South African Journal of Botany 111 (2017) 275–282

Contents lists available at ScienceDirect

South African Journal of Botany

journal homepage: www.elsevier.com/locate/sajb

Essential oil composition and leaf trichomes of *Pegolettia baccharidifolia* and *Pegolettia retrofracta* (Asteraceae)

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ARTICLE INFO

Article history:
Received 10 October 2016
Received in revised form 30 November 2016
Accepted 9 March 2017

Edited by AM Viljoen

Keywords:
Pegolettia baccharidifolia
Pegolettia retrofracta
Asteraceae
Essential oil
Trichomes
β-Caryophyllene
Caryophyllene oxide
Thymyl and isothymyl derivatives

ABSTRACT

The essential oils obtained by hydrodistillation from *Pegolettia baccharidifolia* Less. and *P. retrofracta* (Thunb.) Kies were studied for the first time, using GC/MS and GC/FID techniques. A total of 178 components were identified, representing between 85.3% and 100.0% of the total oils. Some differences were observed in the composition of the essential oils of the two species but pronounced differences were also observed between different provenances of *P. baccharidifolia* from geographically separated populations. Five compounds, δ-cadinene, caryophyllene oxide, T-cadinol, T-muurolol and δ-cadinol (=α-muurolol, torreyol) were present in almost all *Pegolettia* samples. Of interest was the discovery of thymyl and isothymyl derivatives in two populations of *P. baccharidifolia* from the Vanwyksdorp area which were mainly in a dormant growth stage. However, 8,9-dehydrothymol was also present in two samples of *P. baccharidifolia* (0.1 and 0.2% of total yield) that were taken from actively growing plants, and 8,9-dehydrothymol isobutyrate was present in *P. retrofracta* at 2.1%. All other thymyl and isothymyl derivatives (thymol isobutyrate, 8,9-dehydrothymyl isobutyrate, 8,9-dehydrothymol, isothymyl valerate, thymyl valerate, isothymol, 8,9-dehydroisothymol isobutyrate, 10-isobutyryloxy-8,9-dehydrothymol isobutyrate, and 10-acetoxy-8,9-epoxythymol isobutyrate) were found only in the two Vanwyksdorp populations. The two species differ markedly in the shape and margins of the leaves, as well as in their surface appearance (glossy and bright green in *P. baccharidifolia*, dull green in *P. retrofracta*). However, the leaf trichomes (multicellular glands, probably the site of essential oil production and/or accumulation) are very similar in both species.

1. Introduction

The genus *Pegolettia* Cass. (Asteraceae) is a small group of nine species found only in Africa and the Middle East (Leistner, 2000). All nine of the species occur in southern Africa but only one is known thus far to be of practical value in traditional medicine. *Pegolettia baccharidifolia* Less., known locally in the Cape region of South Africa as *ghwarrieson*, *heuningdou* or honeydew (Philander, 2010) is an important medicinal plant in the Little Karoo, an arid region situated at the southern end of the Great Karoo of South Africa. This species is sometimes confused with *P. retrofracta* (Thunb.) Kies, known as *geelbergdraaibos* or *perdebos* (Van Wyk and Van Heerden, 2002); both are highly aromatic with distinctive fragrances but the latter is poisonous to antelope (Philander, 2010) and is implicated in stock losses (Van Wyk and Van Heerden, 2002). The vernacular name *ghwarrieson* is believed to be derived from the similarity of the leaves to those of *ghwarrie* (*Euclea undulata* Thunb.) and *son* (sun), implying that it grows in full sunlight (Van Breda and Barnard, 1991). The alternative common name *heuningdou* ("honey dew") refers to the shiny leaf surface that is characteristic of *P. baccharidifolia* (Anderberg, 1986).

Almost no published information about the chemistry and uses of *Pegolettia baccharidifolia* is available, despite the local importance of the plant in the traditional health care system of the Little Karoo region of South Africa. The leaves are slightly sticky and have a very strong herbal scent when bruised (Van Breda and Barnard, 1991). Animals utilise *Pegolettia baccharidifolia* well and it is considered to have medicinal value for both humans and animals (Van Breda and Barnard, 1991). For kidney and bladder ailments, *ghwarrieson* is steeped in water like a tea (Van Breda and Barnard, 1991). It is prepared the same way to treat oedema and skin cancer and can be used as a tonic (Anonymous, 2001). A tincture prepared from bruised leaves can be used to treat heart ailments, fever and stomach complaints, including intestinal disorders (Anonymous, 2001). The sweet tea made from the leaves aids in the treatment of diabetes and respiratory ailments including tuberculosis (Philander, 2010, 2011). To enhance the healing efficacy for diabetes, an infusion of *Pegolettia baccharidifolia* and *Lessertia* (*Sutherlandia*) *frutescens* (L.) Goldblatt & J.C.Manning leaves can be used (De Jager,

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http://dx.doi.org/10.1016/j.sajb.2017.03.030
0254-6299/

2010). This 'honey-smelling' tea is also used to treat inflammation of the chest or on other parts of the body and helps asthma or breathing problems (Van Wyk et al., 2008). Back ache and high blood pressure are other conditions that are treated by an infusion of its fragrant leaves (De Jager, 2010).

The only publication on the chemistry of *Pegolettia* species appears to be a report on sesquiterpene lactones and other compounds from *P. oxydonta* DC., *P. retrofracta* (Thunb.) Kies and *P. senegalensis* Cass. (Zdero and Bohlmann, 1989). Nothing is apparently known about *P. baccharidifolia*. The essential oil compositions of *Pegolettia baccharidifolia* and *P. retrofracta* are here reported for the first time. We also investigated the leaf trichomes of both species, not only to identify the most likely site of essential oil production but also to see if there are any differences between the two species.

The main aim of this paper is to give a first detailed characterisation of the essential oil composition of an important but hitherto poorly studied traditional medicine of the Cape region of South Africa (*Pegolettia baccharidifolia*). We also compared the leaf trichomes and volatile compounds with those of a related species (*P. retrofracta*) with which *P. baccharidifolia* is sometimes confused.

2. Experimental

2.1. Plant material

Fresh aerial parts (upper thin stems with leaves, as are used in traditional medicine) of both *Pegolettia baccharidifolia* and *P. retrofracta* were collected from five different localities in the Cape region of South Africa (full details, including dates of collection and elevation, are provided in Table 1). Voucher specimens were deposited in the Herbarium of the University of Johannesburg (JRAU).

2.2. Essential oil isolation

The aerial parts (thin stems with leaves) were air-dried and the oils were hydrodistilled using a Clevenger-type apparatus (European Pharmacopoeia, 2005). The oil yield was calculated on a dry weight basis (Tables 1 and 2), dried over anhydrous sodium sulphate, and stored in sealed vials in the refrigerator (4 °C) until GC/FID and GC/MS analyses. In the case of very low yields, the samples were dissolved in hexane to ensure maximum recovery.

2.3. Gas chromatographic analysis

The GC/MS analysis was carried out with an Agilent 5975 GC-MSD system (Agilent, USA; SEM Ltd., Istanbul, Turkey). HP-Innowax FSC column (60 m × 0.25 mm, 0.25 μm film thickness, Agilent, Walt & Jennings Scientific, Wilmington, DE, USA) was used with a helium carrier gas at 0.8 mL/min. GC oven temperature was kept at 60 °C for 10 min and programmed to 220 °C at a rate of 4 °C/min, kept constant for 10 min at 220 °C, and then programmed to increase at a rate of 1 °C/min to 240 °C. The oil was analysed with a split ratio of 40:1. The injector temperature was 250 °C. Mass spectra were taken at 70 eV and the mass range was from m/z 35 to 450. The Gas Chromatography (GC) system used was an Agilent 6890N GC system (SEM Ltd., Istanbul, Turkey) fitted with a FID detector set at a temperature of 300 °C. The use of a duplicate of the same column (HP Innowax) and the same operational conditions allowed us to directly compare the GC/FID and GC/MS results.

2.4. Identification and quantification of compounds

Identification of the compounds was done by comparing their relative retention times with those of authentic reference samples or by comparing their relative retention indices (RRI) calculated using *n*-alkane series. A C_9–C_{40} *n*-alkane standard solution (Fluka, Buchs, Switzerland) was used to spike the samples for the determination of RRI. Identifications were confirmed by computer matching against commercial libraries, including the Wiley GC/MS Library (Wiley, 2012), the Adams Library (Adams, 2007) and the MassFinder 4 Library (Hochmuth, 2008), and also an in-house "Baser Library of Essential Oil Constituents" that was built up with authentic reference compounds and components of known oils, as well as MS literature data. The relative percentages of the separated compounds were calculated from FID chromatograms.

2.5. Leaf trichome morphology

Fresh leaf materials of *Pegolettia baccharidifolia* and *P. retrofracta* were preserved in Formalin: Acetic Acid: Alcohol (FAA) (5:5:90) for at least 24 h. Small leaf portions were treated according to 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

Table 1
Plant materials of *Pegolettia baccharidifolia* (Pb) and *P. retrofracta* (Pr) that were studied and yields of essential oil obtained. Voucher specimens were deposited in the Herbarium of the University of Johannesburg (JRAU).

Sample	Collection date (growth phase)	Populations	Locality	GPS coordinates and elevation	Voucher specimen	Oil yield %
Pb 1	20 Dec 2013 (plants dormant)	A1	Vanwyksdorp district,	33° 48′ 35.6″ S	*Hulley & Van Wyk 24-13*	0.097
Pb 2			Farm Hoogekraal, Grafte se Kloof	21° 39′ 46.3″ E	*Hulley & Van Wyk 25-13*	0.068
Pb 3				273 m	*Hulley & Van Wyk 26-13*	0.039
Pb 4	30 Sep 2015 (plants actively growing)	A2			*Hulley & Van Wyk 24-15*	0.036
Pb 5					*Hulley & Van Wyk 25-15*	0.077
Pb 6					*Hulley & Van Wyk 26-15*	0.052
Pb 7	20 Dec 2013 (dormant)	B1	Vanwyksdorp district,	33° 49′ 22.6″ S	*Hulley & Van Wyk 27-13*	0.037
Pb 8			Farm Hoogekraal, Koedoeskloof	21° 39′ 23.4″ E	*Hulley & Van Wyk 28-13*	0.034
Pb 9				287 m	*Hulley & Van Wyk 29-13*	0.117
Pb 10	30 Sep 2015 (plants actively growing)	B2			*Hulley & Van Wyk 27-15*	0.039
Pb 11					*Hulley & Van Wyk 28-15*	0.039
Pb 12					*Hulley & Van Wyk 29-15*	0.042
Pb 13	11 Apr 2015 (plants starting to grow)	C	Prins Albert district	33° 17′ 48.1″ S	*Hulley &Van Wyk 30-15*	1.72
Pb 14				22° 26′ 52.1″ E	*Hulley &Van Wyk 31-15*	1.23
Pb 15				817 m	*Hulley &Van Wyk 32-15*	0.38
Pb 16	11 Mar 2014 (plants starting to grow)	D	Huisrivier Pass near Calitzdorp	33° 29′ 41.9″ S 21° 34′ 29.8″ E 483 m	*Vlok & Van Wyk 4503a*	0.079
Pr 17	11 Mar 2014 (plants starting to grow)	E	Groenfontein near Calitzdorp	33° 29′ 15.4″ S	*Vlok & Van Wyk 4502a*	0.036
Pr 18				21° 44′ 12.2″ E	*Vlok & Van Wyk 4502b*	0.042
				471 m		

Table 2
Chemical composition of *Pegolettia* essential oils, with major compounds indicated in bold, thymyl and isothymyl derivatives shaded and “t” indicating trace amounts only (i.e. less than 0.05% of total yield). Samples are numbered as in Table 1. Dormant (water-stressed) plants were sampled in December; actively growing plants from the same populations were re-sampled in September.

RRI	Species:	*P. baccharidifolia*																	*P. retrofracta*
	Localities:	Vanwyksdorp												Prins Albert			Calitzdorp		
	Populations:	A1 (dormant)			A2 (active)			B1 (dormant)			B2 (active)			C			D	E	
	Individual plant samples:	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
	Oil yield (mg/g dry wt)	0.97	0.68	0.39	0.36	0.77	0.52	0.37	0.34	1.17	0.39	0.39	0.42	17.2	12.3	3.80	0.79	0.36	0.42
	Compounds:																		
1032	α–Pinene	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2.5	–	–
1174	Myrcene	–	–	–	–	–	–	t	–	–	–	–	–	–	–	–	1.7	–	–
1203	Limonene	–	0.1	–	–	–	–	t	t	t	0.2	t	–	–	–	–	**18.5**	–	–
1213	1,8–Cineole	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	**15.1**	–	–
1220	*cis*–Anhydrolinalool oxide	–	0.1	–	–	–	–	t	–	t	0.2	0.3	–	–	–	–	–	–	–
1244	Amyl furan (*2–Pentyl furan*)	–	–	–	–	–	–	t	–	t	t	–	–	–	–	–	–	–	–
1246	(*Z*)–β–Ocimene	–	0.1	–	–	–	–	t	–	–	t	t	–	–	–	–	–	–	–
1253	*trans*–Anhydrolinalool oxide	–	0.1	–	–	–	–	t	–	t	0.1	t	–	–	–	–	–	–	–
1255	γ–Terinene	–	–	–	–	–	–	–	–	–	t	–	–	–	–	–	–	–	–
1266	(*E*)–β–Ocimene	–	–	–	–	–	–	t	–	–	t	t	–	–	–	–	–	–	–
1280	*p*–Cymene	–	0.1	–	–	–	0.3	t	–	t	0.2	t	–	–	–	–	**8.5**	–	–
1290	Terpinolene	–	0.1	–	–	–	–	t	t	t	–	t	–	–	–	–	–	–	–
1335	(*E*)–2–Heptenal	–	–	–	–	–	–	–	–	–	0.1	–	–	–	–	–	–	–	–
1400	Nonanal	–	–	–	0.3	–	0.2	t	–	–	–	–	t	–	–	–	–	–	–
1454	Siphin–1–ene	1.6	1.9	1.5	–	–	–	0.6	0.5	0.4	–	–	–	–	–	–	–	–	–
1437	α–Thujone	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	t	–	–
1444	7–α–(H)–Silphiperfol–5–ene	–	0.1	–	–	–	–	t	t	t	–	–	–	–	–	–	–	–	–
1450	*trans*–Linalool oxide (*Furanoid*)	–	0.1	–	0.6	0.5	0.5	–	t	t	1.0	1.0	0.4	–	–	–	t	–	–
1452	1–Octen–3–ol	–	–	–	–	–	–	–	–	–	t	t	–	–	–	–	–	–	–
1452	7–β–(H)–Silphiperfol–5–ene	–	0.1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
1466	α–Cubebene	–	t	–	–	–	–	–	t	t	–	t	–	–	–	–	–	–	–
1478	*cis*–Linalool oxide (*Furanoid*)	–	–	–	–	0.4	0.6	–	–	–	1.0	0.9	0.3	–	–	–	t	–	–
1483	Octyl acetate	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1.8	–	–
1492	Cyclosativene	–	–	–	–	–	–	–	t	–	–	–	–	–	–	–	–	–	–
1497	α–Copaene	0.6	0.3	0.2	0.6	–	0.2	0.4	0.4	0.3	–	t	–	–	–	–	–	–	–
1506	Decanal	–	–	–	–	–	–	–	–	–	0.4	0.3	t	–	–	–	–	–	–
1507	(*E,E*)–2,4–Heptadienal	–	–	–	–	–	0.3	–	–	–	–	t	–	–	–	–	–	–	–
1532	Camphor	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	3.6	–	–
1540	Modhephene (=2–Modhephene)	2.0	1.7	1.8	–	–	–	0.9	0.4	0.9	–	–	–	–	–	–	–	–	0.3
1541	Benzaldehyde	–	–	–	–	–	–	–	–	–	0.2	–	–	–	–	–	–	–	–
1544	Cyperene	1.6	0.4	0.6	0.4	–	–	0.5	1.6	1.4	–	–	–	–	–	–	–	–	
1551	α–Isocomene	1.7	2.1	2.1	–	–	–	1.0	1.0	0.6	–	–	–	–	–	–	–	–	0.5
1553	Linalool	**7.5**	**12.6**	7.0	**42.8**	**61.5**	**43.5**	**9.5**	2.8	3.0	**42.4**	**38.6**	**42.0**	–	–	–	**15.8**	–	–
1571	*trans*–p–Menth–2–en–1–ol	–	–	–	–	–	t	–	–	–	–	–	–	–	–	–	–	–	–
1582	*cis*–Chrysanthenyl acetate	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	**14.4**	–	–
1591	β–Isocomene	2.3	2.4	2.6	–	–	–	1.0	–	–	–	–	–	–	–	–	–	–	–
1592	Bornyl acetate	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	t	–	–
1600	Hexadecane	–	–	–	–	–	0.2	–	–	–	–	t	–	–	–	–	–	–	–
1600	β–Elemene	2.5	1.4	2.0	–	–	–	0.5	–	0.6	0.1	–	–	–	–	–	–	–	0.4
1602	β–Copaene	–	t	–	–	–	–	t	–	–	–	–	–	–	–	–	–	–	–
1611	Terpinen–4–ol	–	0.3	–	–	–	2.2	–	–	–	–	–	–	–	–	–	–	–	–
1612	β–Caryophyllene	2.8	4.2	4.7	t	0.8	–	3.2	2.0	3.9	1.8	0.7	–	–	–	–	3.2	**35.7**	8.6
1616	Hotrienol	–	t	–	3.3	3.7	2.6	–	–	–	2.8	4.0	1.5	–	–	–	–	–	–
1655	(*E*)–2–Decenal	–	–	–	–	–	–	–	–	–	0.1	–	–	–	–	–	–	–	–
1655	(*E,E*)–2,5–epoxy–6,8–megastigmadine	–	–	–	–	–	0.4	–	–	–	–	–	–	–	–	–	–	–	–
1661	Alloaromadendrene	2.3	1.5	1.0	0.4	–	–	1.7	2.0	1.8	0.8	1.2	0.6	–	–	–	–	t	0.3
1677	*epi*–Zonarene	–	0.2	–	–	–	–	t	–	0.2	–	–	–	–	–	–	–	–	–
1687	α–Humulene	0.7	0.9	1.0	–	–	–	–	–	0.4	0.5	–	–	–	–	–	–	3.0	0.8
1687	Methyl chavico l	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1.7	–	–
1688	Selina–4,11–diene (=*4,11–Eudesmadiene*)	–	0.1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
1700	p–Mentha–1,8–dien–4–ol (= *Limonen –4–ol*)	–	–	–	–	–	0.3	–	–	–	–	–	–	–	–	–	–	–	–
1704	γ–Muurolene	1.8	1.2	0.9	0.5	–	0.5	1.4	1.8	1.8	0.5	0.8	0.6	–	–	t	–	t	–
1706	α–Terpineol	1.7	2.1	1.5	**5.6**	**11.3**	**6.7**	1.8	–	0.9	4.8	4.7	**6.0**	–	–	–	3.1	–	–
1708	Ledene	–	–	–	–	–	–	–	–	–	–	–	–	–	–	t	–	–	–
1730	Zonarene	–	–	–	–	–	–	–	–	–	0.1	t	t	–	–	–	–	–	–
1736	Bicyclosesquiphellandrene	–	–	–	–	–	–	–	–	0.2	t	–	t	–	–	–	–	–	–
1740	α–Muurolene	3.0	1.9	1.8	1.2	0.3	1.1	2.4	3.2	3.2	0.1	1.7	1.4	–	–	0.1	–	t	–
1740	Valencene	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.9
1740	γ–Amorphene	–	0.2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

(*continued on next page*)

Table 2 (*continued*)

RRI	Species:	***P. baccharidifolia***																***P. retrofracta***	
	Localities:	**Vanwyksdorp**												**Prins Albert**			**Calitzdorp**		
	Populations:	**A1 (dormant)**			**A2 (active)**			**B1 (dormant)**			**B2 (active)**			**C**			**D**	**E**	
	Individual plant samples:	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
	Oil yield (mg/g dry wt)	0.97	0.68	0.39	0.36	0.77	0.52	0.37	0.34	1.17	0.39	0.39	0.42	17.2	12.3	3.80	0.79	0.36	0.42
	Compounds:																		
1742	β–Selinene	–	0.2	0.6	–	–	–	t	–	0.3	–	–	–	–	–	–	–	–	–
1743	α–Cadinene	1.7	1.5	0.4	–	–	–	1.0	1.4	0.5	–	–	–	–	–	–	–	–	–
1744	α–Selinene	–	t	t	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
1755	Bicyclogermacrene	–	–	–	–	–	–	–	–	–	–	–	–	–	t	–	–	t	–
1773	δ–Cadinene	**5.6**	3.9	3.0	4.2	1.2	3.6	**6.2**	**6.1**	**7.3**	3.8	**6.0**	**5.1**	0.2	0.2	0.3	–	0.9	2.3
1776	γ–Cadinene	**5.1**	2.7	2.8	2.3	0.7	1.4	2.4	**5.3**	3.7	1.6	2.4	1.8	0.1	0.1	0.1	–	–	–
1779	(*E,Z*)–2,4–Decadienal	–	–	t	–	–	–	0.2	–	t	–	–	–	–	–	–	–	–	–
1781	Zonarene	–	0.2	–	–	–	–	0.2	–	0.2	–	–	–	–	–	–	–	–	–
1785	ar–Curcumene	–	–	–	–	–	–	–	–	–	–	–	–	–	t	–	–	–	–
1786	Kessane	–	–	–	–	–	–	–	–	–	–	–	–	0.1	–	0.1	–	–	–
1799	Cadina–1,4–diene (=*Cubenene*)	–	–	–	–	–	–	t	–	0.2	–	–	–	–	t	–	–	–	–
1800	Octadecane	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.7
1802	Cumin aldehyde	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	3.1	–	–
1804	Liguloxide	–	–	–	–	–	–	–	–	–	–	–	–	0.1	–	t	–	–	–
1808	Nerol	t	0.1	0.8	1.9	3.0	2.2	0.6	–	0.8	1.8	2.1	2.4	–	–	–	–	–	–
1827	(*E,E*)–2,4–Decadienal	–	0.1	–	0.2	–	0.4	0.2	–	t	0.4	0.3	0.4	–	–	–	–	–	–
1838	(*E*)–β–Damascenone	–	0.1	–	–	–	–	t	–	–	–	–	–	–	–	–	–	–	–
1853	*cis*–Calamenene	1.1	0.8	0.7	0.6	–	0.5	1.0	1.2	1.1	–	t	1.1	t	–	t	–	–	t
1854	Germacrene–B	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1.1	–
1857	Geraniol	2.4	3.4	2.9	4.3	**10.1**	**5.2**	3.4	2.3	1.6	**5.4**	**5.6**	**5.6**	–	–	–	–	–	–
1858	1–Octadecene	–	0.1	–	–	–	–	t	–	–	–	–	–	–	–	–	–	–	t
1864	*p*–Cymen–8–ol	–	–	–	–	–	0.4	–	–	–	0.2	–	–	–	–	–	–	–	–
1868	(*E*)–Geranyl acetone	–	0.1	–	–	–	–	0.2	–	t	–	–	–	–	–	–	–	–	–
1871	Neryl isovalerate	–	–	–	–	–	–	t	–	–	–	–	–	–	–	–	–	–	–
1893	Geranyl isovalerate	–	0.2	t	–	–	–	0.1	–	0.1	–	–	–	–	–	–	–	–	–
1898	1,11–Oxidocalamenene	–	t	–	–	–	–	t	–	t	–	–	–	–	–	–	–	–	–
1900	Nonadecane	–	–	t	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
1900	*epi*–Cubebol	–	t	–	–	–	0.1	t	–	t	0.2	0.3	0.2	0.1	–	0.1	–	–	–
1915	Nonadecene	–	t	–	–	–	–	t	–	t	–	–	–	–	–	–	–	–	–
1918	β–Calacorene	–	0.2	–	–	–	–	t	–	–	–	–	–	–	–	–	–	–	–
1922	Thymol isobutyrate	**5.3**	4.5	**12.3**	–	–	–	3.3	3.1	1.5	–	–	–	–	–	–	–	–	–
1941	α–Calacorene	0.8	0.6	0.5	0.3	–	0.1	0.8	–	0.9	0.2	0.3	0.3	–	–	–	–	–	0.4
1942	5,11–Epoxy–1(10)–cadinene	–	t	–	–	–	–	–	–	0.1	–	–	–	–	–	–	–	–	–
1952	Eudesm–4–en–7–ol	0.1	0.3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
1953	Palustrol	–	–	–	–	–	–	–	–	0.1	–	–	–	–	–	–	–	–	–
1957	Cubebol	–	–	–	–	–	t	–	–	–	0.1	–	–	0.2	–	0.1	–	–	–
1958	(*E*)–β–Ionone	–	0.2	–	–	–	–	0.4	–	–	–	–	–	–	–	–	–	–	–
1961	8,9–Dehydrothymyl isobutyrate	0.1	0.6	–	–	–	–	–	–	0.4	–	–	–	–	–	–	–	–	–
1965	(E)–9–Eicosene	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.1
1969	*cis*–Jasmone	–	t	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
1973	Dodecanol	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.4
1984	γ–Calacorene	0.2	0.1	t	–	–	–	0.1	–	0.1	–	–	–	–	–	–	–	–	–
41.92	8,9–Dehydrothymol	1.3	–	–	–	–	–	–	–	–	0.1	0.2	–	–	–	–	–	–	–
1984	(*E*)–12–Norcaryophyll–5–en–2–one	–	–	–	–	–	–	t	–	0.1	–	–	–	–	–	–	–	–	–
2000	Eicosane	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.6
200 0	Isothymyl valerate (=Isothymyl pentanoate)	1.1	1.2	2.4	–	–	–	1.0	–	0.3	–	–	–	–	–	–	–	–	–
2008	Caryophyllene oxide	0.7	1.9	1.9	–	–	–	1.4	1.4	**5.8**	1.1	0.3	–	0.1	t	–	0.9	**21.6**	**10.9**
2015	Thymyl valerate	–	1.7	2.5	–	–	–	2.9	1.1	–	–	–	–	–	–	–	–	–	–
2019	2,3, 6–Trimethylbenzaldehyde	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1.5	–	–
2041	Pentadecanal	–	–	0.1	0.3	–	0.3	0.1	–	–	0.4	0.3	–	–	–	–	–	–	0.8
2049	Cubeban–11–ol	–	–	–	–	–	–	–	–	–	–	–	–	–	t	t	–	–	–
2050	(*E*)–Nerolidol	–	0.7	0.2	–	–	–	0.8	–	–	–	–	–	–	–	–	–	–	1.9
2051	Gleenol	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
2056	13–Tetradecanolide	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1.9	–
2057	Ledol	0.3	0.7	0.4	–	–	–	0.9	–	1.1	–	–	–	–	–	–	–	–	–
2069	Germacrene D–4β–ol	–	–	–	0.3	–	0.5	–	–	–	0.5	0.7	0.3	–	–	–	–	–	–
2071	Humulene epoxide–II	0.1	0.3	0.3	–	–	–	0.5	–	–	–	–	–	–	–	–	–	2.1	–
2074	Caryophylla–2(12),6(13)–dien–5–one	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.6
2080	Cubenol	1.2	–	0.6	0.6	–	0.5	1.0	1.4	1.0	0.6	0.7	0.8	t	t	0.1	–	–	0.5
2084	Octanoic acid	–	0.1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Table 2 (*continued*)

RRI	Species:	*P. baccharidifolia*																*P. retrofracta*	
	Localities:	**Vanwyksdorp**												**Prins Albert**			**Calitzdorp**		
	Populations:	**A1 (dormant)**			**A2 (active)**			**B1 (dormant)**			**B2 (active)**			**C**			**D**	**E**	
	Individual plant samples:	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
	Oil yield (mg/g dry wt)	0.97	0.68	0.39	0.36	0.77	0.52	0.37	0.34	1.17	0.39	0.39	0.42	17.2	12.3	3.80	0.79	0.36	0.42
	Compounds:																		
2087	1,10–di–epi–Cubenol	–	0.7	0.4	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
2088	1–*epi*–Cubenol	0.8	0.6	–	–	–	–	0.7	–	1.1	0.6	0.5	0.7	–	t	0.1	–	–	0.5
2090	Junenol [= Eudesm–4(15)–en–6–ol]	–	0.2	–	0.9	–	–	–	–	–	0.3	0.3	–	1.3	0.5	0.4	–	–	–
2092	β–Oplopenone	0.5	0.8	0.4	–	–	–	1.0	1.0	1.3	–	t	–	–	–	–	–	–	–
2100	Heneicosane	0.2	0.4	0.5	–	–	–	0.3	0.5	–	–	–	–	–	–	–	–	–	1.3
2104	Viridiflorol	–	–	–	–	–	–	–	–	–	–	–	–	3.1	**12.1**	**20.2**	–	–	–
2131	Hexahydrofarnesyl acetone	–	t	0.5	–	–	0.7	0.5	1.0	–	–	–	–	–	–	0.4	–	0.8	2.5
2144	Spathulenol	0.4	0.5	–	t	–	t	–	–	–	–	–	–	1.1	1.1	–	–	0.4	–
2148	(*Z*)–3–Hexen–1–yl benzoate	–	0.1	t	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
2153	Neointermedeol	–	–	–	0.2	–	–	0.2	–	–	–	–	–	–	–	–	–	–	–
2161	Muurola–4,10(14)dien–1–ol	**2.4**	**2.1**	**1.7**	0.6	–	0.3	**2.5**	**2.9**	**3.2**	0.4	0.2	0.5	0.1	–	–	–	**1.4**	–
2179	Nor–Copaanone	–	–	–	–	–	–	–	–	–	–	–	–	0.1	0.1	–	–	–	–
2181	Isothymol (=*2–Isopropyl–4–methyl phenol*)	1.9	0.8	3.2	–	–	–	1.2	1.1	0.8	–	–	–	–	–	–	–	–	–
2185	γ–Eudesmol	–	–	–	–	–	–	–	–	–	–	–	–	0.1	–	–	–	–	–
2187	T–Cadinol	**4.5**	**2.9**	**2.8**	**3.0**	**0.8**	**2.6**	**3.9**	**5.5**	**5.3**	**2.8**	**3.0**	**4.2**	–	**0.2**	**0.1**	–	**1.4**	**2.4**
2200	Docosane	t	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
2205	Eremoligenol	–	–	–	–	–	–	–	–	–	–	–	–	0.1	0.1	0.1	–	–	–
2209	T–Muurolol	**6.8**	**4.5**	**4.1**	**4.4**	1.2	3.4	**5.6**	**8.6**	**8.6**	3.2	3.7	**5.2**	0.1	0.2	0.2		**3.7**	**3.0**
2219	δ–Cadinol (=α–muurolol, torreyol)	1.9	1.4	1.2	1.2	0.3	1.0	2.1	2.7	2.7	1.0	1.1	1.7	–	–	0.1	–	1.9	2.1
2232	α–Bisabolol	–	–	–	0.2	–	–	–	–	–	0.9	0.6	0.2	–	–	–	–	–	–
2233	2–Himachalen–7–ol	–	–	–	0.2	–	–	–	–	–	0.2	0.1	0.1	–	–	–	–	–	–
2250	α–Eudesmol	–	t	–	–	–	–	0.2	–	–	–	–	–	0.1	0.2	0.1	–	–	–
2255	α–Cadinol	**6.6**	**6.5**	**5.0**	**10.1**	3.0	**10.4**	**5.6**	**13.5**	**8.6**	**8.6**	**10.6**	**12.2**	–	–	–	–	**5.9**	**9.2**
2256	Cadalene	3.1	0.2	1.1	–	–	–	3.3	t	4.3	–	–	–	–	–	–	–	–	–
2257	β–Eudesmol	–	–	–	–	–	–	–	–	–	–	–	–	**92.5**	**84.6**	**76.4**	–	–	–
2260	15–Hexadecanolide	–	–	–	0.2	–	0.7	–	–	–	0.2	t	–	–	–	–	–	1.2	–
2262	Opposito–4(15),7(11)–dien–12–al	–	–	–	–	–	–	1.5	–	–	–	–	–	–	–	–	–	**3.7**	**8.8**
2271	Eicosanal	0.3	0.5	1.1	–	–	–	0.4	0.7	0.5	–	–	–	–	–	–	–	–	–
2272	Muurola–4,10(14)–dien–8a–ol*	–	–	–	–	–	–	–	–	–	0.6	–	–	–	–	–	–	–	–
2273	Selin–	0.4	0.4	0.4	–	–	–	0.5	–	0.5	0.2	–	–	–	–	–	–	–	2.9
2300	Tricosane	0.3	0.4	0.5	–	–	–	–	–	0.5	–	0.2	–	–	0.1	–	–	–	2.2
2312	9–Geranyl–*p*–cymene	–	–	–	0.5	–	0.7	–	–	–	0.5	0.5	0.4	–	–	–	–	–	–
231 6	Caryophylla–2(12),6(13)–dien–5–ol β (=*Caryophylladienol I*)	–	1.1	–	–	–	–	–	–	1.5	–	–	–	–	–	–	–	0.3	1.6
2317	Cadina–1(10),4–dien–8a–ol	–	–	–	–	–	–	–	–	1.6	–	–	–	–	–	–	–	–	–
2320	Eudesma –4(15),7–dien–1–ol* isomer	–	0.3	–	–	–	–	1.2	–	–	–	–	–	–	–	–	–	–	0.9
2324	Caryophylla –2(12),6(13)–dien–5α–ol (=*Caryophylladienol II*)	0.2	–	–	–	–	–	1.1	–	–	0.8	–	–	–	–	–	–	0.7	6.3
2341	Eudesma–4(15),7–dien–1–β–ol	0.5	1.0	–	–	–	–	0.9	–	0.9	0.2	1.1	0.5	–	–	–	2.1	–	**7.6**
2389	Caryophylla–2(12),6–dien–5α–ol (=*Caryophyllenol I*)	–	0.2	0.2	–	–	–	0.4	–	–	–	–	–	–	–	–	–	–	1.4
2392	Caryophylla –2(12),6–dien–5β–ol (=*Caryophyllenol II*)	–	–	–	–	–	–	–	0.3	0.4	–	–	–	–	–	–	–	–	5.0
2393	(Z)–9–Tricosene	–	–	–	–	–	–	–	–	0.1	–	–	–	–	–	–	–	–	–
2400	Tetracosane	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.4
2438	Kaur–16–ene	0.6	0.6	0.9	–	–	–	0.6	0.5	0.4	–	–	–	–	–	–	–	–	–
2513	8,9–Dehydroisothymol isobutyrate	–	–	–	–	–	–	–	0.8	–	–	–	–	–	–	–	–	–	–
2515	8,9–Dehydrothymol isobutyrate	1.2	0.7	–	–	–	–	–	1.4	0.4	–	–	–	–	–	–	–	2.1	–
2537	Methoxy cadinadiene	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	3.5	–
2500	Pentacosane	–	0.1	t	–	–	–	–	–	0.2	0.2	t	–	–	–	–	–	–	0.7
2503	Dodecanoic acid	–	0.1	–	–	–	–	–	–	0.3	–	–	–	–	–	–	–	–	–

(*continued on next page*)

Table 2 (*continued*)

RRI	Species:	*P. baccharidifolia*																*P. retrofracta*	
	Localities:	**Vanwyksdorp**												**Prins Albert**			**Calitzdorp**		
	Populations:	**A1 (dormant)**			**A2 (active)**			**B1 (dormant)**			**B2 (active)**			**C**			**D**	**E**	
	Individual plant samples:	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
	Oil yield (mg/g dry wt)	0.97	0.68	0.39	0.36	0.77	0.52	0.37	0.34	1.17	0.39	0.39	0.42	17.2	12.3	3.80	0.79	0.36	0.42
	Compounds:																		
2509	(*Z,Z*)–9,12–methyl octadecadienoate (=*Methyl linoleate*)	–	0.6	–	–	–	–	0.7	1.1	–	–	–	–	–	–	–	–	–	–
2533	γ–Costol	–	–	t	–	–	–	0.3	–	0.2	–	–	–	–	–	–	–	–	–
2551	8,9–Dehydro isothymol isobutyrate	–	–	1.9	–	–	–	0.9	–	–	–	–	–	–	–	–	–	–	–
2555	10–Isobutyryloxy–8,9–dehydrothymol isobutyrate	–	–	2.9	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
2604	α–Costol	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.5
2606	β–Costol	–	–	t	–	–	0.3	–	–	–	0.4	0.2	–	–	0.3	0.4	–	–	–
2622	Phytol	–	0.1	–	–	–	0.2	–	–	0.7	–	–	–	–	–	–	–	–	–
2655	Benzyl benzoate	–	0.2	–	–	–	–	–	–	0.2	–	–	–	–	–	–	–	–	–
2670	Tetradecanoic acid (= *Myristic acid*)	–	0.2	–	–	–	–	–	–	0.2	–	–	–	–	–	–	–	–	–
2792	1,21–Docosadiene	–	0.4	1.0	–	–	–	0.4	0.6	0.5	–	–	–	–	–	–	–	–	–
2883	10–Acetoxy–8,9–epoxythymol isobutyrate	–	–	–	–	–	–	0.2	1.0	0.4	–	–	–	–	–	–	–	–	–
2931	Hexadecanoic acid	0.7	1.3	2.3	3.2	0.6	1.4	2.0	3.1	1.3	1.1	0.7	1.3	–	–	–	–	2.8	–
	Total	**92.5**	**92.7**	**93.2**	**96.6**	**99.4**	**96.5**	**91.8**	**85.3**	**93.4**	**96.0**	**96.2**	**97.8**	**99.7**	**100.0**	**99.3**	**97.5**	**96.1**	**90.3**

Schiff's reagent and toluidine blue (Feder and O'Brien, 1968) and observed under a light microscope.

2.6. Light microscopy (LM)

The stained microscope slides were studied under a light microscope (Olympus CX41) equipped with a digital camera and a computerised data capturing system (Olympus ColorView Soft Imaging System).

2.7. Scanning electron microscopy (SEM)

Fresh leaf materials were dehydrated through a graded alcohol series and placed in amyl acetate. Critical point drying was performed before the samples were coated with gold in preparation for viewing under a scanning electron microscope using standard settings (SEM; TESCAN, soft – VegaTS and Mira 3 LMU) at the central analytical facility (known as Spectrum) of the University of Johannesburg.

3. Results and discussion

3.1. Leaf morphology

Pegolettia baccharidifolia and *P. retrofracta* differ markedly in their leaf morphological feature and surface appearance of their leaves. The former species can easily be distinguished by the glossy leaf surface secretions (hence the vernacular name *heuningdou* or "honeydew") and the conspicuously dentate leaf margins. *P. retrofracta* has dull green leaves with a glandular rather than glossy appearance and the margins are entire (Fig. 1). If attention is paid to these diagnostic characters then the two species are unlikely to be confused.

3.2. Leaf trichomes

The leaves of *P. baccharidifolia* have a very thin cuticle with multicellular glandular peltate trichomes (Fig. 2A1, A2) occurring within the grooves on both surfaces of the leaf. The glands are biseriate, comprising two rows of cells that gradually widens to a more or less rounded head (Fig. 2). The upper membrane surrounding the head (visible in Fig. 2B4) often ruptures, as seen in Fig. 2A4 (also visible in A3 and B3). It is likely that these trichomes produce and/or accumulate oil. The epidermal layer is similar all around with the outer periclinal cell walls highly cutinized. No other secretory cells are visible. The leaves of *P. retrofracta* are similar to those of *P. baccharidifolia* but the outer periclinal cell walls of the epidermis are more highly cutinized and may exceed the thickness of the epidermal cells. The surface has multicellular peltate trichomes (Fig. 2B1, B2) very similar to those of *P. baccharidifolia*. They are, however, almost twice the size and the heads are oval in outline rather than round as in *P. baccharidifolia*.

3.3. Essential oil composition

The results of the oil analyses are presented in Table 2. Despite very low overall yields, a total of 178 essential oil components could be identified in the 18 samples studied. Large differences were observed between the two species, as well as between different populations of *P. baccharidifolia*. The plant to plant variation (within populations) in both species was relatively low. The major compounds were quite variable overall, with β-caryophyllene largely present in one sample of *P. retrofracta* at 35.7% and the other major compound being caryophyllene oxide (10.9 and 21.6%). Linalool was invariably present in all the *P. baccharidifolia* samples of Calitzdorp and Vanwyksdorp but totally absent from the Prins Albert population. It is interesting to note the very high levels (42.0–61.5%) found in all of the actively growing plants sampled at Vanwykdorp. Viridiflorol (3.1–20.2%) and β-eudesmol (76.4–92.5%) appear to be unique to the Prins Albert population and were present in all three samples.

Five compounds, namely δ-cadinene (0.2–7.3%), caryophyllene oxide (from trace amounts to 21.6%), T-cadinol (0.1–5.5%), T-muurolol (0.1–8.6%) and δ-cadinol (0.1–2.7%), were present in almost all the samples of both species. Of rare occurrence are 1,8-cineole (15.1%) and *cis*-chrysanthenyl acetate (14.4%), present only in the Calitzdorp sample (D) of *P. baccharidifolia*.

Eleven compounds were found to be present only in *P. retrofracta* (valencene, octadecane, germacrene-B, (*E*)-9-eicosene, dodecanol, eicosane, 13-tetradecanolide, caryophylla-2(12),6(13)-dien-5-one, tetracosane, methoxy cadinadiene and α-costol).

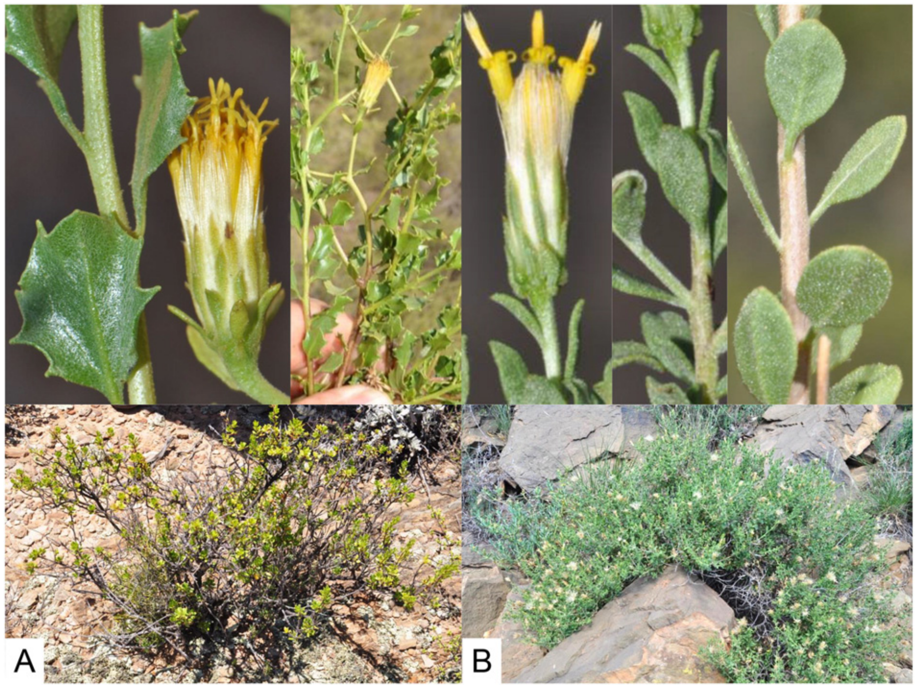

Fig. 1. Morphology of *Pegolettia baccharidifolia* (A) and *P. retrofracta* (B), showing the leaves, flower heads and growth form (habit). Photographs: B.–E. van Wyk.

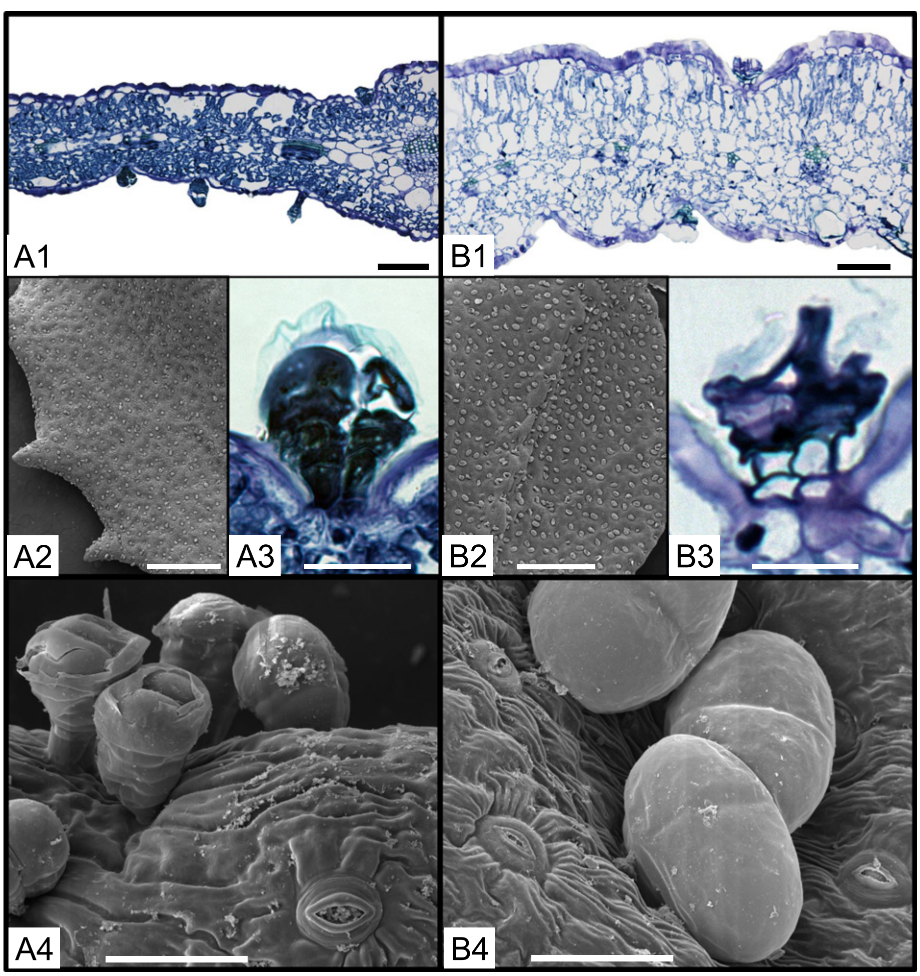

Fig. 2. Leaf trichomes of *Pegolettia baccharidifolia* (A) and *P. retrofracta* (B): A1, B1, transverse section of leaf; A2, B2, Scanning electron micrograph of leaf surface; A3, B3, trichome; A4, B4, Scanning electron micrograph showing trichomes. Scale bars: A1 = 100 μm, B1 = 200 μm; A2, B2 = 1 mm; A3, A4, B3, B4 = 50 μm.

Ten thymyl and isothymyl derivatives were detected in *P. baccharidifolia* (only from Vanwyksdorp and mainly in the dormant, water-stressed plants sampled in December and almost absent from actively growing plants that were sampled in September) and one of these ten in *P. retrofracta*. Pronounced seasonal differences were found between the samples of *P. baccharidifolia* from Vanwyksdorp that were sampled from the same populations while dormant and water stressed (in the dry season, in summer) and when actively growing (in the wet season, in early spring). Fifteen compounds can be singled out as typical of dormant and stressed plants (Table 2), namely siphin-1-ene, cyperene, α-cadinene, thymol isobutyrate, γ-calacorene, isothymyl valerate, thymyl valerate, ledol, β-oplopenone, muurola-4,10(14)dien-1-ol, isothymol, cadalene, eicosanal, kaur-16-ene and 1,21-docosadiene. These compounds may be stress metabolites but it is also possible that they were merely below the threshold of detection in the samples collected in summer, where they may have been masked by the presence of relatively large amounts of other essential oil compounds.

Particularly interesting was the discovery of thymyl and isothymyl derivatives in *P. baccharidifolia*. These compounds are relatively rare but have previously been found in other Asteraceae such as *Doronicum corsicum* (Loisel.) Poir. (Paolini et al., 2007), *Carpesium divaricatum* Siebold & Zucc. (Zee et al., 1998), *Calea nelsonii* B.L.Rob. & Greenm. (Mariano et al., 1987) and *Inula helenium* L. (Stojakowska et al., 2004). The chemotaxonomic significance of the compounds deserves further study, not only because they have not yet been encountered in any thyme or oregano oils where thymol and carvacrol are major constituents, but also because their limited distribution in Asteraceae may indicate a separate (perhaps non-homologous) biosynthetic pathway.

In conclusion, our study has shown that the trichomes of the two species are quite similar but that their essential oils are quite different. The results also demonstrate the importance of seasonal and geographical variation when attempting to characterise the essential oil composition of a species.

Acknowledgement

We wish to thank Mr. A Britz (Hoogekraal Farm, Vanwyksdorp) and Mr. JHJ Vlok (Oudtshoorn) for collecting some of the material used in this study. CapeNature is thanked for providing collecting permits (permit No. 0028-AAA008-00194). Financial support from the University of Johannesburg and the National Research Foundation of South Africa (Grant UID: 88432) is gratefully acknowledged.

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