The Essential oil composition of *Trachymene incisa* subsp. *incisa* Rudge from Australia†

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Abstract: *Trachymene incisa* subsp. *incisa* is an Australian endemic taxon that varies greatly in the abundance and length of the leaf trichomes. The essential oil composition of five populations of this subspecies, three corresponding to the typical glabrous form and two of the particularly hairy variant, has been analyzed in an attempt to determinate if that variability is also reflected in their composition. The oils have been extracted by hydrodistillation and analysed by Gas Chromatography (GC) and Gas Chromatography coupled to Mass Spectrometry (GC-MS). The essential oils of *T. incisa* subsp. *incisa* were characterized by the high amount of sesquiterpenes that were the major fraction. The sesquiterpene hydrocarbons were significantly higher in the hairy variant in comparison to the glabrous one. According to the main compound three different chemotypes were found: I.- β-selinene + bicyclogermacrene and II- γ-bisabolene + α-pinene for the typical glabrous variant and III.- bicyclogermacrene + β-caryophyllene for the hairy variant.

Keywords: *Trachymene incisa*; Apiaceae; Chemotype; β-Selinene; Bicyclogermacrene; γ-Bisabolene; α-Pinene; β-Caryophyllene; Essential oil; Chemical composition.

1. Introduction

The genus *Trachymene* Rudge, belongs to the Apiaceae family and comprises at least 56 species of herbs, distributed in Australia, New Guinea, the Philippine Islands, Borneo, New Caledonia, Vanuatu and Fiji. Of those, 38 species are native to Australia with a wide diversity of habitats ranging from deserts to alpine areas. The taxonomy of this genus has been controversial throughout the history because of the numerous synonyms and the plasticity of some of the species [1]. The type section, *Trachymene* sect. *Trachymene*, includes perennial species with functionally bicarpellate fruits[2].

*Trachymene incisa* Rudge subsp. *incisa* (Figure 1) is an erect or ascending perennial herb to 80 cm high with a thick rootstock. The plants have highly variable dissected basal leaves and one or more rosettes attached to the rootstock. The leaves sparsely hairy to almost glabrous, the lamina broad ovate, 14-65 mm long, 15-76 mm wide; segments elliptic or linear, 0.5-6 mm wide. Plants have bisexual white flowers, clustered into simple umbels with a mean of 72 flowers per umbel. The fruits are broad ovate are 2-4 mm long, 2.5-4.0(-7.5)mm wide, brown; occasionally developing only one mericarp. Mericarps 1.8-2.0(-3.8)mm wide; smooth orbiculate to papillate schizocarps. Flowers most of the year, peaking between September and April. The dispersal takes place from March to October with no obvious peak. It grows commonly in dry eucalypt woodland or scrub, sclerophyll
Research on this genus has focused on medicinal or ecological aspects. In south-west Queensland and north-west New South Wales, different species of *Trachymene* (T. glaucifolia (F.Muell.) Benth., T. cyanantha Boyland and T. ochracea L.A.S.Johnson) seem to be toxic to cattle [10-14]. The only herbarium voucher of them corresponded to T. glaucifolia that seems to be directly correlated to a form of ‘staggers’ in sheep and the deformity ‘bent leg’ in lambs [13]. Further research has correlated the intake of T. ochracea with the limb paresis syndrome, included in the first level of the five progressive clinical groups described of the nervous and muscular locomotor disorders that affect sheep throughout Australia [14]. The genus *Trachymene* has been also reported to show teratogenic compounds that affect the development of an embryo, pregnancy or may cause a birth defect in the child [15]. On the other hand, the niche and competitive ability between T. cyanopetala (F.Muell.) Benth. and T. ornata (Endl.) Druce was evaluated, although little evidences was found under well-watered and water-stressed conditions [16]. *Phytophthora caetorum* (Lebert & Cohn) Schroeter is a pathogen fungus that attack T. caerulea R. Grah., the blue laceflower, that is field-grown for the cut-flower industry although the use hymexazol (35 mg/L) reduced significantly the symptoms [17]. Other species, T. pilosa Sm., has been described as resistant and key symptomless host of a relative pathogen *P. cinnamomi* Rands. However, under laboratory condition this species died [18-20]. The resistance of the exine from the mature pollen grains of this species has been estimated [21]. The regeneration ability of T. coerulae Graham under experimental conditions showed that the centre of the shoot apex produced fewer mature leaves and fewer embryos from the basal tissues than did the flanks [22]. Finally, the importance of this genus is clear when species such T. cussonii (Montrouz.) B.L.Burtt has been described as a coral cay endemic plants representative to the conservation of this type of habitat [23-24].

This species has been previously reported, as being a member of the Woodland endangered ecological community [25]. However, most of the papers focus on its reproductive ecology, pollination and germination [4, 7, 26-27].

The aim of this research was to contribute to the knowledge of the essential oil composition of *Trachymene incisa* subsp. *incisa* and evaluate if the abundance of trichomes can affect the composition. Apart from our preliminary conference report, as far as we know this is the first report about the chemical composition of the essential oil of this species, or the genus.
2. Results and Discussion

The distillation of the aerial parts of five different populations of *T. incisa* subsp. *incisa* yielded small amounts of a pale yellow oil (0.05-0.17%). The amount of oil obtained was reduced in comparison with other members of the Apiaceae that are well known and used as condiment or spices [28-29]. However, it would be of interest to check other species of this genus to contrast this.

The identified constituents from the aerial parts of *T. incisa* subsp. *incisa*, their retention indices and their percentage composition are summarized in Table 1 where all the compounds are arranged in order of their elution from the DB-Wax column. A total of 47 compounds have been identified representing from 88.4% to 93.5% of the total oil. The sesquiterpene fraction predominantes, both quantitatively and qualitatively, in all the samples examined, with a total of 33 constituents. Sesquiterpene hydrocarbons (48.3-74.8%) were more abundant than oxygenated sesquiterpene (6.59-36.2%). It is worth noting the low amount of monoterpene fraction and practically the absence of the oxygenated ones, that only appear in T.in.I (9.0%). The synthesis of terpenoids is correlated from the lower molecular weight to the higher [30-31] so this specie's has increased the metabolism that produce sesquiterpenes using monoterpenes as precursors. That could explain the low amount of monoterpenes detected. However, it should be interesting to make a seasonal study of the population 3 (T.in.I3) to know it the amount of α-pinene is constant throughout the cycle of the plant if it could be conditioned by other factors.

Table 1. Essential oil composition (%) of *Trachymene incisa* subsp. *incisa* variants.

| KLa | KLb | Compound       | C. type | T.in.I1 | T.in.I2 | T.in.I3 | T.in.II1 | T.in.II2 |
|-----|-----|----------------|---------|--------|--------|--------|---------|---------|
| 1007| 932 | α-pinene       | MH      | 7.2    |        | 19.2   | t       | 0.1     |
| 1034| 945 | α-fenchene     | MH      |        |        | 0.8    |         |         |
| 1041| 946 | camphene       | MH      |        |        | t      |         |         |
| 1084| 974 | β-pinene       | MH      | 0.4    |        | 2.2    | t       | 0.1     |
| 1105| 969 | sabinene       | MH      |        |        |        |         |         |
| 1127| 1008| β-3-carene     | MH      |        |        | 1.8    |         |         |
| 1139| 1002| α-phellandrene | MH      |        |        | 1.2    |         |         |
| 1141| 988 | myrcene        | MH      | t      |        |        |         |         |
| 1157| 1014| α-terpinene    | MH      |        |        |        |         |         |
| 1176| 1024| limonene       | MH      | t      |        | 0.8    |         |         |
| 1178| 1025| β-phellandrene | MH      | t      |        |        |         |         |
| 1181| 1026| 1,8-cineole    | OM      | t      |        | 9.0    |         |         |
| 1218| 1032| Z-ocimene      | MH      | t      |        |        |         |         |
| 1224| 1054| γ-terpinene    | MH      | t      |        |        |         |         |
| 1226| 1044| E-β-ocimene    | MH      | t      |        |        |         |         |
| 1249| 1020| p-cymene       | MH      | t      | t      | 0.6    | t       | t       |
| 1260| 1086| terpinolene    | MH      | t      |        | 0.4    | 0.9     |         |
| 1320| 989 | 6-methylhept-5-en-2-one | O   | t      |        | 0.2    | t       | t       |
| 1459| 1335| δ-elemene      | SH      | 0.3    | 0.3    |        | t       |         |
| 1462| 1374| α-copaene      | SH      | t      | 3.4    | t       |         |         |
| 1510| 1387| β-cubebene     | SH      | 0.6    |        | t       |         |         |
| 1523| 1411| α-cis-bergamotene | SH  |        |        | 0.6    | 0.4     |         |
| 1543 | 1432 | α-trans-bergamotene | SH | 0.2 | 2.1 | t |
| 1560 | 1389 | β-elemene | SH | 0.8 | 2.9 | 0.4 | t |
| 1563 | 1417 | β-caryophyllene | SH | 2.5 | 5.4 | 1.2 | 10.8 | 10.4 |
| 1570 | 1439 | aromadendrene | SH | 1.5 | 0.6 | 0.5 | 5.6 | 5.0 |
| 1572 | 1509 | α-bulnesene | SH | 0.5 | t | 1.2 | 0.9 |
| 1632 | 1458 | allo-aromadendrene | SH | 0.2 | 0.4 | 0.4 | 0.4 | 0.4 |
| 1644 | 1454 | (E)-β-farnesene | SH | 0.3 | 1.4 | 0.6 |
| 1653 | 1452 | α-humulene | SH | 0.5 | 1.5 | 0.3 | 1.8 | 1.8 |
| 1658 |  | C15H24 | SH | 0.5 | 1.2 | 0.9 |
| 1661 | 1496 | viridiflorene | SH | 1.9 | 0.8 | 0.6 | 5.4 | 4.7 |
| 1670 | 1484 | germacrene-D | SH | 1.4 | 2.4 | 1.5 | 0.5 |
| 1680 | 1505 | γ-bisabolene | SH | 27.4 |
| 1697 | 1489 | β-selinene | SH | 36.7 | 11.9 | 0.5 | 1.8 |
| 1706 | 1500 | bicyclogermacrene | SH | 28.0 | 21.5 | 7.5 | 34.7 | 24.4 |
| 1722 | 1513 | γ-cadinene | SH | t |
| 1724 | 1522 | δ-cadinene | SH | t | 9.0 | 0.5 | 1.5 | 1.7 |
| 1727 | 1495 | cis-cadina-1,4-diene | SH | 0.3 | t |
| 1734 | 1505 | (E,E)-α-farnesene | SH | t | 1.4 | 2.6 | 1.6 |
| 1749 | 1479 | ar-curcumene | SH | t | t | 0.7 | 1.1 |
| 1886 | 1582 | caryophyllene oxide | OS | 1.6 | 2.3 | 0.2 | 1.0 | 12.8 |
| 1958 | 1602 | ledol | OS | 0.2 | 0.2 | 0.1 | 0.5 | t |
| 2016 | 1645 | cubenol | OS | t | 0.9 | 0.2 | 0.8 | 1.5 |
| 2018 | 1595 | cubeban-11-ol | OS | 0.7 | 0.4 | 0.4 | 1.6 | 1.1 |
| 2034 | 1590 | globulol | OS | 2.0 | 1.5 | 1.3 | 7.0 | 6.9 |
| 2044 | 1592 | viridiflorol | OS | 1.5 | 1.1 | 0.8 | 3.9 | 3.5 |
| 2066 |  | C15H26O | OS | 0.5 | 0.3 | 0.3 | 2.2 | 2.0 |
| 2074 |  | C15H26O | OS | 0.4 | 0.6 | 0.3 | 1.3 | 1.0 |
| 2078 |  | C15H26O | OS | 0.2 | 0.3 |
| 2086 | 1577 | spathulenol | OS | 4.2 | 11.2 | 2.9 | 6.7 | 7.5 |
|     |     | Total |     | 93.5 | 81.7 | 91.0 | 91.1 | 88.4 |

| Monoterpene Hydrocarbon (MH) | 16 | 7.6 | 0 | 27.0 | 0.9 | 0 |
| Oxigenated Monoterpene (OM) | 1 | 0 | 0 | 9.0 | 0 | 0 |
| Sesquiterpene Hydrocarbon (SH) | 23 | 74.8 | 63.0 | 48.3 | 65.4 | 52.0 |
| Oxigenated Sesquiterpene (OS) | 10 | 11.1 | 18.7 | 6.5 | 24.8 | 36.3 |
| Other (O) | 1 | 0 | 0 | 0.2 | 0 | 0 |
| Total | 51.0 | 93.5 | 81.7 | 91.0 | 91.1 | 88.2 |

C. type= Compound type; MH= monoterpene hydrocarbon, OM= oxygenated monoterpene, SH= sesquiterpene hydrocarbon, SO= oxygenated sesquiterpene, O=Other; t=traces (<0.1%); KLa and KLib= linear retention index relative to n-alkanes on DB-Wax column or DB-5 column respectively; T.In.= Trachymene incisa subsp. incisa ; I and II= typical glabrous and hairy variant respectively
According to our results, the abundance of trichomes seems to affect the composition of the essential oils of *Trachymene incisa* subsp. *incisa* (Table 1). Although the terpenoid distribution seems similar between both glabrous and hairy variants, the amount of sesquiterpene hydrocarbons is significantly higher in the hairy one (Figure 2). Only five populations were analyzed and not climatic or biological conditions were registered. So further study should be done to confirm this or to check if the age of the plant, the presence of pathogen or the climatic condition could affect to the chemical composition. Previous reports have confirmed that the phenology of the plant and the growing conditions can affect the essential oils of other species of the same family [33-34]. It has been noted that the density of seeds affects the germination of this species [7], so the competition between growth in the plants could also alter its chemistry as allelopathic compounds do [35-37]. In fact, the competition has been reported to have an effect on the phylogenetic signal and phenotypic plasticity in plant functional traits and to the root biomass [38–39]. More work is required to check under laboratory conditions if any of these aspects are involved in the abundance and length of the leaf trichomes of this subspecies.

![Histograms showing the distribution of compound type of the essential oils of *T. incisa* subsp. *incisa*.](image)

Figure 2. Distribution of the compound type of the essential oils of *T. incisa* subsp. *incisa* (T.In.= *Trachymene incisa* subsp. *Incisa* ; I and II= typical glabrous and hairy variant respectively).

With respect to the chemical composition of the volatile oils, *T. incisa* subsp. *incisa* exhibits the same plasticity in its vegetative and reproductive forms. The typical glabrous variant (T.in.I), showed \(\beta\)-selinene (11.9-36.7\%) and bicyclrogemacrene (21.5-28.0\%) as main compounds in the populations 1 and 2. However, the principal components of the hairy variant (T.in.II) were identified as bicyclogeramcere (24.4-34.7\%) and \(\beta\)-caryophyllene (10.4-10.8\%) in the populations 4 and 5,
Although the population 5 also contained a high amount of caryophyllene oxide (12.8%). According to these results it seems that each variant has a characteristic composition, but the population 3 belonging to the typical glabrous variant (T.in.I) showed other chemotype, with γ-bisabolene (27.4%) and α-pinene (19.2%) being the major compounds. According to the main compound three different chemotypes could be defined: I. β-selinene + bicyclogermacrene and II. γ-bisabolene + α-pinene for the typical glabrous variant and III. bicyclogermacrene + β-caryophyllene for the hairy variant, though more sampling of the species would be needed to confirm this.

With this paper we contribute for the first time to the knowledge of the chemistry of the genus *Trachymene*. It would be interesting to study the effect of climate, light or soil conditions on the chemical composition of this species to confirm the chemotypes described. The diversity of this genus could also be the source of further investigations to characterize their essential oils.

### 3. Materials and Methods

#### 4.1. Plant material

Five different populations of *Trachymene incisa* subsp. *incisa* were gathered at flowering from the Northern Tablelands of New South Wales in 2007 (Table 2). Voucher specimens were lodged at the N.C.W. Beadle Herbarium of the University of New England, Australia (NE).

#### Table 2. Location of the populations of *Trachymene incisa* subsp. *incisa* studied.

| Sample | Voucher nº  | Location                                      | Yield    |
|--------|------------|-----------------------------------------------|----------|
| T.in.I | LMC4175    | Single National Park: (NSW, Australia) 20 km NW of Guyra (24 Mar. 2007) | 0.05%    |
| T.in.I | LMC4176    | Single National Park: (NSW, Australia) 20 km NW of Guyra (24 Mar. 2007) | 0.17%    |
| T.in.I | LMC4211    | Dumasq Dam: (NSW, Australia) Along south-western side of Dumasq Dam, c. 10 km NW of Armidale (14 Apr. 2007) | 0.05%    |
| T.in.II| LMC4210    | Dumasq Dam: (NSW, Australia) Along south-western side of Dumasq Dam, c. 10 km NW of Armidale (14 Apr. 2007) | 0.05%    |
| T.in.II| LMC4234    | Dumasq Dam: (NSW, Australia) Along south-western side of Dumasq Dam, c. 10 km NW of Armidale (14 Apr. 2007) | 0.1%     |

| T.In= Trachymene incisa subsp. incisa ; I and II= typical glabrous and hairy variant respectively. |

#### 4.2. Isolation of volatile oils

The oils were isolated by steam distillation with cohabation for 8 hrs as previously described by Brophy et al [40]. The oils were colourless to pale yellow, with a yield from 0.05% to 0.17% based in dry weight (Table 2).

#### 4.3. Identification of components

Analytical gas chromatography (GC) was carried out on a Shimadzu GC17A gas chromatograph with a Megabore column of DB-Wax (60 m x 0.5 mm x 1 μm) which was programmed from 50°-220°C at 3°Cmin⁻¹ with helium as the carrier gas. GC integrations were performed on a SMAD electronic integrator. GC-MS was carried out on a Shimadzu GCMS-QP5000 mass spectrometer operating at 70 eV ionization energy. The GC column used was a DB-wax (30 m x 0.25 mm x 0.25 μm) programmed from 35° to 220°C at 3°Cmin⁻¹ with helium as the carrier gas. Compounds were identified by their GC retention indices relative to known compounds and by comparison of their mass spectra with either known compounds or published spectra [41-48]. The oil sample was also analyzed on the same GC/MS system and under the same conditions as above except that a DB-5 (30 m x 0.25 mm x 0.25 μm) column was used, programmed from 35° to 250°C at 5°Cmin⁻¹ with helium as the carrier gas.

### 4. Conclusions

According to our results detailed above, the essential oils of *Trachymene incisa* subsp. *incisa* are characterized by a high amount of sesquiterpenes that were the major compounds. The sesquiterpene hydrocarbons were significantly more abundant in the hairy variant in comparison to glabrous form. It may be that three different chemotypes occur, with the typical glabrous variant showing two different chemotypes, characterised by: β-selinene + bicyclogermacrene and γ-
bisabolene + α-pinene, while the hairy variant was characterized by bicyclogermacrene + β-caryophyllene.

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