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**RESEARCH ARTICLE**

# Major volatile compounds in the essential oil of the aromatic culinary herb *Pelargonium crispum* (Geraniaceae)

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**Abstract**

The aromatic culinary herb *Pelargonium crispum* is used as a condiment across the world to confer a lemony aroma to food. The phytochemistry of one cultivar of unknown origin has been tentatively described in earlier studies, with only the dominant volatile components assigned as neral and geranial and with chrysin as the major flavone. In the current study a more detailed chemistry of the essential oils is given, and the major flavanone in the leaves is assigned as (2*S*)-(-)-pinocembrin, a dihydro derivative of chrysin. The predominance of neral and geranial is confirmed and a number of related oxide and phenylpropanoid ester derivatives are assigned. A major outcome is the realization that the wild specimens sampled by us displays different chemistry to the chemotype previously described.

**Keywords**: Neral, geranial, (2*S*)-(-)-pinocembrin, phytochemistry, wild form

## Introduction

*Pelargonium crispum* (L.) L'Her. is a native of South Africa, occurring from Worcester to Bredasdorp in the south of the Western Cape Province. It is a small aromatic shrub that is valued for the lemony aroma that it confers to food or tea (Lim, 2014). It is widely cultivated in herb gardens and several cultivars (some possibly of hybrid origin) are known. Studies have demonstrated that the volatiles are not merely the flavor principle and that tartaric acid that is accumulated in the leaves also contributes a sour taste (Stafford, 1961). Various biosynthetic studies of tartaric acid demonstrated routes from simple sugars via ascorbic acid (Lim, 2014).

As a food condiment, *P. crispum* is recognized for more than its aesthetic contribution to dishes. The flavone chrysin is implicated in therapeutic or health boosting outcomes, particularly related to anti-inflammatory, antiviral and anticancer activities (Lim, 2014). In addition to chrysin, Williams *et al*. (1997) identified a *C*-methyl flavanone that was suggested to be either strobopinin or cryptostrobin, which are structurally very similar to pinocembrin but differ markedly by their masses, due to an aromatic methyl moiety.

An earlier investigation of the chemistry of the essential oils under the vernacular names 'Crispum variegatum' and 'Lemon fancy' merely identified neral, geranial and 'sesquiterpenes' (Lis-Balchin et al., 1998). Antimicrobial activities of these essential oils were measured by agar well diffusion, so it is difficult to generalize about the outcome, but in relative terms, neral and geranial-rich essential oils were among the most active tested in that study (Lis-Balchin et al., 1998).

Evidently, the chemistry of the essential oils needs to be more comprehensively assessed, as there are many essential oils dominated by neral and geranial. It is also necessary to confirm if chrysin predominance in the leaf exudate is consistent across the species distribution, to provide backing to the health boosting claims that are now made for this species when used as a food condiment. Thus, the current study aims to

provide a more comprehensive chemistry to the essential oils from three specimens collected in their endemic distribution. Isolation of the major flavonoid in this population aims to confirm or nullify the occurrence of chrysin in randomly selected specimens.

## Materials and Methods

### Plant material

Plant samples from three different individuals were provided by the property owners of the farm Klipbokkop in the vicinity of Worcester, near Brandvlei Nature Reserve (33.80487093844676˚ S; 19.37562882900238˚ E). The material was collected from strongly aromatic shrubs of ca. 1 m high, growing in rocky sandy soil in mountainous terrain. A herbarium voucher specimen (*Sadgrove 545*) was deposited in the University of Johannesburg Herbarium (JRAU). Careful examination of the material by one of us (B EVW, a taxonomist) confirmed the identity of the species as *P. crispum*, as is evidenced by the small leaves and single-flowered inflorescences.

### Isolation of the essential oil

Hydrodistillations were performed using a Clevenger-type apparatus and essential oils were dried over anhydrous sodium sulfate and stored at 4 °C away from light until analysis.

### Analysis

Essential oils were characterised by GC-MS and NMR, and quantified with GC-FID. Only components higher than 1% by MS were identified.

GC-MS operating conditions were as follows: Shimadzu 2010 with detector interface at 250 °C; ion source 200; injector temperature 200 °C; carrier gas helium; 1 µl injections with a split ratio (1:20); fitted with an OV-1 (WCOT) (non-polar) column; column flow at 1 ml/min; column ramp: 60 °C (no hold), 5 °C per min then held at 280 °C for 5 min. Identification of compounds was made by comparing the mass spectra and retention indices (calculated relative to n-alkanes) with the NIST library and Adams (2007) and some major components confirmed by NMR. GC-FID operating conditions were identical to GC-MS and n-alkanes were used to guide peak assignment. NMR assignments were made using a 500 Mhz Bruker Avance (Bruker, Germany) in $CDCl_3$. Spectra for neral and geranial were matched to shifts included in Kelm et al. (1997) and linalool to shifts in Blanc et al. (2005).

#### *(2S)-(–)-Pinocembrin*

100 g of plant material was extracted in methanol then the volume reduced under pressure. The residue was re-extracted into dichloromethane, filtered and the residue (760 mg) was subjected to column chromatography over silica gel in mobile phase 1:9 ethyl acetate:petroleum ether. A total of 203 mg was isolated and first dissolved into $CDCl_3$ for NMR analysis then re-dissolved into *d*-DMSO for comparison to literature values. The spectra were identical to shifts included in Tanaka *et al*. (1985). Nowhere in the literature are the spectra for (2S)-(–)-pinocembrin given in $CDCl_3$ so $^{13}C$ shifts are provided here; $^{13}C$-NMR ($CDCl_3$) δ: 196.1 (C-4), 164.6', 164.59', 163.4' (C-5, C-7 or C-8a)', 138.5 (C-1'), 129.1 (C-4'), 129 (C-3'and C-5'), 126.3 (C-2'and C-6'), 103.5 (C-4a), 96.9'', 95.6'' (C-6 or C-8)'', 79.4 (C-2), 49.5 (C-3). Optical rotations were calculated on a Polartronic H532 and the negative enantiomer assigned. Absolute stereochemistry was inferred from Napal et al. (2009).

## Results and Discussion

Confirmation of the dominant components in the essential oil, neral and geranial, is unsurprising (Table 1). However, previous reports of 'sesquiterpenes' is not applicable to the chemical character in the current study. Sesquiterpenes were observed but comprise only trivial relative abundance compared to other monoterpenes, such as linalool and hydroxycitronellol, and monoterpene alkane and phenylpropanoid esters, such as geranyl hexanoate or geranyl benzoate respectively. *Z*- and *E*-linalool oxides and epoxy oxides are also present, as well as the two methyl cinnamate isomers. The dominant two sesquiterpenes were β-bourbonene and α-curcumene, but these are in only minor quantities.

The lemony aroma of the essential oil from *P. crispum* is without a doubt derived from the terpenoid mixture of the two dominant components geranial (citral A; α-citral) and neral (citral B; β-citral), which are merely *cis*/*trans* isomers of 3,7-dimethyl-2,6-octadienal. It is noteworthy that geranial and neral were detected in only one of 13 shrubby species of *Pelargonium* studied by Lalli et al. (2006), namely *P. citronellum* (the reported yields respectively 27.2 and 17.4%).

Table 1. Major essential oil compounds in three individual plants (A, B and C) of *Pelargonium crispum* sampled from a natural population of the species.

| | **AI** | **Pub AI** | **A** | **B** | **C** |
|---|---|---|---|---|---|
| **Yield (% g/g wet wt)** | | | 0.56 | 0.49 | 0.55 |
| *p*-Cymene | 1024 | 1020 | 1.8 | 0.5 | 0.9 |
| *Z*-Linalool oxide | 1070 | 1067 | 3.0 | 2.3 | 4.1 |
| *E*-Linalool oxide | 1087 | 1084 | 2.9 | 2.1 | 3.8 |
| Linalool | 1105 | 1100 | 12.1 | 4.5 | 5.0 |
| Nonenol | 1144 | 1164 | - | - | 1.6 |
| Terpinen-4-ol | 1188 | 1174 | 1.7 | 1.9 | 0.9 |
| Neral | 1250 | 1235 | 14.9 | 18.6 | 13.3 |
| Geranial | 1283 | 1264 | 24.3 | 30.9 | 20.7 |
| *Z*-Epoxy-linalool oxide | 1290 | * | 2.0 | 7.5 | 10.9 |
| *E*-Epoxy-linalool oxide | 1295 | * | 2.2 | 5.9 | 9.9 |
| *Z*-Methyl cinnamate | 1312 | 1299 | 2.1 | 1.6 | - |
| Hydroxycitronellol | 1342 | 1359 | 3.4 | 1.5 | - |
| *E*-Methyl cinnamate | 1388 | 1376 | 5.5 | 7.0 | 7.0 |
| β-Bourbonene | 1398 | 1387 | 2.3 | 1.9 | 1.1 |
| Funebrene, 2-epi-β- | 1426 | 1411 | 0.8 | 0.7 | 1.0 |
| β-Cedrene | 1434 | 1419 | 0.5 | 0.8 | 0.5 |
| α-Curcumene | 1490 | 1479 | 1.7 | 2.2 | 3.1 |
| Spathulenol | 1593 | 1577 | 1.0 | 0.3 | 0.6 |
| Globulol | 1600 | 1590 | 0.6 | 1.5 | 0.4 |
| Geranyl tigliate | 1703 | 1696 | 1.6 | - | - |
| Neryl hexanoate | 1730 | 1732 | - | 1.3 | 2.1 |
| Geranyl hexanoate | 1757 | 1755 | 3.4 | 2.3 | 3.2 |
| Neryl benzoate | 1955 | 1946 | 3.3 | 0.5 | 1.8 |
| Geranyl benzoate | 1973 | 1951 | 0.8 | 0.6 | 1.6 |
| **Total** | | | **92.1** | **96.5** | **93.4** |

The mixture of geranial and neral is often called 'citral' or 'lemonal', which earned its name in the 1800s after it was isolated from a lemon Citrus species, but today the major supply of citral comes from *Cymbopogon* (Akhila, 2009). Due to poor separation of the isomers, citral was initially treated as a pure isolate, but was subsequently demonstrated to be a mixture, with their structures established as early as 1947, but confirmed later by NMR (Ohtsuru et al., 1967). The antimicrobial activity of 'citral' is already known and reported to be noteworthy, with the general value of 0.05% v/v (<0.5 mg/ml) across a range of Gram-positive and Gram-negative bacteria (Onawunmi, 1989). Citral is also regarded as a prominent insect pheromone (Kuwahara & Suzuki, 1983) and it is claimed to have antiadipogenic activity in rats (Modak & Mukhopadhaya, 2011).

Trace amounts of similar flavonoids to pinocembrin were observed but could not be isolated in sufficient quantity for proper chemical assignment. However, the assignment of pinocembrin as the overwhelmingly dominant flavonoid is in contrast to previous reports of chrysin as predominating (Williams et al., 1997). This suggests that chemotypes exist within the species or that cultivars grown in Europe may have a hybrid origin.

The realization of pinocembrin as the major flavonoid in the leaves came as a surprise since it was not assigned earlier (Williams et al., 1997). The possibility of a misidentification in the study by Williams et al. (1997) of the *C*-methyl flavone was initially contemplated by the authors of the current study. Since we identified pinocembrin, which is not a *C*-methyl flavanone, there is a possibility that it was observed in the study by Williams et al. (1997), but incorrectly characterized. However, this is not the case because the masses of the compounds do not overlap. It is evident that pinocembrin was not present in the material studied by Williams et al. (1997), which indicates that at least two chemically distinct variants occur within *P. crispum*. It is not yet clear if this will also correlate to essential oil chemotypes, but such an outcome will not be surprising.

Much like chrysin, pinocembrin has also demonstrated biological activities that implicate positive human health benefits. It is present in honey and propolis; but well known plant sources include ginger roots (*Zingiber officinale* Radix), *Eucalyptus sieberi*, wild marjoram (*Origanum vulgare*) and *Eriodictyon californicum*; the latter is from where pinocembrin was first isolated (Lan et al., 2016). In 2008 the compound was approved in China for use in the treatment of stroke, which is thought to at least partly derive from its cognition improving activity via neurovascular unit protection (Liu et al., 2014). Other noteworthy benefits include anti-inflammatory activity, modulation of mitochondrial function, regulation of apoptosis, protection of the blood-brain barrier (Lan et al., 2016; Rasul et al., 2013) and cardioprotection (Lungkaphin, 2015). Furthermore, citral is not the only pheromone identified in the current study. The active antifeedant component in an extract from *Flourensia oolepsis*, which was used against pest larvae of *Epilachna paenulata*, was in fact pinocembrin (Napal et al., 2009).

There is some overlap in biological activities of chrysin and pinocembrin. Chrysin also has neuroprotective and anti-inflammatory effects (Oh, 2016). However, mediation of insulin resistance has been somewhat overlooked for the two flavonoids. It is evident from a study of Chinese propolis that both chrysin and pinocembrin should be examined more comprehensively for management of insulin resistance (Zhao et al., 2014). Due to much overlap in biological activities of both chrysin and pinocembrin, it is feasible that health claims associated with use of *P. crispum* as a food condiment are warranted in both flavonoid chemotypes.

At this stage it is not known if essential oil chemotypes occur in nature but the current study constitutes the first comprehensive investigation of three plants from a wild population of the species, which assigns

mainly geranial/neral (citral), linalool and their related derivatives at a yield of approximately 0.5% g/g wet leaves.

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