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Research Note

Essential oil composition of *Pentzia incana* (Asteraceae), an important natural pasture plant in the Karoo region of South Africa

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***Pentzia incana* is one of the most important of all natural pasture plants in the dry interior (Karoo) region of South Africa. This highly aromatic shrub is thought to be responsible for the distinctive flavour of Karoo lamb (a registered geographical indication), yet the essential oil is here characterised for the first time. Leafy twigs are traditionally chewed for relief of stomach ache. Essential oil is associated with numerous small, multicellular glands. Gas chromatography and gas chromatography/mass spectrometry analysis of 17 essential oil samples from five populations showed considerable variation in both yield (0.12% to 0.88% dry weight) and composition. Of interest was the presence of one major biosynthetic group comprising yomogi alcohol (to 38.9%), artemisia alcohol (to 26.1%), artemisia ketone (to 35.0%) and artemisyl acetate (to 9.4%). High levels of fragranol (26.9%) and fragranyl acetate (27.1%) were observed only in a single specimen. Other main compounds were 1,8-cineole (to 16.7%), santolina alcohol (to 11.3%), camphor (to 47.9%), linalyl acetate (to 17.4%) and bicyclogermacrene (to 11.8%). Six of the 10 major compounds were isolated and their identities confirmed by nuclear magnetic resonance. The volatile compounds may possibly be linked to the medicinal use of *P. incana*, as well as the flavour profile of Karoo lamb.**

Keywords: deciduous, evergreen, leaf phenology, plant functional types, semi-arid

Pentzia incana (Thunb.) Kuntze is one of the best known and most widely distributed aromatic shrubs of the Asteraceae endemic to southern Africa. It is a characteristic element of the Karoo region (both the Nama-Karoo and Succulent Karoo Biomes), as reflected in the Afrikaans vernacular name *karoobossie* (Smith 1966; van Breda and Barnard 1991). It is of considerable ecological value because it counteracts erosion due to the stoloniferous habit, as reflected in another Afrikaans vernacular name *ankerkaroo* (Esler et al. 2006). This name literally means 'anchor karoo' and refers to the drooping branches that form new plants where they touch the ground (Figure 1A).

Although not particularly palatable to sheep, it is nevertheless regarded as one of the most valuable of all Karoo shrubs. This is because it is well utilised during certain times of the year, hence the Afrikaans common name *skaapbossie* (Le Roux et al. 1994; Shearing 1994) or *kortbeenskaapbos* in the Calvinia district (Vlok 1963). The palatability of the plant is known to vary from region to region (Le Roux et al. 1994), suggesting possible chemical variation amongst populations. The relatively low palatability is actually an advantage because it prevents harmful overgrazing and ensures persistence. *Pentzia incana* has been classified as a 'partial leaf-shedder' because it may lose all or most of its leaves during drought but regrows leaves and even flowers within a week after rain (Esler et al. 2006). Many people believe that it is this strongly aromatic shrub that gives Karoo lamb its distinctive flavour (van Breda and Barnard 1991; Esler et al. 2006). Using stable isotope ratio analysis, Erasmus et al. (2016) showed that a clear link can be established between the provenance of Karoo lamb (which is registered and trademarked as a geographical indication) and the diet of the animals. It is noteworthy that *P. incana* was one of the major fodder species that was sampled for all of the typical Karoo provenances that were analysed in this study (Erasmus et al. 2016).

Pentzia incana is widely used in traditional medicine in the Cape region of South Africa. The main use, confirmed by numerous participants during ethnobotanical field studies, is to treat stomach cramps (van Wyk et al. 2008). The fresh material is typically chewed *in situ* ('when out in the veld'); the fibres are spat out and the juice swallowed. It is also used as an infusion for numerous other ailments, especially for respiratory conditions, colds and influenza (IMH et al. unpublished data).

Despite the obvious ecological, agricultural and medicinal importance of this aromatic Karoo bush, the yield and composition of its essential oil has apparently not been published in the scientific literature. Bohlmann and Zdero (1978) reported acetylenic compounds (linalyl acetate and

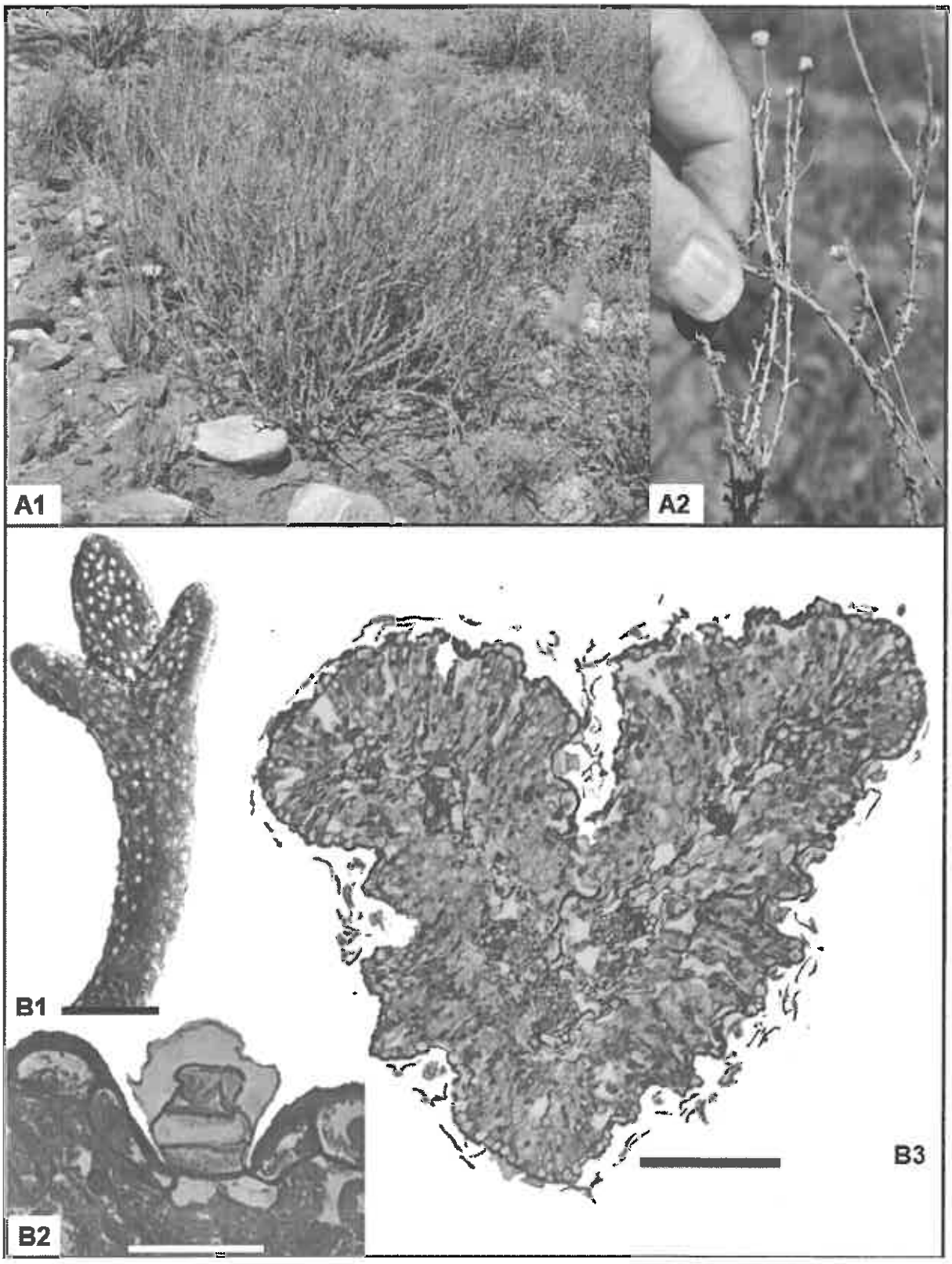

Figure 1: Morphology of *Pentzia incana*, showing the growth form/habit (A1) and the leaves and flower heads (A2) (photographs: B-E van Wyk). Leaf morphology (B1) and leaf anatomy (B2 and B3): transverse section of leaf and gland. Scale bars: B1 = 1 000 µm; B2 = 50 µm; B3 = 200 µm (photographs: IM Hulley)

a mixture of unidentified sesquiterpene lactones) in *Pentzia* species that were very similar to those of *Tanacetum* species. No information could be found on the anatomical details of the leaf glands (presumably the site of origin and/or accumulation of the oil). Anatomical information may also be of value in the botanical identification of plant remains in faecal and rumen samples. This study was focused on analysing and describing the essential oil of *P. incana* for the first time (which could possibly relate to the flavour profile of Karoo lamb), including possible regional variation.

Fresh aerial parts consisting of stems and leaves of *P. incana* were collected from five different localities in the Cape region of South Africa (Table 1). Two bulk samples, comprising a mixture of several individual plants, were collected at Prince Albert in the Great Karoo and at Vanwyksdorp in the Little Karoo. To explore possible differences between individual plants, three samples were collected at each locality, giving a total of 17 samples (Table 1). Voucher specimens were deposited in the Herbarium of the University of Johannesburg (JRAU).

Fresh leaf material was preserved in formalin:acetic acid: alcohol (FAA) (5:5:90) for at least 24 h. Small leaf portions were treated in accordance with the method of Feder and O'Brien (1968) for embedding in glycol methacrylate (GMA). The material was dehydrated using a graded alcohol series and embedded in GMA-containing capsules. The GMA was polymerised at 60 °C for 24 h after which thin sections of about 3 µm thick were cut using a Porter Blüm ultramicrotome. The sections were stained with Schiff's reagent and toluidine blue (Feder and O'Brien 1968) and observed under a light microscope (Olympus CX41) equipped with a digital camera and a computerised data capturing system (Olympus ColorView Soft Imaging System).

The essential oil was isolated from carefully air-dried stems and leaves by continuous hydrodistillation for 3 h, using a Clevenger-type apparatus (European Pharmacopoeia 2005). The oil yield percentage was calculated on a dry weight basis (Table 1). Oil samples were dried over anhydrous sodium sulfate and stored in sealed amber glass vials in a refrigerator (4 °C) until gas chromatography/mass spectrometry (GC/MS) analyses.

The GC and GC/MS conditions were as follows. System: Agilent 5975 GC/MSD system and Agilent 6890N GC system; column: HP-Innowax FSC column (60 m × 0.25 mm, 0.25 µm film thickness); carrier gas: helium (0.8 mL min^{-1}); GC oven temperature: 10 min at 60 °C, 4 °C min^{-1} to 220 °C, 10 min at 220 °C, 1 °C min^{-1} to 240 °C (total 80 min); injection mode: split mode (40:1); injection temperature: 250 °C; mass spectrum: 70 eV; mass range: m/z 35 to 450; GC detector: FID at 300 °C. To obtain the same elution order with GC/MS, simultaneous injection was done by using the same column and appropriate operational conditions.

Identification of the volatile constituents was achieved by parallel comparison of their retention indices and mass spectra with data stored in the Wiley GC/MS Library (Wiley, New York, NY, USA), MassFinder software 4.0 (Dr Hochmuth Scientific Consulting, Hamburg, Germany), Adams Library (2007), NIST Library and the in-house 'Başer Library of Essential Oil Constituents', which includes more than 3 500 genuine compounds with mass spectra and retention data. *n*-Alkanes (C_8–C_{40}) were used as reference points in the calculation of retention indices (RI). Quantification of volatiles components was

Table 1: Voucher specimen details of the 17 samples of plant material of *Pentzia incana* that were studied for their essential oil yields and compositions

Sample no.	Locality and date of collection	Coordinates and elevation	Voucher specimens (all deposited in JRAU)	Date of hydrodistillation	Mass of material distilled (g)
K1	Kamieskroon 18 Feb 2016	30°12'16.1" S, 17°56'31.9" E 763 m	Ruiters and van Wyk 28a	22 Feb 2016	77.9
K2			Ruiters and van Wyk 28b	21 Feb 2016	85.0
K3			Ruiters and van Wyk 28c	22 Feb 2016	65.4
K4			Ruiters and van Wyk 28d	21 Feb 2016	78.5
N5	Nieuwoudtville 5 Aug 2016	31°26'17.6" S, 19°08'36.8" E 705 m	Hulley and van Wyk 37-16a	18 Aug 2016	33.6
N6			Hulley and van Wyk 37-16b		35.4
P7	Prince Albert 18 May 2016	33°20'20.8" S, 23°26'55.0" E 845 m	Hulley and van Wyk 31-16a	27 May 2016	55.9
P8			Hulley and van Wyk 31-16b		59.5
P9			Hulley and van Wyk 31-16c		76.9
P10			Mixture (several plants)	29 May 2016	101.9
O11	Oudtshoorn 16 May 2016	33°34'18.0" S, 22°11'36.5" E 351 m	Hulley and van Wyk 27-16a	26 May 2016	61.3
O12			Hulley and van Wyk 27-16b		92.5
O13			Hulley and van Wyk 27-16c		77.9
V14	Vanwyksdorp 19 May 2016	33°46'37.1" S, 21°39'35.3" E 261 m	Hulley and van Wyk 32-16a	30 May 2016	94.08
V15		33°46'39.2" S, 21°39'52.3" E 279 m	Hulley and van Wyk 32-16b		94.7
V16		33°47'13.1" S, 21°40'10.2" E 224 m	Hulley and van Wyk 32-16c		95.3
V17		33°46'37.1" S, 21°39'35.3" E 270 m	Mixture (several plants)		260.5

performed on the basis of their GC/FID peak areas using integration data.

Nuclear magnetic resonance (NMR) spectroscopy was used to confirm the identity of several of the main compounds isolated in flash chromatography. A Bruker Avance 500 MHz machine was used to produce 1H and 13C spectra, which were matched to published spectra of artemisyl acetate (Sasaki et al. 1971), artemisia alcohol (Bertea et al. 2005), artemisia ketone (Umlauf et al. 2004), fragranol (Weyerstahl et al. 1987), fragranyl acetate (Radulović et al. 2012) and yomogi alcohol (Hethelyi et al. 1981).

The leaves of *P. incana* are conduplicate and amphistomatic, with a deep adaxial groove and numerous small indentations over the entire leaf surface in which small glands are concentrated (Figure 1B1). The leaves are also characterised by a thin cuticle and very highly cutinised outer periclinal epidermal cell walls. Palisade parenchyma is adjacent to the epidermis with spongy parenchyma in the centre. The vascular tissue comprises a main bundle in the centre, surrounded by two or three layers of bundle sheath cells and up to about three smaller bundles on the sides, each with a single layer of bundle sheath cells (Figure 1B3).

The glands have a single basal cell, one or two stalk cells and usually two head cells. They are small, averaging 46 µm long and 37 µm wide (Figure 1B2). These glands are presumably the site for oil production. It appears that the oil accumulates beneath the cuticle of the head cells, which eventually ruptures to release the oil.

The results of the oil analyses are presented in Table 2. The yields were relatively low and varied between ~1.2 mg (0.12%) and almost 9 mg (0.88%) per gram dry weight. Considerable chemical variation was observed between the different populations as well as the individual plants of *P. incana*. The main compounds were (given in descending relative retention index [RRI] order and in bold in Table 2) 1,8-cineole (to 16.7%), artemisia ketone (to 35.0%), yomogi alcohol (to 38.9%), santolina alcohol (to 11.3%), artemisia alcohol (to 26.1%), camphor (to 47.9%), linalyl acetate (to 17.4%), fragranyl acetate (27.1%), bicyclogermacrene (to 11.8%) and fragranol (26.9%). The identity of six of the 10 major compounds was confirmed by NMR spectroscopy and these only occurred in certain populations (Table 2). The major compounds illustrated both qualitative and quantitative variability between individual plants and populations. The results show combinations of several monoterpenoids and sesquiterpenoids. Monoterpene hydrocarbons ranged from trace amounts to 13.7%, oxygenated monoterpenes from 12.4% to 72.8%, sesquiterpene hydrocarbons from 2.3% to 30.8% and oxygenated sesquiterpenes from 9.2% to 42.3%. Fatty acids and their esters were present in small amounts (up to 1.9%). Of special interest is the presence of a major biosynthetic group, comprising yomogi alcohol, artemisia alcohol, artemisia ketone and artemisyl acetate.

Yomogi alcohol was present as a main compound in the Vanwyksdorp population (16.6% to 44.1%) and Oudtshoorn population (1.3% to 29.8%) and to a lesser extent in the Nieuwoudtville population (3.4% to 6.3%). Artemisia alcohol was present in all of the samples from Vanwyksdorp (8.3% to 19.8%), Oudtshoorn (0.4% to 26.1%) and Nieuwoudtville (1.7% to 7.6%) and in two samples from Prince Albert (0.2% to 2.6%). Artemisia ketone dominated in two samples, one from Prince Albert (35.0%) and one from Oudtshoorn (31.9%). It also occurred in all of the Vanwyksdorp samples (0.1% to 5.3%). Artemisyl acetate was found in all samples from Vanwyksdorp (2.5% to 9.4%) and Nieuwoudtville (1.6% to 4.8%) but only in one sample from Prince Albert (6.2%). Fragranol and fragranyl acetate were present in a single sample from Kamieskroon. Fragranol is an irregular plant monoterpenol that was previously reported only from three genera of Asteraceae, namely *Achillea* L., *Artemisia* L. and *Tanacetum* L. Its epimer, grandisol, co-occurs in the majority of species from these genera. However, grandisol was not detected in *Tanacetum coccineum* (Willd.) Grierson and *Achillea umbellata* Sm. (Radulović et al. 2012) nor in *P. incana*. Fragranol and fragranyl acetate occurred only in one sample from Kamieskroon (26.9% and 27.1%, respectively).

Camphor is biosynthetically related to borneol, which was also present in relatively small amounts in several samples. Camphor occurred in practically all samples and was most abundant in three samples from Kamieskroon, at 20.7%, 45.4% and 47.9% of the respective totals. Several compounds showed a universal (or almost universal presence), namely α-pinene, β-pinene, limonene, γ-terpinene, *p*-cymene, terpinen-4-ol, β-caryophyllene, alloaromadendrene, germacrene D, δ-cadinene, caryophyllene oxide, ledol, caryophylla-2(12),6(13)-dien-5-one, globulol, viridiflorol, spathulenol, T-muurolol, *trans*-α-bergamotol, and caryophylladienol I and II.

We used principal component analysis (PCA) in an attempt to distinguish between possible *P. incana* chemical variants based on the essential oil compositions of the 17 samples and the relative amounts of 225 volatile constituents (Table 2) detected in their chromatographic profiles. Quantitative analyses were performed using the 13 major compounds that were abundant enough in the oils to be quantified using the FID detector. Coding of the essential oil constituents was given as follows: I 1,8-cineole, II artemisia ketone, III yomogi alcohol, IV artemisyl acetate, V artemisia alcohol, VI camphor, VII linalool, VIII linalyl acetate, IX fragranyl acetate, X bicyclogermacrene, XI fragranol, XII spathulenol and XIII β-eudesmol. After submitting the normalised data matrix to PCA, it was possible to extract three potential groups of oils: compounds VII, VIII, X, XII and XIII were highly representative for the oils from the P7, P8, P9 and O12 localities. Compounds I, II, VI, IX, XI were representative for the oils from K1, K2, K3, K4 and O14. Compounds III, IV, V and VI were representative for the oils from the V15, V16, V17, O13, N6, P10 and O11 localities. In general, however, some populations were relatively uniform in their main volatile constituents, whereas others showed pronounced intra-population variation. The differences between populations showed no obvious geographical trends in the essential oil composition, perhaps due to the relatively small number of samples that were analysed.

The antispasmodic activity achieved in traditional therapeutic use 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 oils from *P. incana* are chemically similar to many from *Artemisia* (i.e. *A. herba-alba* Asso) and some of these components have been

Table 2: Chemical composition of *Pentzia incana* essential oils. Major compounds are indicated in bold (when at least one sample had more than 10% of total yield). Samples are numbered consistent with Table 1. Values are the percentage calculated from FID data. t = Trace amounts only (i.e. less than 0.05% of total yield). RRI = relative retention indices calculated against *n*-alkanes (C_9–C_{40}) on a HP-Innowax column. ID = Identification method: a = identification based on retention index of authentic compounds on the HP-Innowax column; b = identification on the basis of computer matching of the mass spectra from Başer; c = tentative identification on the basis of computer matching of the mass spectra from the Adams, MassFinder, Wiley, and NIST libraries; d = identification of isolated compound by NMR spectroscopy (see text)

No.	RRI	Compound	Kamieskroon				Nieuwoudt-ville		Prince Albert				Oudtshoorn				Vanwyksdorp			ID
			K1	K2	K3	K4	N5	N6	P7	P8	P9	P10	O11	O12	O13	O14	V15	V16	V17	
Oil yield (mg g^{-1} dry weight)			2.47	5.79	1.07	5.47	2.16	1.93	4.08	8.80	1.07	2.14	1.36	3.96	6.62	6.58	4.71	5.68	4.30	
1	1014	Tricyclene		0.2		t														a,b,c
2	1032	α-Pinene		1.2	0.7	2.2	0.5	0.5	0.7	t	0.3	0.2	0.6		2.0	1.8	0.4	0.3	0.1	a,b,c
3	1035	α-Thujene																	t	a,b,c
4	1043	Santolinatriene		1.2	1.4	1.5	0.2	0.5	t			0.1	0.2		3.7		t	2	t	a,b,c
5	1076	Camphene		1.5	1.5	4	t	t				0.1	t		0.6	1.3	0.2	0.2	0.3	a,b,c
6	1118	β-Pinene		0.5	1.6	1.8	1.5	0.9	4.9	1.6	1.3	0.4	0.5		3.7	4.9	0.4	0.8	1.6	a,b,c
7	1132	Sabinene		0.3	0.6	0.6	0.5	0.4	0.3	t	t	0.2	t		t	0.5	0.2	0.1		a,b,c
8	1136	Isoamyl acetate																	t	a,b,c
9	1174	Myrcene		0.1	t		t	t	0.3	0.6	t	0.1				0.3	0.2			a,b,c
10	1176	α-Phellandrene					t			t							t		t	a,b,c
11	1188	α-Terpinene		5	0.1		0.6	0.7		t	t	t				0.2	t		t	a,b,c
12	1192	Artemisiole		0.2	0.1	0.2		t				t			0.4				t	b,c
13	1198	Isobutyl 3-methyl butyrate (= Isobutyl isovalerate)			t															a,b,c
14	1203	Limonene	t	0.1	0.6	0.5	t	t	0.4	0.5	0.3	0.2	0.6	0.3	t	0.5	0.2		t	a,b,c
15	**1213**	**1,8-Cineole**	**t**	**7.2**	**6.0**	**4.9**	**7.2**	**4.3**	**3.6**	**1.6**	**2.8**	**5.9**	**7.9**	**1.7**	**0.6**	**16.7**	**2.5**	**1.5**	**3.2**	**a,b,c**
16	1220	*cis*-Anhydrolinalool oxide							t	t	t	t							t	a,b,c
17	1225	(*Z*)-3-Hexenal																		a,b,c
18	1244	Amyl furan (= 2-Pentyl furan)			t		t			t		t	t			0.1	0.2		t	a,b,c
19	1246	(*Z*)-β-Ocimene					t		t	t	t								t	a,b,c
20	1255	γ-Terpinene	t	0.3	1.8	0.9	2.4	2.5	0.3	0.5	0.4	0.2	1	0.7	0.7	0.6	0.4		0.6	a,b,c
21	1266	(*E*)-β-Ocimene			t		t		t	0.3	t		t				t		t	a,b,c
22	1280	*p*-Cymene	t	1	4.4	1.6	1.4	3.1	1.5	3.8	1.2	1.2	1.1	0.7	2.2	3.3	0.9	0.4	0.8	a,b,c
23	1286	Isoterpinolene			t															a,b,c
24	1286	2-Methyl butyl 2-methylbutyrate			t															a,b,c
25	1290	Terpinolene		t	t	t	0.4	0.4	t	t	t		t	t		0.2	t		t	a,b,c
26	1299	2-Methylbutyl isovalerate		t	0.1	t														a,b,c
27	1303	Amyl isovalerate								t										a,b,c
28	1312	Santolina epoxide													3.1					c
29	1319	(E)-2,6-Dimethyl-1,3,7-nonatriene			t					t									t	a,b,c
30	1348	6-Methyl-5-hepten-2-one		0.2		t	0.3	0.2					0.2		t					a,b,c
31	**1358**	**Artemisia ketone**	**0.7**				**1.8**	**t**	**t**	**t**	**t**	**35.0**	**0.2**		**0.2**	**31.9**	**5.3**	**0.1**	**0.1**	**a,b,c,d**
32	1380	(*Z*)-3-Hexen-1-ol														0.1				a,b,c
33	1400	Tetradecane												0.1						a,b,c
34	**1403**	**Yomogi alcohol**	**0.7**	**0.1**		**t**	**3.4**	**6.3**	**0.5**		**0.4**	**8.8**	**29.8**	**6.9**	**13.6**	**1.3**	**16.6**	**38.9**	**44.1**	**a,b,c,d**
35	**1405**	**Santolina alcohol**					**t**	**t**	**t**		**t**	**1.3**	**1.4**	**0.3**	**t**		**1.1**	**11.3**	**t**	**a,b,c**
36	1424	7-α-(H)-silphiperfol-5-ene								0.2	t					0.1	0.1	0.1	0.1	a,b,c
37	1430	Artemisyl acetate					1.6	4.8				6.2					2.5	6.4	9.4	a,b,c,d
38	1450	*trans*-Linalool oxide (Furanoid)								t	t									a,b,c
39	1451	Filifolone					t	t												c
40	1451	β-Thujone		t	t	t	t	t								0.1				a,b,c
41	1452	α,*p*-Dimethylstyrene			t											0.1				a,b,c
42	1452	7-β-(H)-silphiperfol-5-ene			0.1		t			t	t	0.4	1.1	1.7	0.2		0.2	0.1	0.2	b,c
43	1466	α-Cubebene	t	t				t		t	t	0.1	t	0.2		t	t			a,b,c
44	1468	Siphin-1-ene								0.7		0.4	0.6	0.8	0.2	0.1				a,b,c
45	1474	*trans*-Sabinene hydrate		0.1			3.9	0.3									1.1			a,b,c
46	1480	Nerol oxide																	0.1	a,b,c
47	1482	(*Z*)-3-Hexenyl-2-methyl butyrate			0.1															a,b,c
48	1494	(*Z*)-3-Hexenyl 3-methyl butyrate (= (*Z*)-3-hexenyl isovalerate)			t	t												1.6		a,b,c
49	1495	Bicycloelemene	t	t		0.1	1.9	t	0.1	0.1	t	t	t				0.1		t	a,b,c
50	1497	α-Copaene	1.6	0.4	0.6	0.1		2.2	t		t	0.1	0.7	0.8	0.4	0.2			t	a,b,c
51	**1510**	**Artemisia alcohol**	**t**	**t**			**1.7**	**7.6**	**0.2**		**t**	**2.6**	**3.5**	**0.9**	**26.1**	**0.4**	**19.8**	**8.3**	**10.9**	**a,b,c,d**
52	1520	Chrysanthenone					t	0.5	0.2											a,b,c
53	**1532**	**Camphor**	**2.0**	**45.4**	**20.7**	**47.9**	**3.3**	**3.2**	**0.2**	**t**	**0.5**	**0.2**	**1.5**	**0.6**	**4.2**	**10.7**	**1.6**	**0.8**		**a,b,c**

Table 2 (cont.)

No.	RRI	Compound	Kamieskroon				Nieuwoudt-ville		Prince Albert				Oudtshoorn				Vanwyksdorp			ID
			K1	K2	K3	K4	N5	N6	P7	P8	P9	P10	O11	O12	O13	O14	V15	V16	V17	
54	1544	Cyperene			0.2															a,b,c
55	1536	Pinocamphone													t					a,b,c
56	1538	*trans*-Chrysanthenyl acetate					0.3		1.2	0.9	2.9									a,b,c
57	1540	Modhephene	t										t	0.3						a,b,c
58	1544	α-Gurjunene	t	t	t	t		t	t			t				0.1				a,b,c
59	1547	Dihydroachillene		t		t														a,b,c
60	1549	β-Cubebene					t	t					0.2	0.1	t					a,b,c
61	1553	Linalool	t	t	0.2	t	2.4	t	9	4	7.1	0.5			t		0.3		1.2	a,b,c
62	1556	Isocomene	t		0.2	t						0.2		0.2		0.1				a,b,c
63	1556	*cis*-Sabinene hydrate		t		t	1	t							t					a,b,c
64	1559	β-Maaliene														0.1				a,b,c
65	1562	Isopinocamphone		0.5	0.5	0.6	0.4	0.4	0.1				0.3			0.5			0.1	a,b,c
66	1565	**Linalyl acetate**					**3.9**		**17.4**	**17.2**	**14.0**	**0.8**					**0.8**		**1.3**	**a,b,c**
67	1571	*trans-p*-Menth-2-en-1-ol	t	0.2			0.6	0.6	t						t		0.2			a,b,c
68	1582	*cis*-Chrysanthenyl acetate		t			1.4	1.1			0.4									a,b,c
69	1585	α-Bergamotene							0.6	0.7										a,b,c
70	1586	Pinocarvone		0.3	0.2	0.2	t	t				0.1	0.8	0.3	0.4	0.2				a,b,c
71	1590	Bornyl acetate	t	0.4	3.3	2.4	t	0.4				0.2	0.1			0.1		0.1		a,b,c
72	1594	*trans*-β-Bergamotene									0.6						0.1			a,b,c
73	1600	β-Elemene	t	0.2	0.1		t	0.3	0.1	t	t	0.1	0.2	0.1	t	0.1	0.1			a,b,c
74	1606	β-Copaene														t				a,b,c
75	1604	Thymol methyl ether												0.2						a,b,c
76	1611	Terpinen-4-ol	0.7	1.7	1	1	8.6	7.5	1.3	0.6	1.4	0.9	1.7	0.4	0.7	2.6	0.8	0.3	0.5	a,b,c
77	1612	β-Caryophyllene	1.3	0.5	1.8	1.9	3.2	1.2	1.3	0.8	1.2	0.6	1.8	3.7	0.5	0.7	2.1	0.2	0.4	a,b,c
78	1616	Hotrienol							1.1	0.5	1.8	0.5					t		0.3	a,b,c
79	1628	Aromadendrene	t	t	0.1				0.3		t	0.3	0.2	0.3		0.4	0.2	0.2	t	a,b,c
80	1635	Cadina-3,5-diene			0.2	t		t					t	0.3					t	b,c
81	1638	*cis-p*-Menth-2-en-1-ol	t	0.2		0.2	0.4	0.4					t						t	a,b,c
82	1639	Selina-5,11-diene								t			t			0.1			t	a,b,c
83	1642	Thuj-3-en10-al					t													a,b,c
84	1648	Myrtenal		0.7			t					0.1	0.3		0.1	0.3				a,b,c
85	1655	Sesquisabinene					t													a,b,c
86	1661	Alloaromadendrene		t	0.2		0.2	0.5	0.3	0.4	0.4	0.3	0.4	0.6	0.2	0.2	0.3	0.4	0.1	a,b,c
87	1670	*trans*-Pinocarveol		0.3	0.2		t	t	0.1				0.6	0.2	0.8	0.3			0.5	a,b,c
88	1672	(*Z*)-β-Farnesene							t			0.2					0.8	0.3	0.6	a,b,c
89	1677	*epi*-Zonarene	t	t	0.3		t	0.3						0.4						a,b,c
90	1680	Lavandulol														0.9				a,b,c
91	1681	(*Z*)-3-Hexenyl tiglate	t	0.2	0.2	0.2														b,c
92	1683	*trans*-Verbenol													0.3					a,b,c
93	1684	*trans*-Chrysanthemol	1.6	1.2																a,b,c
94	1687	Methyl chavicol						0.5												a,b,c
95	1687	α-Humulene			0.7	0.6	0.2	t	0.9	0.5	0.5	0.5	0.6	0.8	0.9		0.7	0.4	0.5	a,b,c
96	1688	Selina-4,11-diene (= 4,11-Eudesmadiene)		0.3	0.1				0.7	0.2		0.2	0.8	0.4	0.2		t			a,b,c
97	1689	*trans*-Piperitol (= *trans-p*-Menth-1-en-3-ol)						0.2												a,b,c
98	1700	*p*-Mentha-1,8-dien-4-ol (= Limonen-4-ol)											t							a,b,c
99	1700	**Fragranyl acetate**	**27.1**																	a,b,c,d
100	1700	Amorpha-4,11-diene															0.6		0.5	a,b,c
101	1704	γ-Curcumene					t													a,b,c
102	1704	Myrtenyl acetate							0.5	0.5	0.5	0.4				0.5				a,b,c
103	1704	γ-Muurolene						t						0.2				0.1	t	a,b,c
104	1706	α-Terpineol			0.2		1.5	0.5	4.4	2.3	3.8				0.3					a,b,c
105	1708	Ledene	t		0.3	0.1						0.7	0.7	0.7	0.2	0.9	0.5	0.2		a,b,c
106	1719	Borneol		3.6	2.5	1.8	0.2	0.3					0.6	0.1	0.5	0.4	0.5	t	0.5	a,b,c
107	1719	α-Amorphene											t							a,b,c
108	1725	Verbenone											0.5							a,b,c
109	1726	1,2-Dehydrosesquicineole					0.4													a,b,c
110	1726	Germacrene D	t	t	0.4	0.2	0.6	0.4	1.1	1.1	1.7	1.7	2.0	0.7	0.5	0.8	1.5		1.4	a,b,c
111	1732	Bicyclosesquipheliandrene (= 4(15),5,11-Muurolatriene)		t	0.1	t		0.2	t					0.3						a,b,c
112	1733	Neryl acetate							1.2	1.5									0.3	a,b,c
113	1740	α-Muurolene		0.1				0.5	t	t	1.1	0.2		0.5		t	0.2	0.2		a,b,c

Table 2 (cont.)

No.	RRI	Compound	Kamieskroon				Nieuwoudt-ville		Prince Albert				Oudtshoorn				Vanwyksdorp			ID
			K1	K2	K3	K4	N5	N6	P7	P8	P9	P10	O11	O12	O13	O14	V15	V16	V17	
114	1741	β-Bisabolene					0.6													a,b,c
115	1742	β-Selinene	3.4	0.2	5.1	1.2		t		t			0.7		0.7	0.4				a,b,c
116	1744	Selina-4(15),7(11)-diene													0.5					b,c
117	1744	α-Selinene		t	1	0.2														a,b,c
118	1747	*trans*-Carvyl acetate	t			0.1							0.6	t						a,b,c
119	1755	**Bicyclogermacrene**	**4.1**	**1.3**	**5.3**	**2.5**	**7.4**	**2.4**	**4.6**	**5.3**	**7**	**4.9**	**5.8**	**11.8**	**1.2**	**1.5**	**4.7**		**4.1**	**a,b,c**
120	1758	*cis*-Piperitol					0.1	0.3										t		a,b,c
121	1758	(*E*,*E*)-α-Farnesene										t		0.5						a,b,c
122	1764	*cis*-Chrysanthenol		0.2			0.5	0.6			0.3									a,b,c
123	1765	Geranyl acetate					0.6		1.7	2.6	2.4	0.2								a,b,c
124	1772	Neryl isobutyrate				0.9														a,b,c
125	1773	γ-Cadinene	2.9	0.3	2.3	1.1	0.9	2.6	0.7	1.0	1.0	1.0	1.3	4.0		0.7	0.4	t	0.4	a,b,c
126	1774	Fragranyl isobutyrate	t																	a,b,c
127	1776	δ-Cadinene		t	0.3	0.1	t	0.2	0.2	0.3	0.3	0.3	0.2	0.3		0.1	t	0.1		a,b,c
128	1782	*cis*-Carvyl acetate		0.2																a,b,c
129	1783	β-Sesquiphellandrene							t											a,b,c
130	1784	Campholene alcohol		3.8	1.0	2.1	t	0.3								0.3				a,b,c
131	1786	Neryl propionate			0.5															a,b,c
132	1786	Kessane							0.2	0.8	0.7	t		0.5			0.4	0.2	2.7	a,b,c
133	1787	Aromadendra-1(10),4(15)-diene								t		0.1					0.2		t	a,b,c
134	1799	Cadina-1,4-diene (=Cubenene)	t		t									0.3						a,b,c
135	1804	Myrtenol	t				t		0.6	0.2	0.4	0.2	0.8	0.5		1.1	t	t		a,b,c
136	1805	α-Campholene alcohol	t	0.6	0.3	0.4		0.3												a,b,c
137	1808	Nerol					t		0.7	0.5	0.7									a,b,c
138	1810	**Fragranol**	**26.9**																	**a,b,c,d**
139	1811	Methyl salicylate																	0.2	a,b,c
140	1814	Liguloxide							0.5	1.9	1.8	0.1	0.5	0.7			0.7	0.4	0.7	a,b,c
141	1827	(*E*,*E*)-2,4-Decadienal						t						t						a,b,c
142	1845	*trans*-Carveol	t	0.3	t	0.2		0.3					0.4	0.3		t	t			a,b,c
143	1849	Cuparene							t											a,b,c
144	1853	*cis*-Calamenene	t	0.1	0.2	0.1		t	0.1	t		0.3		0.5		0.1	t	t	t	a,b,c
145	1854	Fragranyl butyrate	t																	a,b,c
146	1857	Geraniol					0.6		1.8	1.6	1.9	0.1							0.3	a,b,c
147	1864	Fragranyl 2-methylbutyrate	t																	a,b,c
148	1864	p-Cymen-8-ol		0.2				t	0.1				t			t				a,b,c
149	1875	Fragranyl 3-methylbutyrate	t																	a,b,c
150	1882	*cis*-Carveol	t	0.2		0.3	t													a,b,c
151	1889	Ascaridole					t													a,b,c
152	1896	*cis-p*-Mentha-1(7),8-diene-2-ol		t																a,b,c
153	1900	*epi*-Cubebol	1	0.4	0.2	0.1	0.7	1.5	0.1			0.3		0.5			t			a,b,c
154	1930	5,11-epoxy-1(10)-Cadinene																	0.5	a,b,c
155	1941	α-Calacorene						t				0.1		0.3						a,b,c
156	1948	*trans*-Jasmone	t					t												b,c
157	1953	Fragranyl pentanoate (= Fragranyl valerate)	0.3																	a,b,c
158	1953	Palustrol		0.4	0.7	0.2	0.4	0.9	0.5	1.4		0.3				0.8		0.1		a,b,c
159	1957	Cubebol		0.5	0.3	0.3	2.0	2.8	0.2		0.3	0.3	t	0.3	t		0.1		t	a,b,c
160	1969	*cis*-Jasmone		0.7	0.2	0.6	0.6	0.3	t		0.2	0.2		0.3	t	0.1	0.2	0.3	t	b,c
161	1973	Dodecanol												0.5						a,b,c
162	1984	γ-Calacorene		t	0.1												t			a,b,c
163	2001	Isocaryophyllene oxide					0.2	t										0.1		a,b,c
164	2005	*trans*-Sesquisabinene hydrate					t													a,b,c
165	2008	Caryophyllene oxide	2.3	1.1	2.2	1.1	2.6	2.0	2.0	2.1	2.9	0.7	0.7	2.0	0.6	0.3	1.5	1.6	0.5	a,b,c
166	2012	Maaliol	t		0.1				0.3	0.2		0.1		0.2			0.1	0.2	t	a,b,c
167	2014	*epi*-Globulol							t							0.1				a,b,c
168	2030	Methyl eugenol					0.2			0.3					t					a,b,c
169	2050	(*E*)-Nerolidol		0.1	0.3		1	0.8	0.2	0.4	0.4	0.2	0.2		t		t	t	t	a,b,c
170	2051	Gleenol	t																	a,b,c
171	2057	Ledol	t	0.2	0.2		t	0.3	0.1	0.2	0.3	0.1		0.3	0.2	0.1	0.1	0.1	t	a,b,c
172	2057	*p*-Menth-1-en-4,8-diol																	t	a,b,c
173	2069	1(10),5-Germacradien-4β-ol		t	t															a,b,c
174	2071	Humulene epoxide-II							0.3	0.6	0.8	0.2			0.2			0.4	t	a,b,c

Table 2 (cont.)

No.	RRI	Compound	Kamieskroon				Nieuwoudt-ville		Prince Albert				Oudtshoorn				Vanwyksdorp			ID
			K1	K2	K3	K4	N5	N6	P7	P8	P9	P10	O11	O12	O13	O14	V15	V16	V17	
175	2074	Caryophylla-2(12),6(13)-dien-5-one	0.4	0.5	0.7	0.4	1.4	1.0	0.4	t		0.4	0.5	0.8	0.2	0.2		0.5	t	a,b,c
176	2075	Cubeban-11-ol			0.3				0.2	t		0.1				0.2	0.1	0.1	t	a,b,c
177	2080	Cubenol	0.6	0.2	0.6	0.2	t	0.6	t	t		0.2	0.2						t	a,b,c
178	2088	1-*epi*-Cubenol	0.9	0.5		0.3	0.3	1.1		t		0.4	0.3	1.4		0.2			t	a,b,c
179	2092	β-Oplopenone		t	0.4			0.6												a,b,c
180	2093	*cis*-Sesquisabinene hydrate		t		0.5	1.8													a,b,c
181	2096	Elemol															0.9			a,b,c
182	2098	Globulol	0.8	0.5	1.1	0.2	0.5		1.5	1.5	1.4	1.1	0.7	1.9	0.5	1.0		0.7	0.4	a,b,c
183	2104	Viridiflorol	1.1	0.3	0.4	0.3	0.4	0.7	1.0	0.9	1.2	0.5	0.6	1.7	0.7	0.7	0.4	0.4	0.3	a,b,c
184	2110	Caryophylla-2(12),6-dien-5-one					t					0.1					0.2	0.1		a,b,c
185	2113	Cumin alcohol															0.6			a,b,c
186	2131	Hexahydrofarnesyl acetone								0.3										a,b,c
187	2142	Rosifoliol	t		0.1		0.1		0.4	0.1		t				0.3	0.1	0.1	t	a,b,c
188	2143	Silphiperfol-6-en-5-one																0.1		b,c
189	2144	Spathulenol	2.1	2.3	3.8	1.1	2.7	3.6	8.0	7.7	8.6	5.6	3.4	8.9	3.4	2.2	5.5	5.0	1.9	a,b,c
190	2161	Muurola-4,10(14)-dien-1-ol	t	0.2	t			0.3												b,c
191	2174	Fokienol									0.3		0.5							b,c
192	2181	Isothymol		t				0.4												a,b,c
193	2185	γ-Eudesmol										0.3								a,b,c
194	2187	T-Cadinol		0.3	0.4	0.3		1.7	0.6	0.8	0.6		1.3	2.0		0.2		0.5		a,b,c
195	2198	Thymol			t															a,b,c
196	2205	Eremoligenol		0.1		0.1			0.2	0.3			0.6		0.3		0.4	0.4	t	a,b,c
197	2208	Copaborneol						0.3												a,b,c
198	2209	T-Muurolol	6.0	3.1	3.3	1.4	2.5	4.8	1.0	1.5	1.5	0.9	0.7	2.9	0.6	0.5	0.5	0.1	0.5	a,b,c
199	2214	ar-Turmerol					0.2													a,b,c
200	2219	Torreyol (= δ-Cadinol, α-muurolol)	t	0.3	0.3	0.4		0.3												a,b,c
201	2230	Pogostol (= 11-Guaien-10-ol)												1.0						a,b,c
202	2232	α-Bisabolol				0.6				2.0					3.4		0.5			a,b,c
203	2247	*trans*-α-Bergamotol	t	0.3	0.5		0.5	0.5	1.2	1.8	1.0	0.8	0.9	1.4		0.4	1.2	0.4	0.5	a,b,c
204	2250	α-Eudesmol				0.2														a,b,c
205	2255	α-Cadinol		1.0	1.7	1.3	0.7	1.5	t			0.5								a,b,c
206	2257	β-Eudesmol							6.7	14	6.8		6.3	7.3	3.4	1.2	0.7	2.5	1.2	a,b,c
207	2264	Intermedeol							t			0.4		1.4	1.3	0.3	0.2			a,b,c
208	2271	(2*E*,6*E*)-Farnesyl acetate											0.3							a,b,c
209	2273	Selin-11-en-4α-ol	t	0.2	0.5		0.2	t												a,b,c
210	2300	Tricosane			0.3			0.2		t										a,b,c
211	2312	9-Geranyl-*p*-cymene					0.2		0.3	0.8			0.7							a,b,c
212	2316	Caryophylladienol I	5.8	0.3	0.3	0.2	0.7	0.4	t		0.5	t	t	0.9	2.9		0.5	0.2	t	a,b,c
213	2324	Caryophylladienol II		3.0	1.7	0.9	2.1	4.0	3.1	3.1	4.3	0.8	t	5.2		0.5	3.0	1.7	0.9	a,b,c
214	2349	Cadina-4,10(15)-dien-3-one							1.9											a,b,c
215	2389	Caryophyllenol I			0.3		0.5							0.2			0.8			a,b,c
216	2392	Caryophyllenol II		0.4	0.5	0.2		0.4			0.2	t		0.8	2.4		0.6	0.4	t	a,b,c
217	2430	ChamaZulene					0.4	0.4												a,b,c
218	2435	6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol												1.8						c
219	2509	(*Z*,*Z*)-9,12-Methyl octadecadienoate (= Methyl linoleate)					0.4	0.5		0.6			0.3	0.2			0.2			a,b,c
220	2533	γ-Costol		0.2									0.5	0.4						a,b,c
221	2538	Ethyl octadecadienoate (= Ethyl linoleate)			0.8		0.8													a,b,c
222	2606	β-Costol			2.1	0.4				0.3										a,b,c
223	2622	Phytol								0.4	0.3			1.0			t		0.3	a,b,c
224	2670	Tetradecanoic acid (= Myristic acid)			t					0.2									t	a,b,c
225	2931	Hexadecanoic acid			0.6		0.1	0.6		0.8	0.5		0.4					t	t	a,b,c
Total			94.3	99.9	94.4	96.0	92.0	94.0	96.2	97.6	93.2	94.0	92.1	91.5	90.0	98.2	88.3	92.2	95.1	
Monoterpene hydrocarbons			t	11.4	12.7	13.1	7.5	9.0	8.4	7.3	3.5	2.7	4.7	1.7	12.9	13.7	2.9	3.8	3.4	
Oxygenated monoterpenes			60.0	67.6	36.7	63.2	46.0	40.9	45.9	34.0	41.5	64.2	51.0	12.4	51.3	68.4	53.3	68.0	72.8	
Sesquiterpene hydrocarbons			13.3	3.4	19.7	8.2	15.0	10.8	11.0	11.3	13.8	12.7	17.3	30.8	5.7	6.7	12.8	2.3	8.3	
Oxygenated sesquiterpenes			21.0	16.2	20.9	10.3	22.3	30.5	30.6	41.6	33.6	14.4	17.7	42.3	20.1	9.2	19.1	16.3	10.1	
Fatty acids and their esters			0	0	1.6	0	0.5	1.9	0	1.6	0.5	t	0.7	0.2	0	0	0	1.8	0	
Others			t	1.3	2.8	1.2	0.7	0.9	0.3	1.8	0.3	t	0.7	4.1	t	0.2	0.2	t	0.5	

demonstrated to have antispasmodic activity, such as bornyl acetate (Duke 2002). The other major essential oil components these species have in common are artemisia alcohol, artemisyl acetate, artemisia ketone, fragranol, fragranyl acetate and spathulenol. Further research is necessary to confirm if these components are involved in antispasmodic and analgesic effects.

Although the systemic circulation of essential oils in animals, after oral administration, is well established in the literature (Kohlert et al. 2000), it is widely incorrectly believed that bioaccumulation is unlikely, because the majority of components are eliminated after glucuronation and sulphation. The accumulation of preconjugated lipophilic forms in adipose tissue has been mostly ignored. However, Miller et al. (2010) have pointed out that adipose tissue levels of limonene are in the range of 51 to 195 times higher than systemic levels in candidates who consume limonene-rich lemonade. It is therefore feasible that the lipophilic volatiles in *P. incana* are bioaccumulated in the adipose tissues of grazing lambs, which would undoubtedly contribute to the flavour of the lamb.

It is therefore possible that the essential oils partly contribute to the medicinal uses that have been reported for *P. incana* and that they contribute to the flavour of karoo lamb. Furthermore, the observed variation in essential oil provides a plausible explanation for the reported differences in palatability between geographical regions. This aspect deserves further study but will require a much greater sampling density and a rigorous quantification of local palatability (perhaps through feeding experiments). Given that a relation between common pasture plants and the flavour profile of Karoo lamb has already been established using stable isotope ratio analysis, it is tempting to speculate that the essential oil compounds in *P. incana* may contribute to the unique spicy flavour associated with Karoo lamb.

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