

Article

The Essential oil composition of *Trachymene incisa* Rudge 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 orbiberculate 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

forest and cleared areas on sandy soils and on rocky granite outcrops and resprouts from the tuberous taproot after fire. It is distributed widely throughout eastern NSW north from Ulladulla and into Queensland [1, 3-9].

Figure 1. *Trachymene incisa* Rudge subsp. *incisa*. Details of the basal leaves and umbel.



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. coerulea* 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.Burt 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 predominates, both quantitative 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.I₃ (9.0%). The synthesis of terpenoids is correlated from the lower molecular weight to the higher [30-31] so this species has increased the metabolism that produces 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 if 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.

KIa	KIb	Compound	C. type	T.in.I ₁	T.in.I ₂	T.in.I ₃	T.in.II ₄	T.in.II ₅
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	δ -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- β -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		C ₁₅ H ₂₄	SH	0.4	0.3	0.7		
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		C ₁₅ H ₂₆ O	OS	0.5	0.3	0.3	2.2	2.0
2074		C ₁₅ H ₂₆ O	OS	0.4	0.6	0.3	1.3	1.0
2078		C ₁₅ H ₂₆ O	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
Oxygenated Monoterpene (OM)			1	0	0	9.0	0	0
Sesquiterpene Hydrocarbon (SH)			23	74.8	63.0	48.3	65.4	52.0
Oxygenated 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%); KIa and KIb= linear retention index relative to n-alkanes on DB-Wax column or DB-5 column respectively; T.In.= <i>Trachymene incisa</i> subsp. <i>incisa</i> ; I and II= typical glabrous and hairy variant respectively								

According to our results, the abundance of trichomes seems to affect to 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.



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 β -selinene (11.9-36.7%) and bicyclogermacrene (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 bicyclogermacrene (24.4-34.7%) and β -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 n ^o	Location	Yield
T.in.I ₁	LMC4175	Single National Park: (NSW, Australia) 20 km NW of Guyra (24 Mar. 2007)	0.05%
T.in.I ₂	LMC4176		0.17%
T.in.I ₃	LMC4211		0.05%
T.in.II ₄	LMC4210		0.05%
T.in.II ₅	LMC4234	Dumaresq Dam: (NSW, Australia) Along south-western side of Dumaresq Dam, c. 10 km NW of Armidale (14 Apr. 2007)	0.1%
T.In= <i>Trachymene incisa</i> subsp. <i>incisa</i> ; I and II= typical glabrous and hairy variant respectively.			

4.2. Isolation of volatile oils

The oils were isolated by steam distillation with cohobation 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 μ m) 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 μ m) 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 μ m) 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.

Author Contributions: Lachlan M. Copeland was the responsible for gathering and identifying the species. All the authors have contributed equally to the rest of the paper.

Funding: This research received no external funding.

Acknowledgments: We would like to express our deepest gratitude to the late Dr. Robert J. Goldsack for his friendship and collaboration throughout the years. Rest in peace.

Conflicts of Interest: The authors declare no conflict of interest.

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