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The ethnobotany, leaf anatomy, essential oil variation and biological activity of *Pteronia incana* (Asteraceae)

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Abstract

The only available ethnobotanical information on *Pteronia incana* has been recorded by the Montagu Museum in the Western Cape Province of South Africa. It was reported that the plant is used to treat influenza, fever, kidney ailments and backache. In common with other species of *Pteronia*, the plant contains an essential oil reminiscent of pine turpentine oil with β-pinene, limonene, 1,8-cineole, myrcene, spathulenol, *p*-cymene and methyleugenol as main compounds present in all or most of the samples, with smaller amounts of α-pinene, sabinene, γ-terpinene, terpinen-4-ol, biclogermacrene, globulol and α-bisabolol in some of the distillates. We investigated the oil composition of 11 individual plants collected at three geographically distant localities but found limited variation, both within and between populations. Leaf sections of *P. incana* showed that it is anatomically similar to *P. divaricata* in the presence of a secretory duct associated with the main vascular bundle (and often other bundles as well), in addition to glandular and non-glandular trichomes on both leaf surfaces. One yeast (*Cryptococcus neoformans*), two Gram-negative bacteria (*Moraxella catarrhalis* and *Klebsiella pneumoniae*) and one Gram-positive bacterium (*Mycobacterium smegmatis*) were selected for antimicrobial activity studies using the minimum inhibitory concentration (MIC) microtitre plate method. The results showed that methanol: dichloromethane (MeOH:CH_2Cl_2) extracts were active against *M. smegmatis* (lowest MIC values of 0.5–0.8 mg/ml) and *C. neoformans* (lowest MIC values of 0.5–2.0 mg/ml). The essential oil was most active against *C. neoformans* (lowest MIC value of 0.3 mg/ml). These results provide a scientific rationale for the use of *P. incana* in Cape herbal medicine.

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

1. Introduction

Pteronia incana (Burm.) DC. (Asteraceae) is one of the most widely distributed and best-known of the 70 species of *Pteronia* L., a genus of small woody shrubs subendemic to southern Africa (Germishuizen and Meyer, 2003; Leistner, 2000). The species can easily be recognized by the small silvery leaves clothed with a woolly whitish indumentum, the solitary, terminal flower heads (Fig. 1) and the obtuse to acute involucral bracts that are glabrous outside (Harvey and Sonder, 1865; Hutchinson and Phillips, 1917; Le Roux and Schelpe, 1981, 1988; Marloth, 1932). *P. incana* is endemic to South Africa and occurs from west of East London in the Eastern Cape Province, throughout the Western Cape Province and northwards to the Namibian border in the Northern Cape Province (Fig. 2). Curiously, the species has not yet been recorded in Namibia (Craven, 1999; Merxmüller, 1967). The plants are strongly associated with the Cape Floristic Region and more specifically renosterveld vegetation, where it is often one of the characteristic or dominant taxa of the various renosterveld units (Mucina and Rutherford, 2006).

Traditional medicinal uses of *P. incana* are summarised in a leaflet distributed by the Montagu Museum (1998). Mention is made of its uses to treat influenza, fever, kidney ailments and backache.

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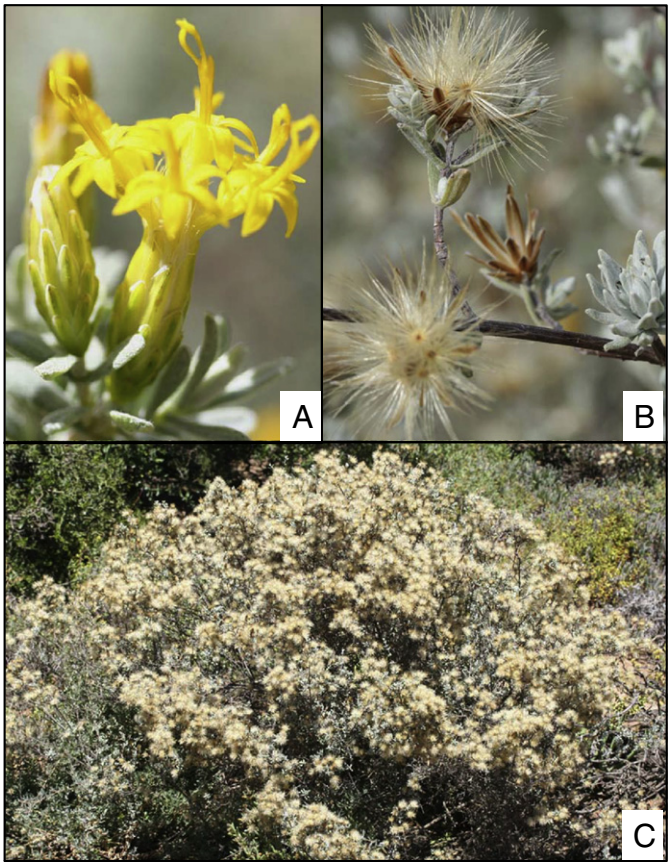

Fig. 1. *Pteronia incana*: leaves and flower heads (A); leaves and fruit (B); habit, showing plant in fruit (C). Photographs taken by B.-E. Van Wyk.

In common with other *Pteronia* species, the plant is highly aromatic and produces a volatile oil (Bruns and Meiertoberens, 1987; Mayekiso et al., 2006; Webber et al., 1999). It is unpalatable to livestock and several studies have shown that over-grazing results in the invasion of natural vegetation (Kakembo et al., 2007 and references cited therein). Mayekiso et al. (2008) studied the morphology and ultrastructure of the glandular and non-glandular trichomes and reported that essential oil was secreted by multicellular glands. Our study of *Pteronia onobromoides* DC. (Hulley et al., 2010a,b) showed that the essential oil is produced in pellucid oil cavities situated within the leaf lamina and not by glandular trichomes on the surface. In a recent study of *P. divaricata* P.J. Bergius, the presence of both oil ducts and glandular trichomes is reported (Hulley et al., 2010a,b in South African Journal of Botany). *P. onobromoides* and *P. divaricata* are important in traditional medicine in South Africa (Hulley et al., 2010a,b).

The aims of our study of *P. incana* were (1) to document accurately the medicinal uses of this species; (2) to explore the leaf anatomy, focussing on secretory structures; (3) to determine the chemical composition and geographical variation of the essential oil; and (4) to investigate possible antimicrobial activity.

2. Material and methods

2.1. Materials studied

Fresh material of *P. incana* was collected at three localities, namely Worcester [33° 19° CB], Herolds Bay [34° 22° AB] and

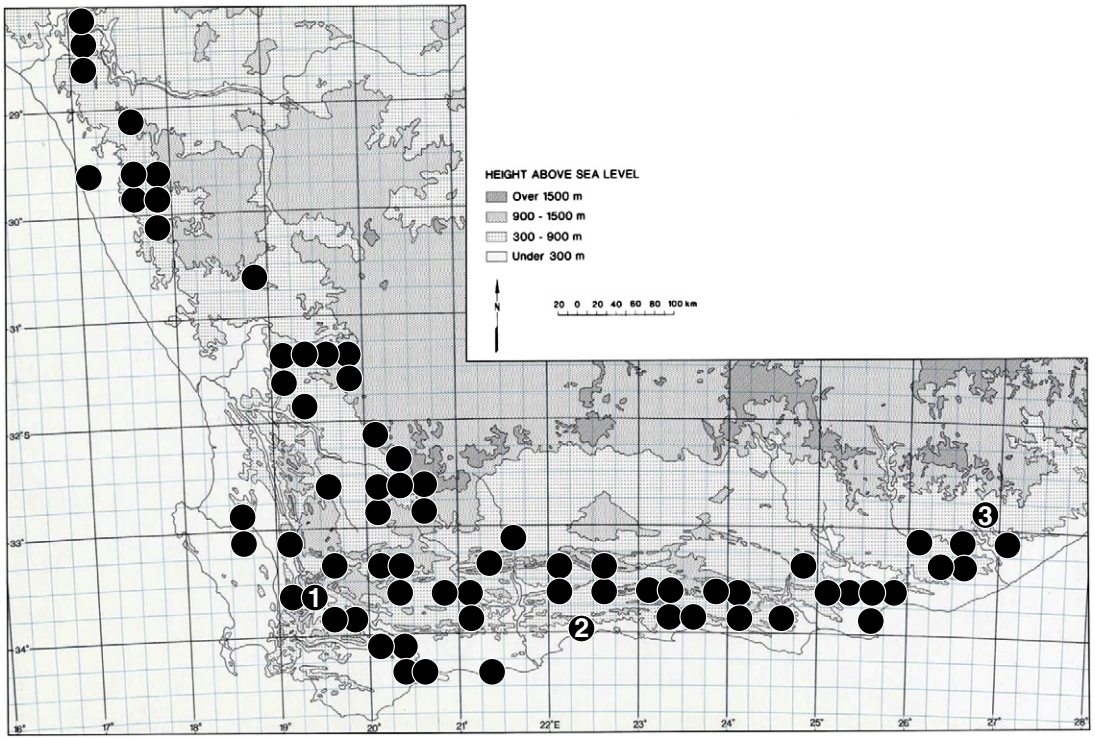

Fig. 2. The recorded geographical distribution of *Pteronia incana*.

near Alice at the University of Fort Hare campus [32° 26° DD]. The samples were carefully air dried. Localities and voucher specimen details are presented in Table 1.

2.2. Anatomical procedures

Dried leaf material was rehydrated and then placed in formalin–acetic acid–alcohol (FAA; 10:5:50) for 24 h after which it was treated according to the glycol methacrylate (GMA) method of Feder and O'Brien (1968). Sections, 3–5 μm thick, were cut using an ultramicrotome. Unstained slides were viewed under a polarizing microscope to observe if any crystals were present. Staining was done with Schiff's reagent and toluidine blue. Stained slides were observed under a light microscope equipped with a digital camera and a computerised data capturing system.

2.3. Distillation and analysis of essential oil

Air-dried leaves of eleven samples (Table 1) were subjected to hydrodistillation for 180 min using a Clevenger-type apparatus. The oils were weighed and stored in sealed vials in the dark, at 4 °C, before analysis.

The oils (20% diluted in hexane) were analysed by a gas chromatograph directly coupled to a mass spectrometer (Agilent 6890N GC system/5973 MS). A volume of 1 μl was injected (using a split ratio of 200:1) with an autosampler at 24.79 psi and an inlet temperature of 250 °C. The GC system equipped with a HP-Innowax polyethylene glycol column (60 m × 250 μm i.d. × 0.25 μm film thickness) was used. The oven temperature program was 60 °C for the first 10 min, rising to 220 °C at a rate of 4 °C/min and held for 10 min and then rising to 240 °C at a rate of 1 °C/min. Helium was used as carrier gas at a constant flow of 1.2 ml/min. Spectra were obtained on electron impact at 70 eV, scanning from 35 to 550 m/z. The percentage compositions of the individual components were obtained from electronic integration measurements using flame ionization detection (FID, 250 °C). We used *n*-alkanes as reference points in the calculation of relative retention indices (RRI). Component identifications (Table 2) were made by comparing mass spectra and retention indices. Library searches were carried out using NIST®, Mass Finder® and Flavour® libraries. Crude extracts and distilled oils were also studied by HP-TLC but to save space the results are not shown here.

2.4. Antimicrobial studies

In view of the reported medicinal uses of *P. incana* for respiratory infections, we decided to test the antimicrobial activity of extracts and essential oil on four respiratory pathogens (Table 3).

The essential oil, water, methanol:water and methanol: dichloromethane extracts were investigated for antimicrobial activity using the minimum inhibitory concentration (MIC) microtitre plate method (Eloff, 1998). Extracts were prepared in the same way as described in a recent publication (Hulley et al., 2010a,b). All MIC assays were undertaken in triplicate. A yeast (*Cryptococcus neoformans* ATCC 90112), two Gram-negative bacteria (*Moraxella catarrhalis* ATCC 23246 and *Klebsiella pneumoniae* ATCC 13883) as well as one Gram-positive bacterium (*Mycobacterium smegmatis* ATCC 14468) were selected as they are all implicated in respiratory infections. *C. neoformans* is associated with lung infection while *M. catarrhalis* is known to cause bronchitis, sinusitis and laryngitis. *Klebsiella pneumoniae*'s most common infection is pneumonia and *M. smegmatis* is a faster growing non-pathogenic mycobacterium strain. Bacterial cultures were subcultured from stock agar plates and grown in Tryptone Soya broth for 18 h. *C. neoformans* was incubated in the same medium for a further 24 h. Middlebrook 7H9 broth (Difco) was the medium for the cultivation of *M. smegmatis* and BBL Middlebrook ADC enrichment was added to the culture media. The methanol:dichloromethane extracts and the essential oils were diluted in acetone and the methanol:water and water extracts were diluted in water. The method of application of these prepared extracts and oils to the microtitre plates, as well as the incubation of cultures is as described in Hulley et al.

Table 1
Voucher specimen details of the plant materials of *Pteronia incana* that were studied. (Samples were collected from single plants at three localities).

Sample number	Locality	Date collected	Voucher specimens all housed in JRAU[a]	Anatomy (A) extracts for MIC studies (MIC) GC–MS
1	Karoo Desert National Botanical Garden, Worcester	07-05-2007	S. Harris s.n. a	A, MIC, GC–MS
2	Karoo Desert National Botanical Garden, Worcester	07-05-2007	S. Harris s.n. b	MIC, GC–MS
3	Herolds Bay near George	19-07-2007	A.M. Viljoen s.n. a	MIC, GC–MS
4	Herolds Bay near George	19-07-2007	A.M. Viljoen s.n. b	GC–MS
5	Herolds Bay near George	19-07-2007	A.M. Viljoen s.n. c	GC–MS
6	Herolds Bay near George	19-07-2007	A.M. Viljoen s.n. d	MIC, GC–MS
7	Herolds Bay near George	19-07-2007	A.M. Viljoen s.n. e	GC–MS
8	Herolds Bay near George	19-07-2007	A.M. Viljoen s.n. f	GC–MS
9	University of Fort Hare, Alice	05-05-2007	M.L. Magwa s.n. a	MIC, GC–MS
10	University of Fort Hare, Alice	05-05-2007	M.L. Magwa s.n. b	MIC, GC–MS
11	University of Fort Hare, Alice	05-05-2007	M.L. Magwa s.n. c	GC–MS

[a] Herbarium of the University of Johannesburg.

Table 2
The main compounds (percentage area) of essential oil samples from eleven individual plants of *Pteronia incana* collected at three localities, as identified by GC–MS. Sample numbers of the individual plants studied are given as in Table 1. Yield figures (shown in bold) are in % w/w. The yields of important compounds (those exceeding 5% of total yield in at least one sample) are shown in bold. (T = tentative identification; RRI = relative retention indices).

	Locality sample number	Worcester		Herolds Bay						Alice		
		1	2	3	4	5	6	7	8	9	10	11
RRI	Major compounds/yield	**0.61**	**0.10**	**1.08**	**0.57**	**0.37**	**0.10**	**0.36**	**0.48**	**0.27**	**0.42**	**0.38**
1017	α-Pinene	**1.8**	**0.9**	**1.7**	**2.1**	**1.6**	–	**0.8**	**1.3**	**5.3**	**6.7**	**6.7**
1020	α-Thujene	0.2	0.1	0.1	0.1	0.2	–	0.1	0.1	0.2	0.1	0.2
1103	β-Pinene	**7.0**	**6.2**	**6.3**	**7.1**	**10.2**	**0.3**	**5.2**	**6.0**	**7.0**	**10.2**	**9.8**
1117	Sabinene	2.1	1.3	1.3	1.5	2.9	0.1	2.5	1.5	2.1	2.1	1.7
1126	Thuja-2,4 (10)-diene	–	–	–	–	–	–	–	–	0.1	–	–
1160	Myrcene	**4.2**	**4.4**	**4.1**	**3.1**	**5.0**	**0.2**	**4.7**	**5.0**	**6.4**	**5.4**	**8.0**
1162	α-Phellandrene	0.5	–	0.5	1.4	0.6	–	–	0.5	–	–	–
1174	α-Terpinene	0.5	0.3	0.3	0.2	0.3	–	0.2	0.1	0.3	0.3	0.2
1193	Limonene	**4.8**	**3.7**	**8.1**	**5.5**	**5.1**	**0.8**	**9.2**	**2.1**	**10.9**	**4.2**	**3.2**
1203	1,8-Cineole	**18.2**	**18.5**	**9.3**	**8.3**	**9.3**	**0.4**	**7.3**	**7.8**	**9.6**	**16.8**	**10.0**
1218	(Z)-β-Ocimene	–	0.1	0.1	–	0.2	–	–	–	–	–	–
1246	γ-Terpinene	0.7	0.6	0.4	0.3	0.5	0.1	0.4	0.2	0.7	0.7	0.5
1258	(E)-β-Ocimene	–	–	–	–	–	–	–	0.2	–	–	–
1263	*p*-Cymene	**36.7**	**27.5**	**16.2**	**15.9**	**18.4**	**4.2**	**33.5**	**29.2**	**13.1**	**14.5**	**13.5**
1290	Terpinolene	0.2	0.2	0.1	0.1	0.2	–	–	0.1	0.2	0.2	0.1
1386	1-Hexanol	1.3	0.7	0.1	0.2	0.3	–	–	0.1	0.1	0.2	0.2
1399	Methyl-octanoate	–	0.2	0.2	–	–	0.1	0.1	0.2	0.2	0.1	0.2
1400	Tetradecane	0.3	3.8	1.1	–	–	–	–	–	1.1	1.1	0.9
1406	Presilhiperfol-7-ene	0.1	0.3	0.8	0.7	0.1	2.3	0.5	0.4	0.1	–	0.6
1424	Perillen	–	–	0.1	–	–	–	–	0.1	0.6	0.4	–
1424	7-(a)-H-Silphiperfol-5-ene	–	0.1	0.1	0.1	0.1	0.3	0.1	0.1	0.1	–	0.1
1500	Pentadecane	0.3	4.1	1.2	–	–	–	–	–	1.1	1.1	1.0
1507	Pinguisene	–	–	0.2	0.2	–	0.6	–	–	–	–	0.2
1512	Dillether	–	0.1	1.8	1.5	–	–	–	–	–	–	–
1532	Modhephene	–	–	–	–	–	–	–	–	0.5	0.1	0.2
1533	Isocomene	0.1	0.2	0.2	–	–	–	–	0.1	–	0.3	0.5
1542	Linalool	–	–	–	–	0.1	–	–	–	–	–	–
1544	*trans-p*-Menth-2-en-1-ol	–	0.2	0.1	–	0.2	–	0.2	–	–	0.2	–
1556	*cis*-Sabinene hydrate	–	–	–	–	–	–	–	1.3	–	–	–
1586	Pinocarvone	–	0.2	0.4	0.1	0.2	–	0.2	0.1	0.9	1.5	0.9
1607	Bergamotene	0.2	0.4	–	0.1	–	–	–	–	–	0.4	0.3
1611	Terpinen-4-ol	**4.2**	**7.1**	**3.2**	**2.4**	**3.5**	–	**2.4**	–	**4.8**	**4.2**	–
1612	β-Caryophyllene	–	–	–	–	–	4.1	–	–	–	–	–
1648	Myrtenal	–	–	0.4	0.1	0.2	–	0.1	0.6	1.1	–	1.1
1661	*trans*-Pinocarveol	0.2	0.3	0.5	0.1	0.3	–	0.2	0.2	1.6	2.8	1.7
1682	d-Terpineol	0.2	0.4	0.2	0.1	0.1	–	0.7	–	–	0.4	0.2
1692	α-Terpineol	0.7	0.5	0.7	0.5	0.7	0.9	–	0.1	0.7	1.1	0.9
1700	Heptadecane	–	2.9	–	–	–	–	–	–	–	–	–
1726	Germacrene D	–	0.2	–	–	0.1	0.3	–	0.4	0.3	0.3	0.8
1738	Eudesma4(14)-11-diene	–	0.1	–	–	–	0.2	–	–	–	–	–
1740	α-Muurolene	–	–	–	–	0.1	–	–	–	–	–	–
1755	Biclogermacrene	0.6	1.6	0.5	1.5	2.2	4.5	0.7	3.0	0.5	0.4	2.0
1768	Carvone	–	–	0.3	–	–	–	–	–	0.9	0.4	–
1773	δ-Cadinene	–	–	0.1	–	0.3	–	–	–	0.2	0.3	–
1776	γ-Cadinene	–	–	–	–	0.1	–	–	–	0.1	–	0.9
1800	Octadecane	–	–	1.2	–	–	–	–	–	1.0	–	–
1804	Myrtenol	0.2	0.3	0.5	0.2	0.2	0.3	0.2	0.3	0.9	1.6	–
1809	*Ar* curcumene	–	–	–	–	–	–	–	–	–	–	1.0
1864	*p*-Cymen-8-ol	0.3	0.2	0.3	0.1	0.2	0.2	–	0.2	0.4	0.6	0.4
1868	Geranylacetone	0.1	–	0.2	0.2	0.1	0.3	–	0.2	2.8	–	–
2008	Caryophyllene oxide	0.5	1.4	2.0	3.0	1.1	4.7	1.0	0.8	–	2.0	1.7
2030	Methyleugenol	**2.3**	**1.0**	**4.3**	**4.9**	**10.9**	–	**8.9**	**6.3**	**4.0**	**1.6**	**4.1**
2037	(E)-Nerolidol	0.7	0.4	0.2	0.3	0.5	2.7	–	0.7	0.3	0.3	0.3
2098	Globulol	–	–	**1.8**	**2.0**	–	**8.3**	**1.6**	**1.5**	–	–	**0.8**
2104	Viridiflorol	0.2	0.2	0.6	0.3	0.6	0.9	–	0.4	0.7	0.8	0.6
2144	Spathulenol	**3.0**	**2.9**	**6.3**	**10.5**	**6.2**	**22.9**	**7.2**	**10.6**	**8.7**	**5.5**	**7.6**
2214	Carvacrol	1.8	1.1	0.4	0.3	–	–	1.2	0.3	–	–	–
2228	Isoledene (T)	0.2	0.2	1.1	1.2	3.7	3.1	0.7	–	–	–	–
2232	Zingiberenol (T)	–	–	–	–	–	–	–	–	0.6	–	0.8

(continued on next page)

Table 2 (*continued*)

	Locality sample number	Worcester		Herolds Bay						Alice		
		1	2	3	4	5	6	7	8	9	10	11
2232	α-Bisabolol	–	–	**3.3**	**2.6**	–	**13.5**	**4.5**	**10.7**	**0.5**	**0.3**	**0.4**
2361	Bisabolol-1-one (T)	–	–	0.5	–	–	–	–	–	0.6	0.4	1.3
2470	Isoelemicine	–	–	**8.5**	**14.8**	**8.2**	–	–	–	–	–	–
Total		**91.3**	**94.9**	**94.8**	**91.9**	**93.6**	**92.8**	**76.3**	**94.4**	**89.6**	**94.4**	**85.6**

Table 3
Minimum inhibitory concentrations (MIC's) for extracts and essential oils of *Pteronia incana*, tested on a yeast (*Cryptococcus neoformans*), two Gram-negative bacteria (*Moraxella catarrhalis* and *Klebsiella pneumoniae*) and one Gram-positive bacteria (*Mycobacterium smegmatis*).

Sample	Sample number (as in Table 1)	MIC (mg/ml)			
		M. catarrhalis ATCC 23246	*M. smegmatis* ATCC 14468	*C. neoformans* ATCC 90112	*K. pneumoniae* ATCC 13883
H_2O extract	1	>8	>8	1.6	>8
H_2O extract	2	>8	>8	2.0	>8
H_2O extract	3	>8	>8	3.5	>8
H_2O extract	6	>8	>8	1.0	>8
H_2O extract	9	>8	>8	1.5	>8
H_2O extract	10	>8	>8	2.7	>8
MeOH:H_2O extract	1	>8	>8	0.1	>8
MeOH:H_2O extract	2	>8	>8	>8	>8
MeOH:H_2O extract	3	>8	>8	0.8	2.0
MeOH:H_2O extract	6	>8	>8	0.5	1.5
MeOH:H_2O extract	9	>8	>8	0.1	>8
MeOH:H_2O extract	10	>8	>8	0.3	>8
MeOH:CH_2Cl_2 extract	1	2.0	0.5	0.5	1.7
MeOH:CH_2Cl_2 extract	2	2.0	0.8	0.5	1.3
MeOH:CH_2Cl_2 extract	3	3.0	0.5	2.0	2.0
MeOH:CH_2Cl_2 extract	6	2.0	0.5	1.0	2.0
MeOH:CH_2Cl_2 extract	9	2.0	0.5	0.5	1.7
MeOH:CH_2Cl_2 extract	10	2.0	0.5	1.0	2.3
Essential oil	1	4.0	1.3	0.8	4.0
Essential oil	2	4.0	1.3	0.3	4.0
Essential oil	3	6.0	1.0	0.8	4.0
Essential oil	6	4.0	1.3	0.8	4.0
Essential oil	9	5.3	1.3	1.0	2.0
Essential oil	10	5.3	1.3	1.3	4.0
Positive control (ciprofloxacin)		0.625 μg	0.625 μg	2.5 μg	0.078 μg

(2010a,b). In brief, 100 μl extract/essential oil was applied to the first row of the microtitre plates at starting concentrations of 32 mg/ml (extracts) and 64 mg/ml (essential oils). The cultures were diluted to an approximate inoculum size of 1×10^8 colony forming units (CFU)/ml and then introduced (100 μl) 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 bacterial test pathogens, and 0.1 mg/ml amphotericin B was used for *C. neoformans*. Negative controls were included to determine the antimicrobial effects of the solvents used. The microtitre plates were sealed with sterile adhesives 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 for all the bacterial pathogens and 24 h for the yeast. The MIC value was determined as the lowest dilution having no evidence of microbial growth.

3. Results and discussion

3.1. Ethnobotany

P. incana is a very common and well-known plant with a wide distribution in southern Africa and it is therefore not surprising that several vernacular names have been recorded. From herbarium specimens, we recorded *ribbokbos* [1936, Calvinia district, *A.A. Schmidt 581* (PRE)] and *bloubos* [December 1938, Peddie district, *Anon. s.n. sub* PRE *41722* (PRE)]. Published vernacular names for the species are

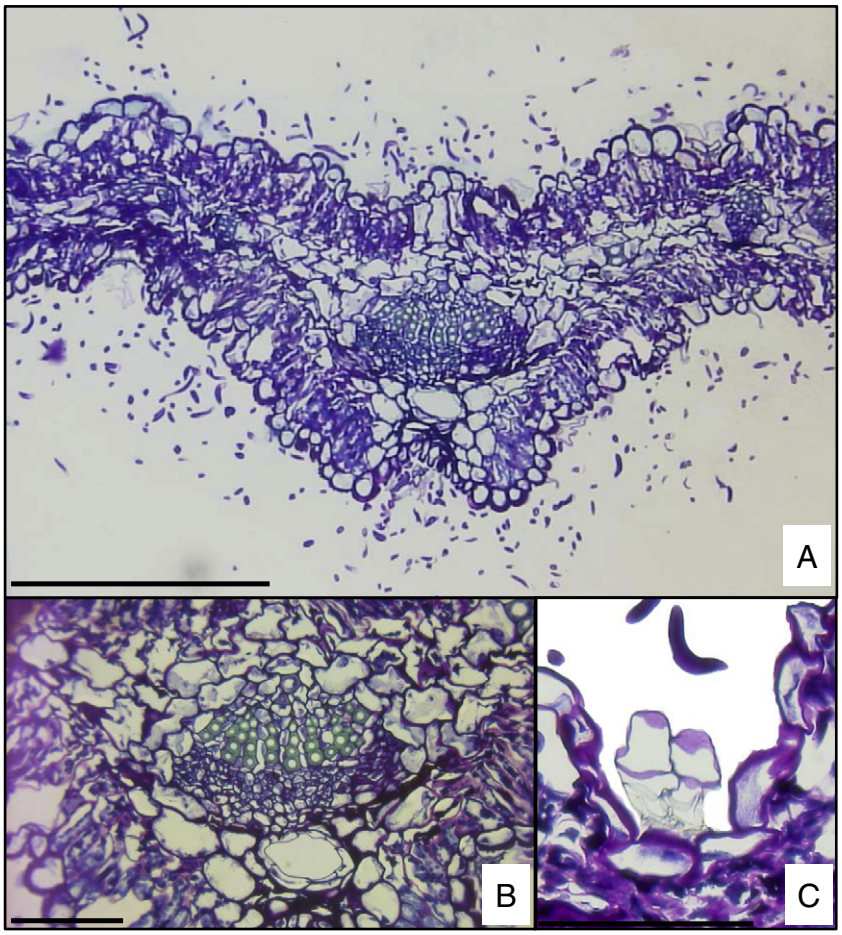

Fig. 3. *Pteronia incana*: transverse sections of a leaf showing a secretory duct and surface trichomes (A); single secretory duct in midrib area (B); multicellular glandular trichome (C). Scale bars: A = 0.2 mm; B and C = 0.07 mm.

laventelbossie, *leventelbossie*, *asbossie*, *perdebossie*, *vaalbossie*, *vaalbloubossie* (Smith, 1966), *bees'karroo* (Von Reis Altschul, 1973), *t'kaibebos* (Le Roux and Schelpe, 1981, 1988), *vaalkaroo*, *tinktinkiebos* (Montagu Museum, 1998), *laventelbossie* (Van Wyk and Gericke, 2000) and *kraakbos*, *laventelbos*, *scholtzbos*, *teebos*, *vaalbos*, wild lavender bush (Powrie, 2004). In the Eastern Cape Province, the plant is widely known as blue bush, blue dog or *bloubos* (Kakembo et al., 2007; Margaris et al., 1982; Webber et al., 1999).

What is surprising, however, is that no published ethnobotanical data is available for the species in the scientific literature (e.g. Arnold et al., 2002; Neuwinger, 2000; Watt and Breyer-Brandwijk, 1962). The only available evidence indicating that the plant has traditional medicinal uses can be found in a leaflet distributed by the Montagu Museum (1998). *P. incana* is reported to be used as a treatment for influenza, fever, kidney ailments and backache. Although the information from the Montagu Museum is usually ascribed to the late Mr Kobus Kriel, who collected anecdotes on medicinal plants over many years, the revised list of 1998 has been a team effort by Mr Pieter Moore Burger and several local experts, some of Khoi-San origin (e.g. a well-known traditional healer or *bossiedokter*, the late Doortjie Dories) (P.M. Burger, pers. comm. to B-EVW). Unfortunately, the exact origin of the anecdotes was not recorded.

3.2. Anatomy

Situated within each of the frequent depressions on both surfaces of the leaf is a multicellular glandular trichome (Fig. 3) as described in detail by Mayekiso et al. (2008). Uniseriate, non-glandular trichomes occur in large numbers all over the leaf surface (Mayekiso et al., 2008). The leaves are amphistomatic and have a thin cuticle of about 2 μm in thickness. The cell walls of the epidermal cells are slightly cutinized. The mesophyll is differentiated into palisade parenchyma, two or three layers thick, and loosely arranged spongy parenchyma. There is a solitary secretory canal below the midrib and often also below other vascular bundles. Elongated calcium oxalate crystals can be observed within the palisade parenchyma.

3.3. Essential oil composition

A number of diterpenes and other phenolic compounds have been isolated from *P. incana* and several other *Pteronia* species (Zdero et al., 1990), including an unusual lactone called

incanapteroniolide, which was found in *P. incana*. The plant is also known to produce essential oil (Bruns and Meiertoberens, 1987; Mayekiso et al., 2006; Webber et al., 1999) but possible regional variation has not yet been investigated.

The essential oil yields of eleven individual plants of *P. incana* collected from three different localities (Worcester, Herolds Bay and Alice) are listed in Table 2. The yields were exceptionally variable, ranging from 0.01% to 1.08% of dry weight. The variation seems to be unrelated to provenance and date of collection.

A total of 62 volatile components were identified in the eleven samples studied. The major compounds are several monoterpenes as well as sesquiterpenes, listed in Table 2. β-Pinene, limonene, 1,8-cineole, myrcene, spathulenol, *p*-cymene and methyleugenol are main compounds present in all or most of the samples, with smaller amounts of α-pinene, sabinene, γ-terpinene, terpinen-4-ol, biclogermacrene, globulol and α-bisabolol in several of the samples. The rare occurrence of isoelemicine is noteworthy, as it was present in three of the six samples from Herolds Bay but undetected in all other samples. Despite considerable quantitative variation the eleven samples, taken from two, three and six plants of three populations, were fairly uniform. The three populations are geographically widely separate so that the similarity in the essential oil composition (Table 2) is somewhat unexpected.

The first studies of the essential oil of *P. incana* were done by E.H. Graven at Fort Hare University as part of the pioneering Ciskei Essential Oil Project. The main compounds of 'Blue Bush Oil' or 'Blue Dog' were reported to be α-pinene (14.2%), β-pinene + sabinene (29.8%), myrcene (17.7%), limonene + 1,8-cineole (14.0%), *p*-cymene (2.3%) and terpinolene (9.4%). These values were cited by Margaris et al. (1982), Bruns and Meiertoberens (1987) and also by Webber et al. (1999), who confirmed that α-pinene, β-pinene, sabinene, myrcene, limonene, 1,8-cineole and *p*-cymene are the main compounds. Small amounts of several sesquiterpenoids were also reported and the oil was considered to resemble juniper berry oil in fragrance (Margaris et al., 1982) and pine turpentine oil in qualitative composition (Bruns and Meiertoberens, 1987). The qualitative and quantitative data given by these authors are in close agreement with the results reported here. It is noteworthy that the oil yield reported by Bruns and Meiertoberens (1987), namely between 0.4% and 1.1%, as well as the yields from 0.2% to 0.6% obtained by Webber et al. (1999), is similar to our values. Mangena and Muyima (1999) reported that, in addition to *p*-cymene, limonene, 1,8-cineole, myrcene, α-pinene and β-pinene, *P. incana* also contained *o*-cymene amongst its constituents. The essential oil was studied by Mayekiso et al. (2006), who showed that it contains a high percentage of myrcene, α-pinene and β-pinene, together with smaller amounts of sabinene, α-terpinene, 1.8-cineole and limonene. It was found that there is some seasonal variation in the composition of main compounds, with α-pinene, β-pinene, β-myrcene, sabinene and 1,8-cineole present in winter (June). At other times of the year, these main constituents were accompanied by other minor compounds such as β-thujene, d-L-limonene, α-terpinene and δ-4-carene. Our variation study (Table 2) showed that α-pinene, β-pinene, sabinene, myrcene, limonene, 1,8-cineole, *p*-cymene, terpinen-4-ol, methyleugenol, globulol, spathulenol, α-bisabolol and isoelemicine are present in several of the distillates. The reported occurrence of α-pinene, β-pinene, β-myrcene, sabinene and 1,8-cineole as the main compounds of *P. incana* is confirmed by our results. The only point of disagreement appears to be the high levels of *p*-cymene found in all of our samples. This compound should be added to the list of main ingredients of the essential oil of *P. incana*. It is interesting that the composition of the essential oil reported here for *P. incana* is very similar to that of *P. onobromoides* (Hulley et al., 2010a,b) despite obvious morphological and anatomical differences, including the absence of glandular trichomes in the latter.

3.4. Antimicrobial activity

The limited information on the medicinal uses of *P. incana* (Montagu Museum, 1998) indicates that the plant may have antimicrobial and anti-inflammatory activity. A comparative study of the antimicrobial activities of the essential oils of *Artemisia afra*, *P. incana* and *Rosmarinus officinalis* was done by Mangena and Muyima (1999) using the agar diffusion assay method and selecting bacterial and yeast strains on the basis of their significance in food spoilage and/or poisoning and also as common human and plant pathogens. According to Mangena and Muyima (1999), *P. incana* oil displayed a fairly broad spectrum of antibacterial activity, particularly at high concentrations but was not tested against any of the respiratory pathogens used in this study. As a first step towards evaluating the medicinal properties of this species, we examined possible antimicrobial activity against a selection of four respiratory pathogens.

The results showed that the methanol:dichloromethane (MeOH:CH_2Cl_2) extracts were the most active against *M. smegmatis*, a Gram-positive bacterium with MIC values as low as 0.5–0.8 mg/ml as well as *C. neoformans*, a yeast pathogen with MIC values as low as 0.5–2.0 mg/ml. The essential oils showed highest antimicrobial activity against *C. neoformans* with the most noteworthy activity of 0.3 mg/ml (sample 2).

Water and methanol:water extracts showed low or no activity at the highest concentration tested against any of the organisms studied. For instance, *K. pneumoniae* was moderately susceptible, with MIC values ranging between 1.5 and 2.0 mg/ml for two MeOH:H_2O extracts (samples 3 and 6). An exception is evident for *C. neoformans*, with MIC values ranging between 0.1 (MeOH:H_2O extracts, samples 1 and 9) and 3.5 (H_2O extracts, sample 3) mg/ml.

4. Conclusions

P. incana is poorly known as a medicinal plant but it has been used for the treatment of influenza, fever, kidney ailments and backache. These uses have remained unrecorded in the scientific literature. The anatomical study by Mayekiso et al. (2008) suggested that the essential oil is produced in the external glandular trichomes and is either released through the

pores of the cuticle or, more likely, after the rupturing of the cuticular sacs, but our study has revealed the presence of secretory ducts below the midrib and some other vascular bundles of the leaves in addition to the glandular trichomes. The type of secretion of each of these structures needs to be established. The oil is variable both in yield and in the levels of the main constituents but the combination of β-pinene, sabinene, limonene, 1,8-cineole, myrcene, spathulenol and *p*-cymene appears to be characteristic for the species. The antimicrobial activity of the methanol:dichloromethane extracts against the Gram-positive and two Gram-negative bacteria as well as all of the extracts against the yeast pathogen, suggests that the plant may have efficacy in the treatment of respiratory infections.

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