8	>8	>8
MeOH:H_2O extract	1	>8	>8	8.0	>8
MeOH:H_2O extract	2	>8	>8	>8	>8
MeOH:H_2O extract	3	6.0	>8	>8	>8
MeOH:H_2O extract	4	>8	>8	>8	>8
MeOH:H_2O extract	5	>8	>8	>8	>8
MeOH:H_2O extract	6	>8	>8	>8	>8
MeOH:H_2O extract	8	>8	>8	>8	>8
MeOH:H_2O extract	9	>8	>8	>8	>8
MeOH:H_2O extract	12	8.0	8.0	>8	>8
MeOH:H_2O extract	13	6.7	>8	>8	>8
MeOH:H_2O extract	14	8.0	>8	>8	>8
MeOH:H_2O extract	15	8.0	>8	>8	>8
MeOH:H_2O extract	16	>8	>8	>8	>8
MeOH:H_2O extract	17	>8	>8	>8	>8
MeOH: CH_2Cl_2 extract	1	1.0	3.3	6.0	2.0
MeOH: CH_2Cl_2 extract	2	1.0	2.3	4.0	2.0
MeOH:CH_2Cl_2 extract	3	1.0	3.0	4.0	2.0
MeOH:CH_2Cl_2 extract	4	1.0	3.0	4.0	2.0
MeOH:CH_2Cl_2 extract	5	1.0	2.0	4.0	2.0
MeOH:CH_2Cl_2 extract	6	1.0	2.0	4.0	2.0
MeOH:CH_2Cl_2 extract	7	1.0	4.0	4.0	2.0
MeOH:CH_2Cl_2 extract	8	1.0	4.0	4.0	2.0
MeOH:CH_2Cl_2 extract	9	1.0	4.0	4.0	2.0
MeOH:CH_2Cl_2 extract	12	0.5	2.0	2.0	2.0
MeOH:CH_2Cl_2 extract	13	0.8	1.0	2.0	2.0
MeOH:CH_2Cl_2 extract	14	1.0	2.0	8.0	8.0
MeOH:CH_2Cl_2 extract	15	1.0	4.0	1.5	2.0
MeOH:CH_2Cl_2 extract	16	1.0	4.0	1.0	2.0
MeOH:CH_2Cl_2 extract	17	1.0	4.0	2.0	2.0
Essential oil	7	2.0	8.0	8.0	4.0
Essential oil	8	2.0	8.0	12.0	6.0
Essential oil	9	2.0	6.0	6.0	6.0
Essential oil	12	2.0	6.0	8.0	6.0
Essential oil	13	2.0	4.0	8.0	3.0
Essential oil	14	2.0	6.0	8.0	4.0
Essential oil	15	4.0	6.0	3.0	4.0
Essential oil	16	2.0	4.0	3.3	3.0
Essential oil	17	2.0	4.0	3.3	3.0
Positive control (ciprofloxacin in H_2O)		0.06 μg	1.25 μg	0.94 μg	0.24 μg

Water and methanol extracts showed no activity at the highest concentration tested against any of the organisms studied. The essential oils showed some antimicrobial activity where the most noteworthy activity was observed for *B. cereus* having MIC values ranging from 2.0 to 4.0 mg/ml. Antibacterial activity was also demonstrated by Coovadia (2007) for various extracts and essential oils of *Pteronia* species including *P. adenocarpa*, *P. elongata* Thunb., *P. fasciculata* L.f., *P. flexicaulis* L.f., *P. glomerata* L.f. and *P. paniculata* Thunb. and, by Hulley et al. (2010), for *P. onobromoides*.

In view of the traditional use against inflammation, it would be interesting to examine *P. divaricata* for possible anti-inflammatory activity.

4. Conclusions

P. divaricata is undoubtedly an important Khoi-San traditional medicinal plant and it is remarkable that its medicinal uses (the treatment of colds, fever, influenza, stomach pain, diarrhoea, back pain, chest ailments, high blood pressure and tuberculosis) have remained unrecorded in the scientific literature. The vernacular names *flip-se-bos*, *inflammasiebos*, *pylbos*, *dassiepisbos* and *boegabos* also appear to be published here for the first time. The anatomical study showed that the essential oil is produced in single secretory ducts in the midrib area. The oil is variable both in yield and in the levels of the main constituents but the combination of sabinene, myrcene, pentadecane, terpinen-4-ol, β-caryophyllene and bicyclogermacrene appears to be characteristic for the species (although valeranone was identified in one population only). The antimicrobial activity of the methanol:dichloromethane extracts against both Gram-positive and Gram-negative bacteria suggests that the plant may have efficacy in the treatment of respiratory and intestinal infections.

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