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The ethnobotany, leaf anatomy and major essential oil compounds of *Leysera gnaphalodes* (Asteraceae), a poorly known aromatic herbal tea endemic to southern Africa

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ABSTRACT

Leysera gnaphalodes (L.) L. (viz., *Leyssera gnaphalioides* L.) is an aromatic, medicinal Cape herbal tea endemic to southern Africa. The plant is known by many different vernacular names, most relating to its use as tea: *geelblommetjiestee*, *duinetee*(*bossie*), *skilpadtee*(*bossie*), *teringtee*(*bossie*), *hongertee* and *geeltee*. According to historical literature and recent ethnobotanical surveys in the Little Karoo, this aromatic shrublet is a treatment for numerous respiratory ailments, especially '*tering*' (tuberculosis). Previous studies have reported the presence of several diterpenes and triterpenes, but the main essential oil compounds have remained unknown. These were identified as α-pinene, β-pinene, *p*-cymene, carvacrol and bicyclogermacrene. Multicellular glandular trichomes are present on the leaf surface, which may be the site of oil production and/or accumulation. *Leysera gnaphalodes* has been reported to exhibit significant anti-mycobacterial activity against three *Mycobacterium* micro-organisms. The traditional claim of its efficacy as a treatment against tuberculosis, as reflected by the common name *teringtee* ('tuberculosis tea') therefore appears plausible. This activity seems to be primarily associated with the presence of the well-known pentacyclic triterpenoids, oleanolic acid and ursolic acid.

1. Introduction

Leysera (Asteraceae) was named for the German botanist Friederich Wilhelm von Leysser (1731–1815), who was the author of the Flora Halensis in 1761 (Bremer, 1978). The genus consists of five species, two of which are endemic to southern Africa, namely *L. gnaphalodes* (L.) L. and *L. tenella* DC. *Leysera gnaphalodes* is a short-lived perennial shrublet of ca. 40 cm high that often occurs in large numbers on roadsides and in other disturbed places (Conradie and Van Rooyen, 2005). The species has a wide distribution range in southern Namibia and South Africa, from Namaqualand southwards to the Cape Peninsula, and eastwards to the Winterberg and Stormberg in the Eastern Cape (Von Staden, 2014). *Leysera tenella* is morphologically very similar to *L. gnaphalodes* but is a much smaller plant of up to 20 cm high (Manning and Goldblatt, 2012). It too has a wide distribution across the dry western interior of South Africa, extending northwards to Namibia (Von Staden, 2014).

The correct orthography is *Leysera gnaphalodes* (and not *Leyssera gnaphaloides*, the spelling used in the older literature), as stated by Manning and Goldblatt (2012) and Van Wyk and Gorelik (2017). *Leysera gnaphalodes* has many vernacular names, indicating that this poorly studied Cape plant must have been much more prominent than its current obscurity would suggest. The common names mostly relate to its use as a traditional herbal tea: *geelblommetjiestee* (Watt and Breyer-Brandwijk, 1962), *duinetee (bossie)*, *skilpadtee (bossie)*, *teringtee (bossie)* (Smith, 1966), *hongerbos* (Von Koenen, 1977, 2001), *hongertee* (Smith, 1966; Ellis, 1989; Rood, 1994; Powrie, 2004; De Jager, 2010) and *geeltee* (Powrie, 2004). Several medicinal uses have been recorded, including the treatment of '*tering*' (tuberculosis) – perhaps as appetite stimulant – and gastro-intestinal complaints.

Leysera gnaphalodes exhibited significant *in vitro* anti-mycobacterial activity against *Mycobacterium microti*, *M. avium* and *M. scrofulaceum* (Bamuamba et al., 2008). The traditional claim of its efficacy as a treatment against tuberculosis, as reflected by the common name *teringtee* ('tuberculosis tea'), therefore appears plausible. The anti-mycobacterial activity seems to be primarily associated with the presence of the well-known pentacyclic triterpenoids oleanolic acid and ursolic acid (Bamuamba et al., 2008).

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In this study, the identity of the main essential oil compounds of the species is reported for the first time. The leaf anatomy was explored in an attempt to find potential sites of essential oil formation and/or accumulation. We also reviewed and summarised the rather scanty ethnobotanical data for the species, as recorded in historical and some contemporary literature sources.

2. Experimental

2.1. Plant material studied

Fresh aerial parts consisting of stems, leaves and flower heads of *L. gnaphalodes* were collected from three different localities in the Western Cape Province of South Africa. Voucher specimens were deposited in the Herbarium of the University of Johannesburg (JRAU).

Bulk samples, each comprising 15–20 individual plants in full flower, were collected at the following three localities (arranged from north to south and east to west).

Clanwilliam (32°10′54.3″ S, 18°52′46.8″ E, 178 m); date of collection 21 September 2015; date of hydrodistillation 29 September 2015; voucher specimen *Hulley & Van Wyk 02-15*.

Vredenburg (32°55′13.9″ S, 17°58′41.2″ E, 161 m), date of collection 22 September 2015, date of hydrodistillation 20 July 2016, voucher specimen *Hulley & Van Wyk 08-15*.

Barrydale (33°54′27.4″ S, 20°43′05.5″ E, 368 m), date of collection 23 September 2015, date of hydrodistillation 10 November 2015, voucher specimen *Hulley & Van Wyk 11-15*.

2.2. Leaf anatomy

Upon collection, fresh aerial material of *L. gnaphalodes* was preserved in formalin:acetic acid:alcohol (FAA) (5:5:90) for a minimum period of 24 h. The method of Feder and O'Brien (1968) was applied to small leaf portions in preparation for glycol methacrylate (GMA) embedding. The leaf material was first dehydrated using a graded alcohol series and then 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. Schiff's reagent and toluidine blue (Feder and O'Brien, 1968) were used to stain the sections which were then observed under a light microscope (Olympus CX41) equipped with a digital camera and a computerised data capturing system (Olympus ColorView Soft Imaging System).

2.3. Essential oil distillation

The essential oil was isolated from air-dried aerial parts by hydrodistillation using a Clevenger-type apparatus (European Pharmacopoeia, 2005).

The oil samples were dried over anhydrous sodium sulfate before the oil yield percentages were determined on a dry weight basis. The oils were stored in sealed amber glass vials in the refrigerator (4 °C) until GC/MS analyses.

2.4. Gas chromatographic analysis

The three essential oil samples were analysed with a GC/MS 2010 (Shimadzu) system equipped with an auto-sampler. The parameters were set as follows: detector interface at 250 °C; ion source 200, injector temperature at 200 °C; carrier gas, helium; 1 μl injections with a split ratio (1:20). Column flow set at 1 ml/min; column ramp from 60 °C without hold at 5 °C/min to 280 °C, hold for 5 min. Compounds were identified by comparing the mass spectra and retention indices (calculated relative to n-alkanes) with the NIST library and Adams (2007).

3. Results and discussion

3.1. Ethnobotany

In the published database of literature on southern African medicinal plants, Arnold et al. (2002) referred to five publications: Watt and Breyer-Brandwijk (1962), Smith (1966), Von Koenen (1977), Ellis (1989) and Rood (1994). These references include vernacular names and/or uses (see Table 1). The first mentioned vernacular names and uses are indicated in bold in Table 1. A recent and more comprehensive review of the ethnobotanical literature on *L. gnaphalodes* is that of Van Wyk and Gorelik (2017). Pappe (1847, 1850, 1857, 1868) first reported the popularity of *L. gnaphalodes* as a Cape herbal tea (hence the vernacular name *geele bloemetjies-thee*) and as a treatment against catarrh, cough and consumption. Marloth (1909) designated *L. gnaphalodes* as the 'Tea of the Cederbergen' (Van Wyk and Gorelik, 2017) and named it *geel blommetjies tee*, *honger tee* and *skilpadtee* (Marloth, 1917). Kling (1923) was the first to record the use of *L. gnaphalodes* to treat whooping cough and bronchitis, in addition to the uses given by Pappe. Watt and Breyer-Brandwijk (1962) documented the use of the leaf and flower infusion as an appetite stimulant. The vernacular names *duinetee*(*bossie*) and *teringtee*(*bossie*) were first mentioned by Smith (1966); he was also the first to record its uses as a medicinal tea in the treatment of chest complaints and as a remedy for dyspepsia. Smith recorded this information near the south-western district and from Bredasdorp to Riversdale (Smith, 1966). Three types of tea were used near Piketberg in the Aurora area according to Coetzee (1969) of which *hongerdoodtee* seemed to be referring to *L. gnaphalodes* (Van Wyk and Gorelik, 2017). Von Koenen (1977, 2001) referred to the same uses as recorded in Pappe (1847, 1850, 1857, 1868) as well as the vernacular names that appear in Marloth (1917). Rood's Afrikaans book 'Uit die veld-apteek' (directly translated as 'Out of the field-pharmacy') referred to previously recorded vernacular names and medicinal uses against chest ailments, loss of appetite and indigestion. Ellis (1989) mentioned that the leaf, stem, flower and fruit are used as a traditional medicine against colds and flu. In the Montagu Museum's booklet (Anonymous, 1998, 2001), *L. gnaphalodes* is recorded as a herbal remedy against several previously recorded ailments (tuberculosis, influenza, colds and chest complaints), but can also be used as a treatment for unspecified stomach ailments. Other published sources (Powrie, 2004; Van Wyk, 2008, 2011a, 2011b; Van Wyk et al., 2009; De Jager, 2010; Manning and Goldblatt, 2012; Vlok and Schutte-Vlok, 2016; Van Wyk and Gorelik, 2017) refer to previous records for vernacular names and uses (Table 1). A PhD thesis by Philander (2010), mentioned that it is easy to confuse the two local *Leysera* species (*L. gnaphalodes* and *L. tenella*). Van Wyk (2011a, 2011b) and Vlok and Schutte-Vlok (2016) recorded the use of the leaves and flowers of *L. gnaphalodes* as a health tea.

Recent ethnobotanical surveys conducted in the Little Karoo (Hulley, 2019; Hulley and Van Wyk, 2019) resulted in the recording of new vernacular names for *L. gnaphalodes*: *TB-bossie*, *teetee*, *skaaptee* and *vaaltee,* of which the last-mentioned also refers to *L. tenella*. The medicinal uses of *L. gnaphalodes* against respiratory ailments with emphasis on tuberculosis and as an appetite stimulant were again confirmed by numerous participants during these Little Karoo surveys (Table 1).

It is important to note that the name *geelblommetjie* was incorrectly used by Kling (1923) for two different species, namely *Leysera gnaphalodes* (Asteraceae) and *Jamesbrittenia atropurpurea* (Benth.) Hilliard (Scrophulariaceae). Under the synonym *Lyperia crocea* Eckl., Pappe (1847, 1850, 1857, 1868), the latter was reported to be used medicinally to treat febrile convulsions in infants. Kling (1923) followed Pappe but used the same name (*geelblommetjie*) for both species, while Pappe distinguished between '*Geele bloemetjes-thee*' (as '*Leyssera gnaphalioides*') and *Geele bloemetjes* (as '*Lyperia crocea*').

Table 1
Summary of vernacular names and ethnobotanical anecdotes for *Leysera gnaphalodes*. First mentioned vernacular names and original uses are indicated in bold.

Vernacular names (first records in bold)	Anecdotes (original data in bold)
Literature	
geele bloemetjes-thee: Pappe (1847, 1850, 1857, 1868)	'Very few of our indigenous plants are so much in domestic use as this one, known as *Geele bloemetjes-thee*. When pounded, or rubbed between the fingers, it gives an agreeable scent, and the infusion has a pleasant, sweetish taste. It is emollient, and for that reason is highly commended in **catarrh**, **cough**, and even **consumption**. Some of our apothecaries have added this plant to the species pectoralis' (Pappe, 1847, 1850, 1857, 1868).
name not given	'In the Cedarbergen the people do not use the Cyclopia, but quite a different plant, viz., *Leyssera gnaphalioides* L., a composite with needle-shaped leaves. No chemical investigation of this plant has been made as yet, to my knowledge, but the **beverage** prepared from it has a pleasant aromatic flavour without any stimulating action' (Marloth, 1909).
geel blommetjies tee, ***honger tee***, ***skilpadtee***: Marloth (1917)	name only (no anecdotes)
geelblommetjie: Kling (1923)	'Longpypkatar, kinkhoes, verslyming, bronkaitis, hoes' – 'catarrh of the trachea, **whooping cough**, phlegm, **bronchitis**, cough'
name not given: Leipoldt (2007) – the book was written in the early 1930's	'...most of the valley folk contented themselves with tea made from a local composite plant which was pleasant to the taste, contained much less tannin than the imported article, was healthier to drink and had the added advantage of being procurable by all those who took the trouble to gather it from the hillside' (Leipoldt, 2007).
geelblommetjiestee: Watt and Breyer-Brandwijk (1962)	'*Leyssera gnaphalioides* L. has an agreeable odour and an infusion has a pleasant sweetish taste (P3). It is emollient and has therefore been much used in catarrh, coughs and pulmonary tuberculosis (P3). Nowadays the European and the African take an infusion of the leaf and flower as a tonic in **loss of appetite**. In the Cederberg the plant is used as a tea, the infusion being pleasant and aromatic in flavour and stimulating in action (M 8)'.
***duinetee*(*bossie*)**, *geelblommetjies-*(—), *skilpad-*(—), *hongertee*, ***tering-*(—)**: Smith (1966)	'When bruised or otherwise crushed, the plant emits an aromatic scent and on this account was formerly used in infusions as a medicinal tea in the treatment of **chest complaints**. The vernacular name is in allusion to the yellow (Afr.: *geel*) florets and the use of the plants as a tea (Aft.: *tee*). A decoction of the plants was formerly in wide use as a remedy for **dyspepsia** to stimulate the appetite, literally to promote hunger (Afr.: *honger*), whence the vernacular name. Used medicinally as a tea in the treatment of consumption (Afr.: *tering*), whence the vernacular name; the tea is also used for other chest troubles'.
hongerdoodtee: Coetzee (1969)	name only (no anecdotes)
hongerbos, skilpadtee: Von Koenen (1977, 2001)	'An aromatic tea can be brewed from the plant, which is taken for upper respiratory tract catarrh, coughs and pulmonary tuberculosis. It also stimulates the appetite' (Von Koenen, 2001).
duinetee, *hongertee, geelblommetjiestee*, *teringtee*, *skilpadtee*: Rood (1994)	'*Aftreksels van die plant is as medisinale tee vir borskwale aanbeveel. 'n Brousel van die hongertee was vroeër algemeen bekend as 'n middel teen verlies van aptyt – vandaar die naam – en teen slegte spysvertering*' (Rood, 1994).
hongertee: Ellis (1989)	'Parts used: Leaf/stem, flower/fruit; culinary: tea; traditional medicinal use: **colds**, **flu**, tonic' (Ellis, 1989)
hongertee, duinetee, teringtee: Van Wyk and Gericke (2000, 2018)	'An infusion of the aboveground portions of the plant can be enjoyed as a general health tea. The tea can be taken two to three times daily as an appetite stimulant in thin, wasted individuals. It has also been used to treat **rheumatic fever** (Dawid and Mrs. Bester, pers. comm.). The plant has been used for coughs, bronchitis and tuberculosis, and has potential for new crop development' (Van Wyk and Gericke, 2000, 2018)
geelblommetjie teebos, *hongerteebos*, *teringteebos*, honey tea: Anonymous (1998, 2001)	'*tering, verkoue, griep, borskwale, maagkwale – trek soos tee, drink ½ koppie 3x per dag*'. – 'tuberculosis, influenza, colds, chest complaints, **stomach ailments** – draw like tea' (Anonymous, 1998, 2001).
hongertee, geeltee: Powrie (2004)	name only (no anecdotes)
geelblommetjiestee, duinetee, hongertee: Van Wyk (2008), Van Wyk et al. (1997, 2009)	'Tea made from *Leysera gnaphalodes* is taken for catarrh, cough and tuberculosis, consumption' (Van Wyk, 2008; Van Wyk et al., 1997, 2009)
naaldetee, *hongertee*: De Jager (2010)	'An infusion of the aboveground parts of the plant can be taken as an aromatic tonic drink. The tea can be taken 3 times daily as an appetite-stimulant by thin, wasted persons. This infusion can treat coughs, bronchitis and tuberculosis. It can be given as a general tonic to recuperating patients. Apparently, it has been used to treat rheumatic fever' (De Jager, 2010).
hongertee,teebos, duinetee, geelblommetjietee, skilpadteebossie, teringtee: Van der Merwe and Van Rooyen (2010)	'The plant is used medicinally for loss of appetite and various chest ailments' (Van der Merwe and Van Rooyen, 2010).
geelbloometjieste, duinetee, hongertee: Philander (2010)	'While I identified this plant as *Leysera tenella* used as a tea for chest ailments, it has been identified in other sources as *Leysera gnaphalodes*. It is used to treat coughs, colds, and tuberculosis. This short-lived perennial is listed as a weedy species in Europe but should be easy to cultivate in a garden where it is native. It is one of the plants that grows when they allow a pasture to lie fallow or in disturbed areas' (Philander, 2010).
duinetee, hongertee: Van Wyk (2011a, 2011b)	'health tea, coughs, appetite stimulant' (Van Wyk, 2011a), 'tea, health drinks, appetite stimulant' (Van Wyk, 2011b)
teebos,skilpadteebossie, teringteebossie: Manning and Goldblatt (2012)	name only (no anecdotes)
hongertee: Hübsch et al. (2014)	'Some plants from which herbal teas are prepared include *Leyssera gnaphalioides* (*hongertee*)' (Hübsch et al., 2014).
teebossie: Le Roux (2015)	name only (no anecdotes)
teringteebos: Vlok and Schutte-Vlok (2016)	'In the past, the leaves and flowers of *L. gnaphalodes* were used to brew a pleasant, sweet tea and also for medicinal purposes, especially to ease the cough of people with tuberculosis' (Vlok and Schutte-Vlok, 2016).
geele bloemetjies-thee (p1–4,6); *duinetee(bossie), eelblommetjiestee(bossie), hongertee*	'Twigs and leaves used as tea. Also as emollient to treat lung ailments – catarrh, cough

Table 1 (*continued*)

Vernacular names (first records in bold)	Anecdotes (original data in bold)
(bossie), kilpadtee(bossie), teringtee(bossie) (s2); *skilpadteebossie, teringteebossie* (m2): Van Wyk and Gorelik (2017)	and consumption' (p1–4,6; s2)
Ethnobotanical survey information (Hulley, 2019; Hulley and Van Wyk, 2019)	
teringtee: Barrydale participant (JB)	JB: 'drink as tee saam suiker en melk' (JB: drink as tea with sugar and milk)
teringtee: Vanwyksdorp participants (AB, CB, MO, ED, EC, EW, JC, MS, AO, MC, MJ, SJ, RP, MM, JM, JM1, AC, WC, KJ, MW), *TB-bossie*: (EW, JC, MS, AO, MC), *teetee*: (PW)	CB, MO, ED, EC: '*medisyne – drink vir tering en* ***geelsug'*** (CB, MO, ED, EC: medicine - drink for tuberculosis and **jaundice**); AB, EW, JC, MS, AO, MC*: 'drink vir TB, drink ook as tee vir die lekker'* (EW, JC, MS, AO, MC: drink for TB, drink also as a tea for enjoyment); MJ, SJ, RP, MM, JM, JM1*: 'TB, drink soos tee vir opbou (tonikum)'* (MJ, SJ, RP, MM, JM, JM1: TB, drink like tea [i.e. like black tea] for convalescence (tonic); AC*: 'tee vir die lekker en vir TB'* (AC: tea for enjoyment and for TB); WC*: 'trek saam met nasgal vir TB, twee doppies kondensmelk en pafieka en meng en sit in yskas en gee vir gat op long van TB'* (WC: infuse together with nasgal (*Solanum retroflexum*) for TB, mix two caps of condensed milk, *pafieka* (*Bulbine frutescens*) and *tellington* [Turlington, a Lennon Cape Dutch medicine] and put in the fridge and drink to treat a hole on the lung due to TB); KJ*: 'TB, drink net as tee'* (KJ: TB, drink only as tea); MW*: 'drink swart'* (MW: drink black [i.e. without milk])
teringtee: Prince Albert participant (CM)	CM*: 'groei nie hier nie, seisoen plant, gebruik eers deur San en toe die Khoi - het dit geëet aangesien hulle nie potte gehad het om tee te maak nie'* (CM: does not grow here, seasonal plant, first used by the San and then the Khoi – they ate it since they had no pots to make tea)
vaaltee, skaaptee: Haarlem participants (AC1, EM, SR)	EM: *'trek af as tee, pophuis gespeel daarmee'* (EM: infuse as a tea, played dollhouse with it)
skaaptee: Uniondale participant (JB1)	JB1*: 'drink as 'n tee'* (JB1: drink as a tea)

3.2. Leaf anatomy

The needle-like leaves of *L. gnaphalodes* are amphistomatic with multicellular glandular trichomes only on the margins (Fig. 1: B1). They have a basal cell, several stalk cells and a multicellular head. These trichome glands are presumably the site for oil production. Characters of the leaves include epidermal cells with a very thin cuticle and a highly cutinised outer periclinal cell wall. There are up to three layers of palisade parenchyma with large sub-stomatal air chambers. The spongy parenchyma similarly has large sub-stomatal air chambers. The vascular tissue consists of a central, highly sclerified bundle and

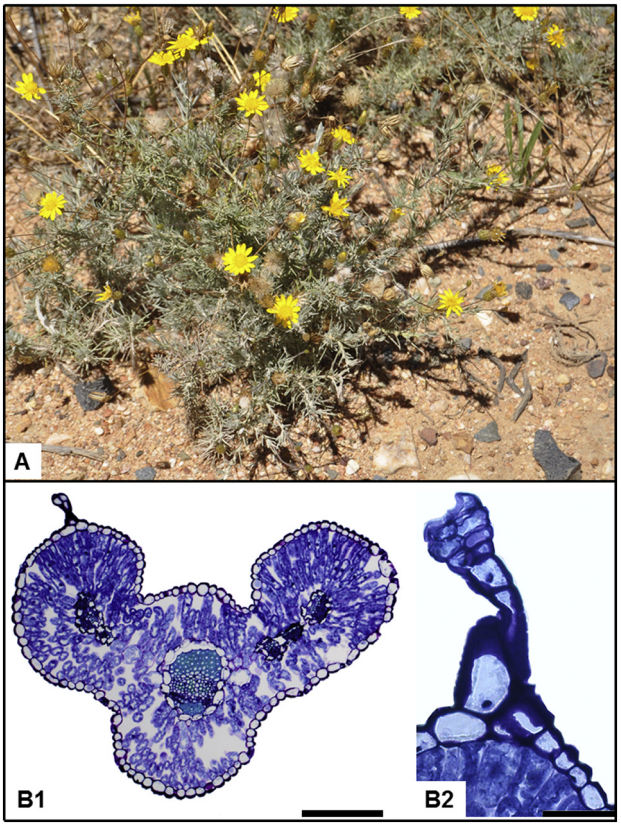

Fig. 1. Morphology and anatomy of *Leysera gnaphalodes*. A, growth form (habit) of a plant in full flower (photograph: B.-E. van Wyk); B, Leaf anatomy. B1, transverse section of leaf; B2, multicellular gland. Scale bars: B1 = 200 μm and B2 = 50 μm) (photographs: I.M. Hulley).

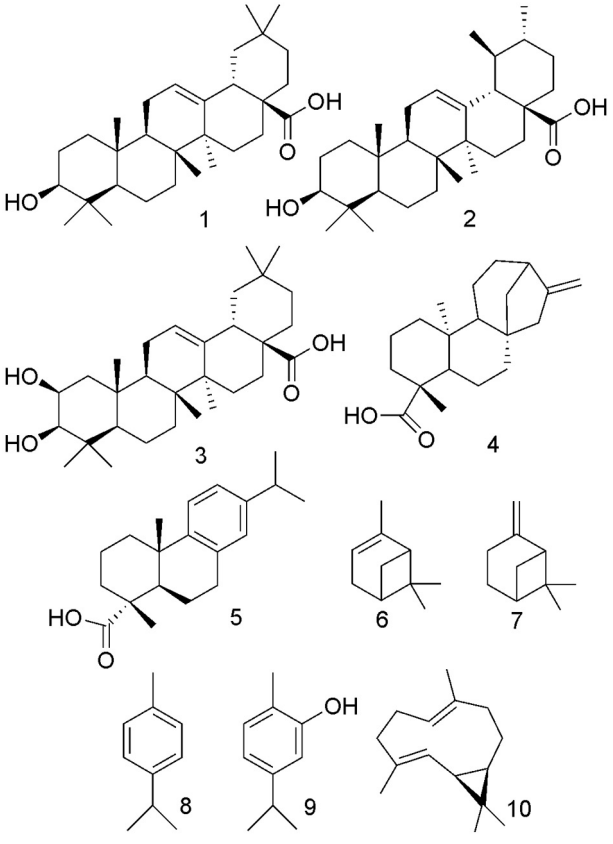

Fig. 2. Examples of known chemical compounds (**1**–**5**) and major essential oil compounds (**6**–**10**) of *Leysera gnaphalodes*. **1**, oleanolic acid; **2**, ursolic acid; **3**, 2α-hydroxyoleanolic acid; **4**, kauren-18-oic acid; **5**, dehydroabietic acid; **6**, α-pinene; **7**, β-pinene; **8**, *p*-cymene; **9**, carvacrol; **10**, bicyclogermagrene.

usually two or three smaller lateral bundles. A layer of chloroplast-containing cells surrounds the vascular bundles (Kranz anatomy; indicative of C4 photosynthesis).

3.3. Known chemical compounds in L. gnaphalodes

Bohlmann and Zdero (1972) reported triterpenes (oleanolic acid and benzofuran derivatives) in the roots of *L. gnaphalodes* (Fig. 2). An aldehyde was also isolated from both *L. gnaphalodes* and *L. tenella* (Bohlmann and Zdero, 1972). Tsichritzis and Jakupovic (1991) listed several terpenes (triterpenes and diterpenes as well as labdane and kaurene derivatives) present in the aerial parts of *L. gnaphalodes*, including ursolic acid, oleanolic acid, 2α-hydroxyoleanolic acid, kauren-18-oic acid, dehydroabietic acid, four labdane diterpenoids, two 13-epimanoyloxide derivatives and a kaur-15-ene derivative (Fig. 2). Dementzos and Dimas (2001) also recorded the presence of unspecified labdanes in *L. gnaphalodes*.

3.4. Essential oil composition

The volatile compounds in *L. gnaphalodes* appear to be unknown as no published information could be found. Our analyses show variable mixtures of monoterpenes and sesquiterpenes. A total of 40 chemical compounds were detected in the essential oils of *L. gnaphalodes* (Table 2). The compounds can be categorised into three major groups, namely (1) aliphatic monoterpenes, (2) alkylbenzenes and (3) sesquiterpenes. The total percentage of each group that occurred in each sample respectively was 33.4, 10.7, 26.4% in the Vredenburg sample, 2.6, 15.3, 52.9% in the Clanwilliam sample and 72.2, 12.9 and 12.2% in the Barrydale sample (Table 2). Note that monoterpenes dominated in the Barrydale sample, while more than half of the Clanwilliam sample comprised sesquiterpenes.

The major monoterpenes were aliphatic monoterpenes and related alkylbenzenes, namely *p*-cymene, thymol and carvacrol. Aliphatic terpenes were of both monoterpenoid and sesquiterpenoid classes, including α- and β-pinene plus limonene as monoterpenes, and caryophyllene and bicyclogermacrene as the dominant macrocyclic sesquiterpenes.

Other sesquiterpenes are decahydronapthalenic, such as viridiflorol. This compound is anti-inflammatory and antioxidant and showed activity against *Mycobacterium tuberculosis* (Trevizan et al., 2016). Another sesquiterpene is spathulenol (that can be prepared from aromadendrene) and also occurs in the essential oils of *Eucalyptus spathulata* Hook. subsp. *grandiflora* (Benth.) L.A.S.Johnson & Blaxell and chamomile (Van Lier et al., 1985). Five compounds are here shown to be major constituents in *L. gnaphalodes,* with more than 10% present in at least one sample (see Fig. 2). α-Pinene (23.4% in only the Barrydale sample) is an anti-inflammatory (Russo, 2011) and antimicrobial (Nissen et al., 2010) but also contributes to many different botanical flavour profiles (Mediavilla and Steinemann, 1997). β-Pinene (16.5 and 26.0% in the Vredenburg and Barrydale samples respectively) is usually associated with α-pinene and can be found in allspice, where it is a flavour ingredient (Human Metabolome Database, 2019). A third main component is *p*-cymene (12.1 and 30.0% in both the Barrydale and Vredenburg samples) which has insecticidal activities and low antifungal activity according to Kordali et al. (2008). Carvacrol is a natural monoterpene derivative of cymene that inhibits bacterial growth and acts as a flavouring agent (Tariq et al., 2019), and is only present in the Clanwilliam sample (11.8%). The fifth and last major component is bicyclogermacrene, a sesquiterpene which is also present in the pungent extract of *Plagiochila ovalifolia* Mitt. and *P. yokogurensis* Stephani, which also contained aromadendrene derivatives that may contribute towards the pungency (Asarawa et al., 1980). Aromadendrene and its derivatives are known from a wide range of well-known culinary spices such as *Origanum vulgare* L. (Gao et al., 2014), *Salvia officinalis* L. (Craft et al., 2017), *Zingiber officinale* Roscoe (Shareef et al., 2016) and *Prunus persica* L. (Koprivica et al., 2018). Bicyclogermacrene occurs in the essential oils of many other species such as *Artemisia campestris* L. (Bellomaria et al., 2001), *Citrus junos* Siebold ex Tanaka (Song et al., 2000) and *Fortunella japonica* (Thunb.) Swingle (now *Citrus japonica* Thunb.) (Choi, 2005) amongst others. This main component is present in all three *Leysera* samples, at levels of 3.4–23.5%. Other compounds that may possibly also contribute to the flavour of *L. gnaphalodes* tea include α-terpinene, an antioxidant also present in tea tree oil (Rudbäck et al., 2012) and three aromadendrene derivatives. Some variation was found in the expression of aliphatic monoterpenes (2.6–72.2%), as well as that of the sesquiterpenes (12.2–52.9%), this is a common feature of intra-population variation. The alkylbenzenes have a similar percentage between the three samples (10.7–15.3).

Table 2
Chemical composition of the essential oil in three bulk samples of *Leysera gnaphalodes* (each sample representing 15–20 individual plants). Major compounds are indicated in bold (interpreted as those representing 10% or more in at least one sample); n.d. = not identified, AI = Arithmetic Index.

Localities of plant samples				Clanwilliam	Vredenburg	Barrydale
Oil yield (mg/g dry wt)				0.35	1.54	0.71
No	Compounds	AI	Published AI	%	%	%
1	α-Thujene	920	924	–	–	0.2
2	**α-Pinene**	**928**	**932**	–	–	**23.4**
3	Sabinene	965	964	–	–	2.9
4	**β-Pinene**	**977**	**974**	–	**16.5**	**26.0**
5	β-Myrcene	980	988	–	–	2.6
6	α-Terpinene	1005	1001	–	9.5	8.0
7	***p*-Cymene**	**1022**	**1020**	–	**30.0**	**12.1**
8	Limonene	1028	1024	–	3.9	6.8
9	γ-Terpinene	1054	1054	–	0.8	1.0
10	Dehydro-linalool	1086	1088	1.5	0.7	0.9
11	Terpinen-4-ol	1179	1174	1.1	2.0	0.5
12	Thymol, methyl ether	1222	1232	–	1.5	0.5
13	Carvacrol, methyl ether	1227	1241	–	1.1	0.3
14	Thymol	1288	1289	3.4	2.1	–
15	**Carvacrol**	**1297**	**1298**	**11.8**	–	–
16	n.d.	1303	–	2.3	–	–
17	δ-Elemene	1331	1335	0.8	0.4	
18	α-Ylangene	1374	1373	0.9	0.1	0.2
19	Geranyl acetate	1376	1379	1.1	–	–
20	β-Patchoulene	1381	1379	1.3	2.7	0.2
21	n.d.	1387	–	3.7	1.2	0.3
22	*Z*-Caryophyllene	1410	1408	0.4	0.2	–
23	*E*-Caryophyllene	1419	1417	2.8	0.8	2.6
24	Aromadendrene	1437	1439	0.3	0.1	0.1
25	Neryl acetone	1445	1434	1.0	0.6	t
26	α-Caryophyllene	1454	1452	0.7	0.3	0.2
27	Alloaromadendrene	1459	1458	0.2	0.2	0.1
28	Germacrene D	1473	1480	1.2	0.6	–
29	β-Selinene	1488	1490	0.7	0.1	–
30	δ-Selinene	1490	1491	0.8	0.1	0.3
31	**Bicyclogermacrene**	**1495**	**1559**	**23.5**	**10.8**	**3.4**
32	Viridiflorene	1495	1496	–	–	2.7
33	δ-Cadinene	1516	1518	1.3	1.3	0.2
34	α-Calacorene	1539	1540	–	1.0	–
35	n.d.	1552	–	–	3.0	–
36	Spathulenol	1576	1577	8.2	2.0	0.6
37	Caryophyllene oxide	1581	1582	0.7	0.2	0.3
38	Globulol	1584	1590	0.6	0.1	–
39	Viridiflorol	1593	1592	1.4	0.8	1.1
40	Aromadendrene epoxide	1631	1639	1.3	–	–
	Total %			**73.0**	**94.5**	**97.2**
Aliphatic monoterpenes				2.6	33.4	72.2
Alkylbenzenes				15.3	10.7	12.9
Sesquiterpenes				52.9	26.4	12.2

4. Conclusions

It is curious that *L. gnaphalodes* and its traditional uses have remained poorly recorded and studied, given its historical importance. It was the most popular tea in the Cederberg region during the 19th century, before rooibos tea [*Aspalathus linearis* (Burm.f.) R.Dahlgren] gained prominence (only in the 20th century – see Van Wyk and Gorelik, 2017). The pleasant taste, the recorded medicinal benefits and weedy tendencies suggest that the plant may have potential for development as a new crop and a novel functional food product.

The flowering aboveground parts of *L. gnaphalodes* (i.e., the material traditionally used to make tea) contain low yields of essential oil, to which the agreeable aromatic flavour of the tea can be ascribed. The site of oil accumulation appears to be the marginal multicellular glands. Considerable variation in the chemical composition of the essential oil was observed, with striking differences between populations. The differences, and especially the apparent total absence of some compounds, are noteworthy when considering that several individual plants were pooled into bulk samples, so that the analytic results represent population 'averages'. It will therefore be interesting to do a geographical variation study of this widely distributed species and to examine individual plants separately (our samples were mixtures of ca. 15–20 plants). The main compounds appear to be α-pinene, β-pinene, *p*-cymene, carvacrol and bicyclogermacrene but several minor compounds, such as aromadendrene and its derivatives (found in well-known spices such as ginger, sage and oregano) may also contribute to the flavour of the tea.

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