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Essential oil composition of a medicinally important Cape species: *Pentzia punctata* (Asteraceae)

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A B S T R A C T

Pentzia punctata Harv. (Asteraceae) is one of 27 species occurring in southern and North Africa. It is a Cape species that is used in traditional medicine to treat stomach ailments and respiratory complaints. *Pentzia punctata* is locally known in the Cape as *bergwildeals* and is considered to be a more potent medicine than the well-known *wildeals* (*Artemisia afra*). An infusion of the leaves can be taken to treat colds. The leaf surfaces of this species are furnished with secretory glands that are probably the site of oil accumulation. The essential oils were hydrodistilled and analysed by Gas Chromatography−Mass Spectrometry (GC/MS) and Flame-Ionisation Detectors (GC/FID) for the first time. High yields of oil were obtained − 0.85−2.45% dry wt. The oils are dominated by monoterpenes, oxygenated monoterpenes and sesquiterpenes. Camphor is a major component present, along with chamazulene which is responsible for the royal blue colour of the essential oil. Other azulene derivatives are also present. Of interest was the presence of five major compounds: camphor (up to 27.3%), chamazulene (up to 19.8%), sabinene (up to 10.8%), spathulenol (up to 11.8%) and yomogi alcohol (to 22.6%).

1. Introduction

Pentzia punctata Harv. (Asteraceae) is one of about 27 species of aromatic African shrubs, 19 of which occur in southern Africa. The plants are locally dominant in many parts of the Karoo region of South Africa and some are important sources of fodder for sheep. Despite their highly aromatic leaves and popular use in traditional medicine, almost no information could be found in the literature about the essential oil of *Pentzia* species, and nothing whatsoever for *P. punctata*. This species is known as *vaalkaroo* and is used to treat fevers, typhoid and convulsions (Shearing, 1994). According to Van Wyk et al. (2008), it is used in the Great Karoo of South Africa, where it is known locally as *bergwildeals*, literally “the *wildeals* [*Artemisia afra*] from the mountain”. Two anecdotes from very knowledgeable persons were recorded by Van Wyk et al. (2008): (1) “colds and stomach problems − drink an infusion” – “better than *mak* wildeals, used in the Sneeuberg area”; and (2) “used against colds” – “stronger than *wildeals*”. Recent ethnobotanical studies (Van Wyk, unpublished data) revealed that *P. punctata* is one of the most popular and well-known medicinal plants of the southern parts of the Great Karoo region of South Africa.

This appears to be the first report on the composition of the essential oil of this highly aromatic and valuable medicinal plant. We also studied the leaf anatomy of *P. punctata* to determine the possible site of oil production and/or oil accumulation.

2. Experimental

2.1. Plant material

Fresh aerial parts consisting of stems and leaves of *Pentzia punctata* shrubs (Fig. 1) were collected from two different localities in the Cape of South Africa. Three samples were collected at each locality to explore possible chemical differences between individual plants. Voucher specimens were deposited in the Herbarium of the University of Johannesburg (JRAU).

Samples were collected at the following two localities:

Colesberg (30° 49′ 5.0″ S, 24° 57′ 34.6″ E, 1429 m); date of collection 31 March 2015; date of hydrodistillation 08 March 2016; Mass

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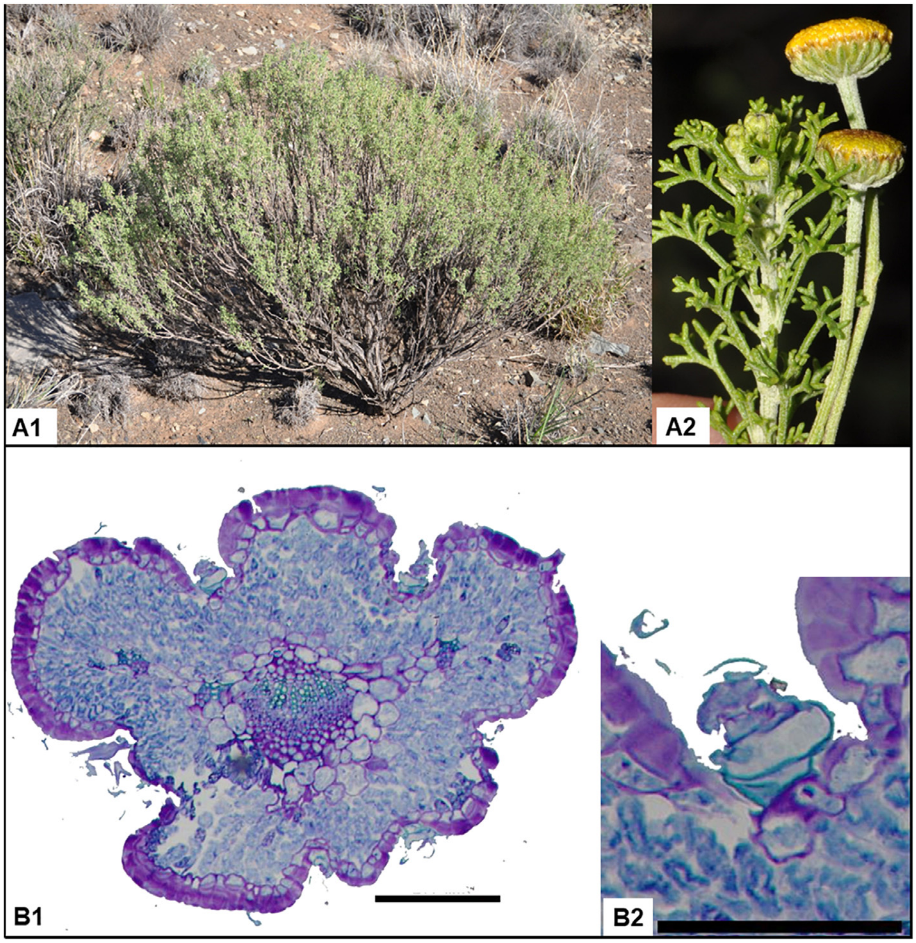

Fig. 1. Morphology of *Pentzia punctata*, showing the growth form/habit (A1) and the leaves and flower heads (A2). Photographs: B.-E. van Wyk. Leaf anatomy (B1 and B2): transverse section of leaf and gland (note the ruptured head). Scale bars: B1 = 200 μm; B2 = 100 μm. Photographs: I.M. Hulley.

of plant material distilled (g) sample 1-51.69, 2-53.23, 3-25.16, voucher specimens (three plants) Hulley & Van Wyk 36-16a, 36-16b, 36-16c (JRAU).

Hutchinson (31° 34′ 03.8′′ S, 23° 15′ 57.8′′ E, 1365 m); date of collection 31 March 2015; date of hydrodistillation 07 March 2016; Mass of plant material distilled (g) sample 4-97.24, 5-91.32, 6-88.24, voucher specimens (three plants) Hulley & Van Wyk 35-16a, 35-16b, 35-16c (JRAU).

2.2. Methodology

Fresh leaf material was preserved and treated according to the method of Feder and O'Brien (1968) as described in Hulley et al. (2018).

Isolation of the essential oil from the air-dried aerial parts was done by hydro-distillation for three hours, using a Clevenger-type apparatus (European Pharmacopoeia, 2005). The oil yield percentage was calculated on a dry weight basis (Table 1), dried over anhydrous sodium sulfate, and stored in sealed amber glass vials in the refrigerator (4 °C) until GC/MS analyses.

The chemical analysis of the essential oil of six samples were performed on a GC (Agilent 6890N GC system) for quantification and a GC/MS (Agilent 5975 GC/MSD system) for identification, using the same conditions as described in Hulley et al. (2018).

Volatile constituents were identified by parallel comparison of their retention indices and mass spectra using the same data bases as stated in Hulley et al. (2018).

Nuclear Magnetic Resonance (NMR) spectroscopy was used to confirm the identity of three of the compounds isolated with flash chromatography. A Bruker Avance 500 MHz machine was used to produce ^{1}H and ^{13}C spectra which were matched to published spectra of artemisyl acetate (Hethelyi et al., 1981), artemisia alcohol (Bertea et al., 2005) and yomogi alcohol (Sasaki et al., 1971).

3. Results and discussion

3.1. Leaf trichome morphology

The leaves of *P. punctata* are amphistomatic with a few deep indentations in which small glands are concentrated. Other characters of the leaves are the very thin cuticle and highly cutinized outer periclinal cell walls. The parenchyma cells are relatively short and consist of several cell layers. The mesophyll cells are unevenly thickened on the outside of the xylem and phloem and surrounded by very prominent bundle sheath cells around the central vascular bundle with up to two smaller bundles on either side.

The glands are peltate with a single basal cell, one to two stalk cells and a head cell. They are small, with an average length of 67 μm and width of 54 μm (Fig. 1: B2). These glands are presumably the site for oil production. It appears that the oil accumulates beneath the cuticle of the head cells and eventually ruptures to release the oil.

Table 1
Essential oil composition of six samples of *Pentzia punctata* from two localities in the Great Karoo, South Africa. The main compounds are indicated in bold (when at least one sample had more than 10% of the total yield).

No	RRI	Compound	Hutchinson			Colesberg		
			H1	H2	H3	C1	C2	C3
Oil yield (mg/g dry weight)			21.7	24.5	22.7	8.6	9.8	17.6
1.	1014	Tricyclene	0.2	0.3	t	t	t	t
2.	1032	α-Pinene	1.6	2.4	1.5	0.8	0.4	0.4
3.	1035	α-Thujene	0.1	0.1	0.2	0.4	0.2	t
4.	1043	Santolinatriene	t	t	–	t	t	t
5.	1076	Camphene	3.8	6.4	3.1	0.9	1.2	–
6.	1118	β-Pinene	2.1	2.4	1.7	0.8	0.3	0.4
7.	1132	**Sabinene**	**5.7**	**3.6**	**8.1**	**10.8**	**4.4**	**2.6**
8.	1174	Myrcene	2.0	1.0	1.7	5.5	2.4	1.2
9.	1176	α-Phellandrene	–	1.1	1.4	2.4	2.3	1.1
10.	1188	α-Terpinene	0.5	0.3	0.7	1.0	1.1	0.2
11.	1203	Limonene	0.6	0.7	0.6	0.9	0.7	0.5
14.	1213	1,8-Cineole	0.9	0.4	3.7	1.2	1.1	0.8
15.	1218	β-Phellandrene	–	t	–	–	–	–
16.	1246	(*Z*)-β-Ocimene	0.1	0.2	0.4	1.7	0.9	0.5
17.	1255	γ-Terpinene	1.2	1.2	2.1	3.2	–	0.8
18.	1266	(*E*)-β-Ocimene	–	–	–	0.2	–	0.3
19.	1280	*p*-Cymene	1.4	2.9	3.7	6.7	6.3	1.8
20.	1290	Terpinolene	0.2	0.1	0.3	0.4	0.5	0.1
21.	1348	6-Methyl-5-hepten-2-one	–	t	–	t	t	–
22.	1358	Artemisiaketone	–	0.1	–	0.1	0.3	0.4
23.	1403	**Yomogi alcohol**	**14.8**	**14.6**	**14.7**	**3.6**	**14.8**	**22.6**
24.	1430	Artemisyl acetate	0.9	0.6	0.7	0.2	0.6	1.0
25.	1450	*trans*-Linalool oxide (Furanoid)	–	–	–	t	–	–
26.	1474	*trans*-Sabinene hydrate	–	–	–	t	–	–
27.	1495	Bicycloelemene	–	–	–	t	t	t
28.	1497	α-Copaene	–	–	0.2	–	0.2	0.2
29.	1499	α-Campholene aldehyde	–	0.1	–	–	–	–
30.	1510	Artemisia alcohol	2.8	2.2	2.5	0.8	3.6	4.3
31.	1528	α-Bourbonene	t	–	–	t	t	t
32.	1532	**Camphor**	**19.4**	**27.3**	**13.3**	**0.8**	**0.2**	**0.2**
33.	1535	β-Bourbonene	t	–	–	–	0.3	0.1
34.	1541	Benzaldehyde	–	–	–	0.1	t	–
35.	1544	α-Gurjunene	–	–	–	–	–	0.3
36.	1549	β-Cubebene	–	–	–	–	t	–
37.	1551	Theaspirane B	–	–	–	–	t	–
38.	1553	Linalool	0.2	0.2	0.2	0.2	0.2	2
39.	1556	*cis*-Sabinene hydrate	–	–	–	t	–	–
40.	1562	Isopinocamphone	–	–	–	–	–	0.2
41.	1565	Linalyl acetate	0.1	–	0.1	0.2	–	3.2
42.	1571	*trans*-*p*-Menth-2-en-1-ol	0.1	–	0.2	0.2	0.3	0.2
43.	1586	Pinocarvone	t	–	–	–	–	–
44.	1589	Acetoxy linalooloxide	–	–	–	–	t	t
45.	1589	β-Ylangene	–	–	–	–	t	–
46.	1590	Bornyl acetate	0.2	0.7	0.5	1.0	0.2	0.1
47.	1600	β-Elemene	0.1	0.2	0.1	0.2	0.2	–
48.	1606	β-Copaene	–	–	–	–	t	–
49.	1611	Terpinen-4-ol	2.6	1.4	3.7	4.6	6.7	1.8
50.	1612	β-Caryophyllene	0.4	0.2	0.1	0.2	0.3	3.0
51.	1628	Aromadendrene	0.1	t	–	0.2	0.1	0.1
52.	1635	Cadina-3,5-diene	–	–	–	–	t	–
53.	1636	2-Methyl-6-methylene-3,7-octadiene-2-ol[a]	0.1	t	–	0.1	t	–
54.	1638	*cis*-*p*-Menth-2-en-1-ol	0.1	t	0.1	0.3	0.2	0.1
55.	1648	Myrtenal	–	t	–	–	–	–
56.	1651	Sabinaketone	–	–	–	0.3	0.1	–
57.	1661	Alloaromadendrene	0.2	0.1	0.1	t	0.2	–
58.	1670	*trans*-Pinocarveol	–	–	–	t	–	–
59.	1674	*p*-Mentha-1,5-dien-8-ol	–	t	–	–	–	–
60.	1677	epi-Zonarene	–	–	–	–	0.1	0.1
61.	1684	*trans*-Chrysanthemol	–	t	t	–	0.2	–
62.	1686	Lavandulol	–	0.5	0.2	0.4	–	0.9
63.	1687	α-Humulene	0.3	–	–	–	0.3	t
64.	1688	Selina-4,11-diene (=*4,11-Eudesmadiene*)	–	–	–	–	–	0.4
65.	1689	*trans*-Piperitol	–	–	t	–	0.1	t
66.	1704	δ-Selinene	–	–	–	–	–	0.1
67.	1705	γ-Muurolene	t	–	1.2	–	0.1	t
68.	1706	α-Terpineol	0.3	–	0.3	0.3	t	1.1
69.	1708	Ledene	0.2	0.2	t	0.3	0.5	–
70.	1719	Borneol	1.0	2.8	0.7	3.1	2.9	1.7
71.	1726	Germacrene D	1.2	1.0	–	1.0	0.6	0.8
72.	1726	α-Zingiberene	0.2	–	–	–	–	–
73.	1732	Bicyclosesquiphellandrene	–	–	t	–	0.1	0.4
74.	1733	Neryl acetate	–	–	–	–	0.2	0.3
75.	1740	α-Muurolene	–	–	t	–	0.1	0.3
76.	1742	β-Selinene	0.3	0.3	0.3	–	0.1	–
77.	1743	α-Cadinene	–	–	–	–	t	–

(*continued*)

Table 1 (*Continued*)

No	RRI	Compound	Hutchinson			Colesberg		
			H1	H2	H3	C1	C2	C3
78.	1755	Bicyclogermacrene	6.0	3.2	3.7	7.9	4.0	2.8
79.	1758	*cis*-Piperitol	–	t	t	0.1	0.1	–
80.	1765	Geranyl acetate	–	–	–	0.1	0.1	0.4
81.	1773	δ-Cadinene	0.1	0.1	0.5	0.6	1.2	2.1
82.	1776	γ-Cadinene	–	t	–	0.1	0.1	0.2
83.	1783	β-Sesquiphellandrene	0.3	–	–	–	–	–
84.	1784	Campholene alcohol	–	t	–	0.3	–	0.2
85.	1786	Neryl propionate	–	t	–	–	–	–
86.	1786	ar-Curcumene	0.1	–	–	0.2	–	–
87.	1787	Aromadendra-1(10),4(15)-diene	0.1	t	t	–	0.1	–
88.	1788	Linalyl-3-methyl butanoate[a]	0.1	–	–	–	–	–
89.	1799	Cadina-1,4-diene (=*Cubenene*)	–	–	–	–	t	t
90.	1802	Cuminaldehyde	–	t	t	0.1	0.1	–
91.	1804	Myrtenol	0.1	t	–	–	–	–
92.	1808	Nerol	–	–	–	–	–	0.2
93.	1811	*p*-Mentha-1,3-dien-7-al	–	–	–	0.1	t	–
94.	1814	*p*-Mentha-1,5-dien-7-ol	–	–	t	t	t	–
95.	1814	Liguloxide	0.4	0.5	1.1	–	–	–
96.	1823	*p*-Mentha-1(7),5-dien-2-ol	–	0.1	0.1	0.2	0.2	0.1
97.	1853	*cis*-Calamenene	–	–	–	–	t	t
98.	1857	Geraniol	–	–	–	–	–	0.4
99.	1864	*p*-Cymen-8-ol	t	t	0.2	0.1	0.1	t
100.	1882	*cis*-Carveol	–	–	–	–	–	t
101.	1900	*epi*-Cubebol	–	–	0.1	–	0.1	0.1
102.	1921	α-Phellandrene epoxide	–	0.1	t	t	–	–
103.	1953	Palustrol	0.1	–	0.1	0.4	0.4	0.4
104.	1957	Cubebol	–	–	0.2	0.3	0.2	0.3
105.	1969	*cis*-Jasmone	–	0.1	–	0.1	t	t
106.	2008	Caryophyllene oxide	0.1	0.2	0.2	–	0.2	0.6
107.	2012	Maaliol	0.1	–	–	0.2	0.1	–
108.	2050	(*E*)-Nerolidol	–	–	–	–	–	0.3
109.	2057	Ledol	0.1	t	–	0.2	0.1	0.1
110.	2069	1(10),5-Germacradien-4β-ol	–	–	–	–	–	0.4
111.	2073	*p*-Mentha-1,4-dien-7-ol	0.1		0.2	0.2	0.2	–
112.	2078	Cubeban-11-ol	–	t	t	–	–	–
113.	2080	Cubenol	–	–	0.1	–	0.2	0.2
114.	2088	1-*epi*-Cubenol	–	–	–	–	0.2	0.4
115.	2095	Junenol (= *Eudesm-4(15)-en-6-ol*)	–	–	–	0.1	–	–
116.	2096	Elemol	–	1.1	–	1.1	1.6	–
117.	2098	Globulol	–	–	–	–	–	0.2
118.	2103	Guaiol	–	0.2	–	–	–	–
119.	2104	Viridiflorol	–	–	0.2	0.5	0.5	0.3
120.	2113	Cumin alcohol	–	–	–	0.1	0.1	–
121.	2144	Rosifoliol	–	–	–	0.1	0.2	–
122.	2144	**Spathulenol**	**10.0**	**6.1**	**8.6**	**11.8**	**10.1**	**3.6**
123.	2185	γ-Eudesmol	0.2	0.3	–	1.8	1.1	–
124.	2186	Eugenol	–	–	0.1	–	–	–
125.	2187	T-Cadinol	–	–	0.2	–	–	0.5
126.	2198	Thymol	–	–	–	0.1	–	–
127.	204	Eremoligenol	–	–	–	0.1	–	–
128.	2209	T-Muurolol	–	–	1.4	0.7	3.9	3.4
129.	2219	δ-Cadinol (=*α-Muurolol*)	–	–	–	–	–	0.2
130.	2228	Isospathulenol	0.1	–	–	0.1	–	–
131.	2230	Pogostol (=*11-Guaien-10-ol*)	0.2	0.3	–	–	–	–
132.	2247	*trans*-α-Bergamotol	0.4	0.6	0.4	1.1	1.1	0.3
133.	2255	α-Cadinol	–	–	1.3	0.6	2	2.4
134.	2257	β-Eudesmol	3.9	2.3	t	1.8	1.8	–
135.	2265	Guaia-6,10(14)-dien-4 β-ol	–	0.3	–	0.1	–	–
136.	2273	Selin-11-en-4α-ol	–	–	–	–	t	–
137.	2312	9-Geranyl-*p*-cymene	–	0.1	–	–	–	–
138.	2389	Caryophyllenol I	–	0.1	–	–	–	–
139.	2392	Caryophyllenol II	–	–	–	0.1	–	–
140.	2400	Eudesma-4(15),7-dien-1-β-ol	–	–	–	0.1	–	–
141.	2430	**Chamazulene**	**4.6**	**3.8**	**5.0**	**10.8**	**9.3**	**19.8**
Total			**93.1**	**95.1**	**92.1**	**97.4**	**95.0**	**96.5**
Monoterpene hydrocarbons			19.5	22.8	25.5	35.7	20.7	9.9
Oxygenated monoterpenes			43.8	51.2	41.4	18.7	32.4	42.2
Sesquiterpene hydrocarbons			14.2	9.1	11.2	21.5	17.9	30.7
Oxygenated sesquiterpenes			15.6	12.0	13.9	21.4	24	13.7
Others			0.0	0.0	0.1	0.1	0.0	0.0

RRI Relative retention indices calculated against n-alkanes.
% calculated from FID data.
t Trace (< 0,1%).
[a] Tentatively identified from the Wiley database.

3.2. Essential oil composition

The results of the oil analyses are presented in Table 1. The yields were relatively high and varied between *ca.* 9 mg (0.86%) and almost 25 mg (2.45%) per g dry weight.

The royal blue colour of the oil is due to chamazulene, present in all samples of both populations (3.8 to 19.8%). Despite considerable chemical variation, the two populations are quite similar, except for the distinct quantitative difference in camphor – 13.3 to 27.3% in the Hutchinson population and only 0.2–0.8% in the Colesberg population. In addition to chamazulene and camphor, three other major compounds (above 10% concentration in at least one sample) are present, namely sabinene (2.6–10.8%), yomogi alcohol (3.6–22.6%) and spathulenol (3.6–11.8%).

We have recently examined the essential oil composition of *Pentzia incana* (Thunb.) Kuntze, the only other species of *Pentzia* for which essential oil data are available (Hulley et al., 2018). This oil was not blue and only 2 samples out of 17 studied had very small amounts of chamazulene (0.4% in both). The two oils agree in the presence of camphor and yomogi alcohol as major constituents. Sabinene occurs in almost all samples of *P. incana* but is invariably a minor compound (below 0.6%); spathulenol occurs in all 17 samples at levels of 1.1–8.9%. Amongst the 225 compounds identified in *P. incana*, additional major compounds were 1,8-cineole, artemisia ketone, artemisia alcohol, linalyl actetate, fragranyl acetate, bicyclogermacrene and fragranol (Hulley et al., 2018).

4. Conclusions

Chamazulene, one of the major components, has demonstrated anti-inflammatory activity *in vivo* (Safayhi et al., 1994). The antispasmodic activity suggested by the traditional medicinal uses might be derived from the same chemical homologues as those present in species of *Artemisia* that are known for antispasmodic effects (Abad et al., 2012; Yashphe et al., 1987). Essential oil from *P. punctata* are chemically similar to many species of *Artemisia* (such as *A. herba-alba* Asso). It is noteworthy that the vernacular name for *P. punctata* (*bergwildeals*) also suggests a similarity to *Artemsia afra* (*wildeals*). Local people in the Karoo claim that it is similar in action to *A. afra* but only much stronger. The relatively high yields of essential oil may also be a contributing factor in the perceived efficacy of this poorly known Cape herbal medicine.

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