PERGAMON

Phytochemistry 50 (1998) 407–415

PHYTOCHEMISTRY

Chemical, physical and antimicrobial properties of essential oils of *Leptospermum scoparium* and *Kunzea ericoides*

Noel G. Porter[a,*], Alistair L. Wilkins[b]

[a]*New Zealand Institute for Crop and Food Research Ltd., Private Bag 4704, Christchurch, New Zealand*
[b]*Department of Chemistry, University of Waikato, Private Bag 3105, Hamilton, New Zealand*

Revised 30 June 1998

Abstract

The major components of commercial New Zealand essential oils of *Leptospermum scoparium* (manuka) and *Kunzea ericoides* (kanuka) are identified. In the manuka oil, monoterpenes are present at low levels (≤3%). Sesquiterpene hydrocarbons are predominant (≥60%) and include groups possessing cubebene/copaene, elemene, gurjunene/aromadendrene, farnesene/caryophyllene, selinene, calamenene and cadinene skeletons. Oxygenated sesquiterpenes and triketones are present (≤30%). The antimicrobial activity of the manuka oil was associated with a fraction containing three major and three trace triketones, two of the latter were previously unreported. Kanuka oil was characterized by high levels of α-pinene (>50%) and lower levels (<10%) of viridiflorol and viridiflorene. GC-MS and GC-FID detector responses to the same components were noticeably different for some major components, including the triketones. Non-commercial manuka oils from different sites differed widely in composition and could be separated into four groups by the presence and levels of distinctive components. The density and refractive index of manuka and kanuka oils were closely correlated with the total sesquiterpene levels. The density of the commercial manuka oil was closely correlated with the level of the triketones. Simple density measurements enabled discrimination between the commercial oil and oils from other sites, and prediction of antimicrobial activity.

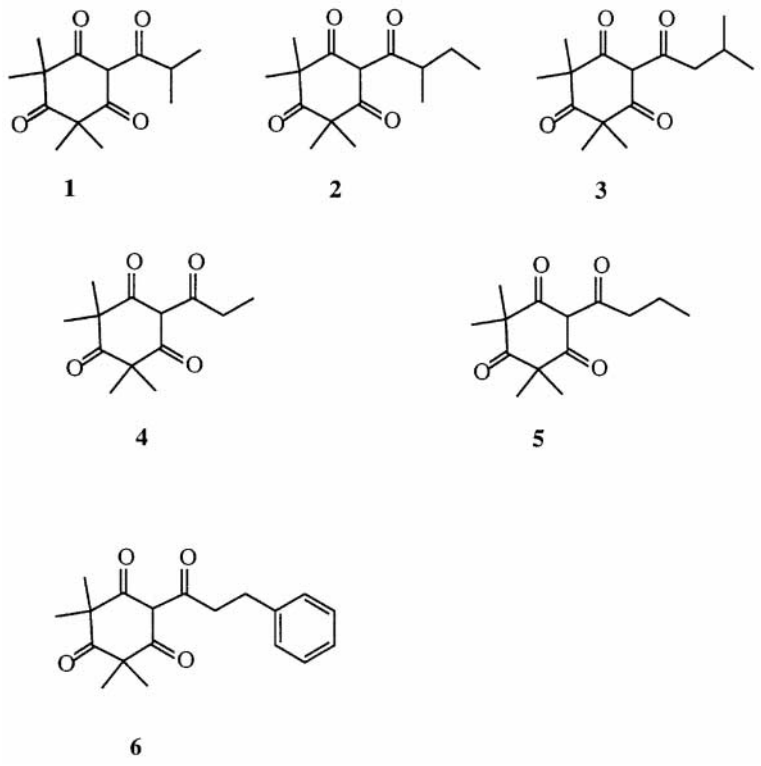
Keywords: *Leptospermum scoparium*; *Kunzea ericoides*; Myrtaceae; Manuka; Kanuka; Essential oil; Triketone; Leptospermone; *Iso*-leptospermone; Flavesone; Bioactivity

1. Introduction

Leptospermum scoparium J.R. Et G. Forst. (manuka) (Allan, 1961) and *Kunzea ericoides* A. Rich. (kanuka) (Connor & Edgar, 1985) Myrtaceae are widely distributed as small trees or shrubs in scattered or dense populations in widely differing climatic and altitudinal zones in New Zealand (Yin Ronghua, Mark, & Wilson, 1984; Wardle, 1991). Early New Zealand records report that the bark, leaves, sap and seed capsules of manuka were used in beverages or medicinal preparations (Best, 1905; Brooker, Cambie, & Cooper, 1981. Leptospermol was isolated first in Australia from the leaves of *L. flavescens* (Penfold, 1921) and soon after in New Zealand from *L. scoparium* (Gardner, 1924, 1925). It was later re-named (Short, 1926) and reported (Briggs, Penfold, & Short, 1938; Briggs, Hassall, & Short, 1945; Brooker, Cain, & Cambie, 1963) as leptospermone (**3**). Other triketones have been reported in some Australian myrtaceous species (Hellyer, 1968; Brophy, Goldsack, Forster, Clarkson, & Fookes, 1996) and components of oils from three Australian *Leptospermum* species have been identified (Flynn, Lassak, & Smyth, 1979). More recently, flavesone (**1**), *iso*-leptospermone (**2**), leptospermone (**3**) (Joulain, 1996; Perry, Brennan, Van Klink, Brennan, Harris, Douglas et al., 1997a), cadina-3,5-diene and δ-amorphene (Melching, Bulow, Wihstutz, Jung, & Konig, 1997) have been reported in *L. scoparium* oils. Published reports (Briggs et al., 1938; Briggs, 1947; Atkinson & Brice, 1955) of biological activity of manuka oil components were supported by our preliminary testing, suggesting that these components have potential for use in pharmaceutical products. Over the last 10 years, renewed interest in

* Corresponding author.

0031-9422/98/$ - see front matter
PII: S0031-9422(98)00548-2



manuka and kanuka oil has developed into commercial production in New Zealand. There is marked variation in the composition of oils from different provenances of *L. scoparium* and *K. ericoides* (Perry et al., 1997a,b) and between individual plants of a single population (Porter, Smale, Nelson, Hay, Van Klink, & Dean, 1998). Essential oils of these species have been used for chemotaxonomic studies (Harris, Porter, & Dawson, 1992; Perry, Van Klink, Brennan, Harris, Anderson, Douglas et al., 1997b) and major components have been identified (Flynn et al., 1979; Perry et al., 1997a,b), but comprehensive data on the composition of oils of *K. ericoides* or *L. scoparium* have not previously been published. This paper presents unpublished results of commercially sensitive work (1989–95) which have led to the development of commercial production of manuka and kanuka oils. We now report the detailed chemical composition and the basis for the antimicrobial properties of the manuka oil obtained from a distinct chemotype found in the East Cape area of the North Island of New Zealand (trade name Manex). We also report details of the chemical composition of kanuka oil from the same region (trade name Kanex). Previously unreported compounds are identified in both oils. Relationships are established between the chemical composition, physical parameters and antimicrobial activity of a range of manuka oils.

2. Results and discussion

2.1. Identification of antimicrobial components of Manex oil

Manex oil (East Cape, North Island) inhibited the growth of a range of test micro-organisms (Table 1). Silica gel CC of Manex oil gave good separation into a non-polar fraction of mainly sesquiterpene hydrocarbons and a polar fraction which consisted largely of three components (≥90%) and retained all the biological activity (Table 1). Another manuka oil (Kaiteriteri, South Island), containing significant levels of monoterpenes but no triketones, was similarly fractionated and tested. No significant inhibition of any of the organisms was obtained with either the oil or its fractions. These results confirm the antimicrobial activity of East Cape manuka oils seen in preliminary tests and add to the activity reported by Perry et al. (1997a). The lack of activity in the non-polar fraction, which contains the remaining components of the oil, shows clearly that the activity is associated with the polar triketones. Activity is also associated with the presence of triketones in manuka oils from other areas (Porter, unpublished data). These results identify a consistent basis for the activity, which is more powerful and selective than that reported by Lis-Balchin, Deans, and Hart (1996). Results of subsequent tests, confirming the activity of Manex oil against a wider range of test organisms, will be published in a subsequent paper.

Table 1
Minimum bactericidal concentrations (MBC) for Manex oil and the non-polar and polar chromatographic fractions against five test micro-organisms. MBC are expressed as the minimum % (w/v) of oil required to inhibit growth

	Organism, strain[a]				
	Staphylococcus aureus, 147	*Staphylococcus aureus*, MRSA NS	*Escherichia coli*, 5934	*Pseudomonas aeruginosa*, 997	*Candida albicans*, 1212
Whole oil	0.039	0.0195	1.25	1.25	0.31
Non-polar fraction	1.25	1.25	1.25	1.25	1.25
Polar fraction	0.039	0.0195	1.25	1.25	0.31

[a]New Zealand Communicable Disease Centre numbers.

The three principal polar components were identified from GC-MS and ^{1}H and ^{13}C NMR spectral data as the triketones flavesone (**1**), *iso*-leptospermone (**2**) and leptospermone (**3**) (Joulain, pers. comm., 1991) and later confirmed by Wilkins (unpublished data, 1993), Joulain (1996) and Perry et al. (1997a). The chromatographically separated triketone fraction was shown by GC-MS to contain the dominant triketones **1**, **2** and **3** and trace levels of three other related triketones **4**, **5** and **6**. These were tentatively identified as 2(1-oxopropyl)-4,4,6,6-tetramethylcyclohexan-1,3,5-trione (**4**), 2-(1-oxobutyl)-4,4,6,6-tetramethylcyclohexan-1,3,5-trione (**5**) and 2(1-oxo-3-benzyl-propyl)-4,4,6,6-tetramethylcyclohexan-1,3,5-trione (**6**). The MS of **5**, which eluted from the GC column after **1** and before **2**, was essentially similar to that of **1**. Prominent $[M-70]^{+}$ fragments, attributable to the loss of dimethylketene, $(H_3C)_2C{=}C{=}O$, were observed in the MS of all compounds (**1**–**6**). Compound **6** has previously been reported as grandiflorone (Hellyer, 1968; Brophy et al., 1996). Compounds **4** and **5** do not appear to have been reported previously in the literature.

2.2. Manex oil composition

Commercial interest in Manex oil, and the need to satisfy toxicological compliance testing, required a more extensive description of the composition of the oil. The identities of components of a representative Manex oil sample and their % contribution to the GC-MS total ion current are given in Table 2. There are several notable features. The monoterpenes, which are all commonly found in other essential oils, are present in Manex at low levels compared with other manuka oils. Esters (mainly C_{10}) are present at low levels. Sesquiterpene hydrocarbons are the predominant class, typically comprising over half of the total oil and including groups of chemically related components or isomeric forms, e.g. compounds possessing cubebene/copaene, elemene, gurjunene/aromadendrene, farnesene/caryophyllene, selinene, calamenene and cadinene skeletons. The triketones are commercially the most important group since they are responsible for the demonstrated antimicrobial activity, but since only $<0.03\%$ of **4**, **5** and **6** were typically present in Manex oil samples, the levels of these compounds are not given in Table 2. The GC data of the East Cape chemotype of Perry et al. (1997a) contains major peaks corresponding to all the major sesquiterpene and triketone peaks shown in Table 2, confirming the major features of oil composition and allowing more complete identification of components in their profile by comparison of retention and GC-MS data. The identification of grandiflorone (**6**) extends the occurrence of this component from *L. flavescens* in Australia (Brooker et al., 1963) to *L. scoparium* in New Zealand, as has been reported with leptospermone (Penfold, 1921; Gardner, 1924, 1925; Short, 1926) and more recently flavesone (Flynn et al., 1979; Joulain, 1996; Perry et al., 1997a). The other triketones, **4** and **5**, do not appear to have been reported previously. Melching et al. (1997) also report identification of other major sesquiterpenoid components, but not the triketones. The identification of cadina-3,5-diene, zonarene and δ-amorphene, comprising 5–10% of Manex oil (Table 2), confirms the first reports of these compounds by Melching et al. (1997).

Manuka oil is often compared and confused with oil from the Australian species, *Melaleuca alternifolia*, because both oils have antimicrobial properties and are both called 'tea tree' in their countries of origin. These oils differ markedly in their chemistry and in their range of antimicrobial activity. The major component of commercial *M. alternifolia* oils (Brophy, Davies, Southwell, Stiff, & Williams, 1989), terpinen-4-ol, is not found as a significant component of manuka oils. However, many of the minor sesquiterpene components in *M. alternifolia* oils e.g. α-cubebene, α-copaene, β-elemene, calamenene, δ-cadinene, are found as major sesquiterpene components in Manex and other manuka oils (Table 2). Brophy et al. (1989) reported three unidentified $C_{15}H_{24}$ compounds in *M. alternifolia* oils which may be cadina-3,5-diene, zonarene and δ-amorphene.

During analysis of the same samples of oils using GC-MS and GC-FID, differences in the % peak con-

Table 2
GC-MS identification and % peak area contributions of components of commercial Manex and Kanex oils

RT (min)	Compound	Manex	Kanex
4.50	α-thujene	0.03	0.62
4.66	α-pinene	1.31	55.51
4.97	camphene	nd[a]	0.08
5.05		nd	0.06
5.56	β-pinene	0.12	0.63
5.88	myrcene	0.24	0.1
6.58	α-terpinene	nd	0.08
6.64	*p*-cymene	0.16	3.41
6.83	1,8-cineole	0.22	3.94
6.89	limonene	0.10	3.94
7.43	*trans*-ocimene	nd	0.33
7.69	γ-terpinene	0.16	2.53
7.92		nd	0.26
8.42		nd	0.15
8.57	terpinolene	0.05	0.51
8.77	linalool	0.10	1.52
8.82	*iso*-amyl-2-methyl butyrate	0.05	nd
8.97	*iso*-amyl *iso*-valerate	0.13	nd
9.04	2-methylbutyl *iso*-valerate	0.05	nd
9.24	*iso*-prenyl *iso*-valerate	0.24	nd
9.34		nd	0.51
10.37		nd	0.23
11.15	terpinen-4-ol	0.04	0.24
11.50	α-terpineol	0.09	0.91
11.69	*iso*-amyl tiglate	0.09	nd
11.73		nd	0.10
11.90	*iso*-prenyl tiglate	0.03	nd
12.38		nd	0.15
17.31	α-cubebene	3.95	0.21
17.98	α-ylangene	0.32	nd
18.15	α-copaene	5.86	0.63
18.23		nd	0.06
18.56	β-elemene	0.55	nd
19.21	α-gurjunene	1.02	0.43
19.43	β-caryophyllene	2.63	0.48
19.64		0.11	nd
19.73		0.11	nd
19.85		0.13	nd
19.92		0.03	nd
20.06	aromadendrene	2.09	0.47
20.13		0.33	nd
20.33	cadina-3,5-diene	4.88	0.53
20.46	α-humulene	0.37	0.17
20.68	allo-aromadendrene	0.80	0.70
20.97		0.04	nd
21.09	δ-amorphene	3.81	0.49
21.16		1.28	nd
21.27	α-amorphene	0.33	nd
21.45	β-selinene	3.67	0.31
21.43		nd	0.10
21.57		1.04	nd
21.77	α-selinene (+viridiflorene)	4.35	2.39
21.88	α-muurolene	0.77	0.20
22.00		0.16	nd
22.07		0.07	nd
22.15	α-farnesene	0.84	nd
22.26		0.67	0.09
22.43	calamenene	14.42	3.02
22.55	δ-cadinene	6.02	0.95
22.85	flavesone	4.91	nd
22.85	cadina-1,4-diene	5.94	1.27
22.94		0.63	nd
23.53		0.07	nd
23.65	β-nerolidol	0.24	1.79
23.82		0.07	0.58
23.91	spathulenol	0.52	1.33
24.06	caryophyllene epoxide	0.25	0.16
24.23		1.53	0.21
24.42	viridiflorol	0.46	7.23
24.50		0.15	nd
24.76	ledol	0.23	1.86
24.97		0.05	nd
25.13	*iso*-leptospermone	4.62	nd
25.11		nd	0.11
25.39	leptospermone	15.54	nd
25.47		nd	0.15
25.51		0.83	nd
25.67		0.18	nd
25.81		0.18	0.08
25.94		0.99	0.21
26.08		0.26	nd
26.61		0.06	nd
26.83		0.07	nd
27.78		0.09	nd

[a]Not detected at >0.03%.

tributions of some of the components, especially the triketones, were noted. The GC-FID and GC-MS detected peak area % contributions were determined for two of the major hydrocarbons (α-copaene and calamenene) and three triketones in six Manex samples from different commercial production batches. Variations in corresponding hydrocarbon and triketone levels were observed. Experience in our laboratories is that variations in detector response across different classes of compounds are generally greater for the quadropole GC-MSD than for the GC-FID, depending on the state of the MSD source and the tuning parameters. The data indicate that the GC-MSD and GC-FID response factors of sesquiterpene hydrocarbons such as α-copaene (a sesquiterpene alkene) are similar. The GC-MSD response factor of calamenene (an aromatic sesquiterpene) appears to be smaller, but those of flavesone, *iso*-leptospermone and leptospermone (triketones) appear to be greater than the GC-FID response factors for these compounds. These observations prompted the investigation of an alternative procedure for the reliable determination of the % triketone contribution (below).

2.3. Kanex oil composition

Kanuka oil is also produced commercially but because it has lower antimicrobial activity and its major oil component, α-pinene, is obtained more cheaply from other sources, it is of less commercial importance and has not been well characterized. The

identities of components of a representative Kanex oil sample and their % contribution to the GC-MS total ion current are given in Table 2.

Most of the kanuka oils examined to date in our laboratories exhibited similar GC-MS and GC-FID profiles. The major features of these profiles were elevated levels of monoterpene hydrocarbons, predominantly α-pinene, and the presence of modest levels of some sesquiterpenes e.g. viridiflorene, calamenene, viridiflorol and ledol. These compounds are also present in *M. alternifolia* oil (Brophy et al., 1989). This commercial oil, harvested across an extensive area near East Cape, had a lower α-pinene level (55.5%) than two kanuka oils from a more restricted area in Coromandel (67.8%). Its composition probably represents a mean within considerable genotypic variation across the harvesting area. Substantial differences were found even between single plants derived from seed collected near East Cape (Perry et al., 1997a).

2.4. Variations in manuka oil compositions

Since the work on manuka oils began in 1987, numerous oil samples from a wide range of geographical sites have been analyzed in our laboratories (supplementary detail on individual samples available on request). South Island sites include three in the Canterbury foothills, one from coastal North Otago, two in the Marlborough Sounds, one in Maruia (West Coast), four in Tasman Bay (Nelson). North Island sites include two in East Cape, one on Great Barrier Island, and one in the Coromandel region. During our investigations, it became increasingly obvious that the oil composition varied from one site to another. Manuka oil samples examined in our laboratories to date can be separated simply into four groupings based on the presence of distinguishing oil components at significant levels: (i) triketone rich, e.g. Manex type from East Cape; (ii) linalool and eudesmol rich, e.g. Kaiteriteri, Nelson; (iii) pinene rich, Otaio and Woodstock, Canterbury; and (iv) triketone, linalool and eudesmol deficient, found in a range of North and South Island sites. Typical levels of distinguishing oil components characteristic of the four types from different sites are presented in Table 3.

These findings are largely in agreement with Perry et al. (1997a) who compared oil composition in plants derived from seed from different sites and suggested three chemotypes which correspond to (i), (iii) and (iv). Their unpublished data also contain a profile corresponding to (ii). Continuing sampling of previously untested sites is revealing further chemical variation (Porter, unpublished data) and suggesting other possible chemical groupings within manuka. It is unlikely that the four groupings suggested here present a complete picture of the geographical variation of manuka oil chemistry in New Zealand. Further, both sets of manuka samples show an extremely wide range of α-pinene levels with the highest levels resembling those of kanuka oils (Table 2). Harris et al. (1992) report an oil profile which combines characteristics of both manuka and kanuka. These findings, and the number of components that are common to both the kanuka and manuka oils, suggest that it may not be possible to distinguish between manuka and kanuka oils solely on compositional data as easily and clearly as would be desired for commercial or taxonomic purposes. However, the most obvious and confirmed distinguishing feature of the variation between sites found so far is the presence or absence of the three major triketone components. This validates the recognition of East Cape manuka as a distinct chemotype. The registered trade name Manex is now applied to the oil from this chemotype to distinguish it from other manuka oils, which do not contain triketones as major components or possess the same level of antimicrobial activity. Before further well-defined chemotypes can be properly established within the range of variation currently being revealed in these two species, a systematic analysis of their full geographical and chemical range in New Zealand is required. Any definition of a chemotype must accommodate the variation in oil composition at each site such as that found between seasons and between individual manuka plants derived from seed collected at a single site (Porter et al., 1998).

2.5. Variation in physical parameters of manuka and kanuka oils

Further variability of oil samples can easily arise by confusion of the two species during field sampling or inclusion during commercial wild harvesting to give mixed foliage and oil samples. Manuka and kanuka often grow in mixed stands and both species can exhibit significant variation in morphological characteristics between individual plants giving rise to superficial similarities at a single site. Oils from mixed kanuka and manuka foliage will have obviously variable composition, physical properties and antimicrobial activity. Such variability may make the oil unacceptable as a commercial oil if it is being produced to guaranteed chemical or antimicrobial activity specifications. Some commercial samples of manuka oil obtained for analysis appeared to be such mixtures, inferring different antimicrobial activity. This illustrates the need for practical methods of discriminating between oils. Having established that Manex oil had superior antimicrobial activity due to the presence of the triketones, we examined a range of manuka, kanuka and mixed oils to develop methods for predicting antimicrobial activity and quality assurance other than analysis of oil composition. We anticipated that

there would be a consistent, logical relationship between the chemical composition and physical properties of the manuka and kanuka oils, based on the assumption that the densities of sesquiterpenes and triketones are greater than those of the monoterpene hydrocarbons. For example, in a typical Manex oil the triketone fraction density was 1.065 g/ml, while that of the residue was 0.918 g/ml. Density, refractive index and mono- and sesquiterpene compositional data (GC-MS detection) for 10 North Island manuka (type (iv) above) and kanuka samples are presented in Table 4. Triketones were not detected (>0.03%) in any of these oils. Density, refractive index, and levels of total sesquiterpenes were strongly correlated,

$$\text{density} = 0.846 + 8.322e^{-4}\% \text{ total sesquiterpenes},$$

$$R^2 = 0.974,$$

$$\text{refractive index} = 1.461 + 4.167e^{-4}\% \text{ total sesquiterpenes},$$

$$R^2 = 0.971,$$

$$\text{density} = 2.058 + 1.988 \text{ refractive index}, \quad R^2 = 0.994.$$

The mono- and sesquiterpene levels, oil density and refractive index data from oil samples out of one geographical area can be used to distinguish the group of mixed foliage oils (E, F, G and H) from kanuka (A, B and D), or manuka (C, I and J) oils (e.g. Fig. 1). Such relationships offer a simple check of the consistency of the chemical and physical parameters with the purity of type (ii) and (iv) manuka oils.

Density can also be used to predict the level of antimicrobial activity in manuka oils. A Manex oil sample (density 0.956 ± 0.001 g/ml) was separated into a triketone concentrate (96% purity by GC-FID analyses, density 1.065 ± 0.003 g/ml) and a residue (density 0.918 ± 0.001 g/ml). The density of the triketone-free, non-active residue is comparable to that for manuka oil samples I and J (Table 4), density 0.916 and 0.917 g/ml, respectively. On the basis of currently available data, it is most unlikely that manuka oils whose density is less than 0.92 g/ml will have significant levels of the antimicrobial activity associated with the triketones. Density can also be used as a simple check on the triketone levels of Manex oils. A series of oil blends (10–40% triketones) was generated from the concentrate and residue covering the range of triketone levels seen in the Manex samples. Fig. 2 shows the correlation between density and the triketone levels of the series of blends. The correlation equation, $\%\text{ triketone} = 570.8 \times \text{density} - 520.1$ ($R^2 = 0.996$) can be used to predict the % triketone contribution, and therefore the antimicrobial activity, of a Manex oil based only on the knowledge of the density of the oil sample. In samples of two recent commercial production batches, oil densities predicted triketone levels of 26.3 and 27.7%, compared with values of 26.8 and 27.9% respectively, from GC-FID analysis. This relationship also provides a check on the purity of the foliage and

Table 3
GC-FID % peak area contribution of distinguishing components in manuka oils of types i–iv from different sites

Component	Site (type)			
	Manex (i)	Kaiteriteri (ii)	Takaka Hill (iii)	Woodstock (iv)
α-Pinene	0.5	1.2	2.3	63.0
Cineole	0.3	1.1	nd[a]	4.2
Linalool	0.1	4.3	nd	3.0
α-Cubebene	4.0	6.8	2.6	0.4
β-Elemene	0.6	6.6	7.5	0.5
β-Caryophyllene	2.6	6.8	2.6	0.6
β-Selinene	3.6	3.1	1.8	0.4
α-Selinene	4.5	5.9	2.9	0.8
Calamenene	11.9	8.0	4.3	1.9
δ-Cadinene	6.1	3.2	1.9	0.6
Total triketones	24.5	nd	nd	nd
Total eudesmols	nd	16.8	5.9	nd

[a]Not detected at >0.1%.

Table 4
Summary of variation in GC-MS determined % composition of North Island type iv manuka (C, I and J), kanuka oils (A, B and D) and mixed foliage oils (E, F, G and H)

	Sample									
	A	B	C	D	E	F	G	H	I	J
Total monoterpenes	86.18	82.67	25.91	86.33	61.61	62.80	69.44	55.48	17.20	15.80
Total sesquiterpenes	13.82	17.33	74.09	13.67	38.39	37.20	30.56	44.52	82.80	84.20
Oil density (20°C) (g/ml)	0.860	0.863	0.910	0.857	0.873	0.875	0.876	0.876	0.915	0.916
Refractive index (20°C)	1.469	1.469	1.494	1.467	1.474	1.474	1.474	1.475	1.496	1.496

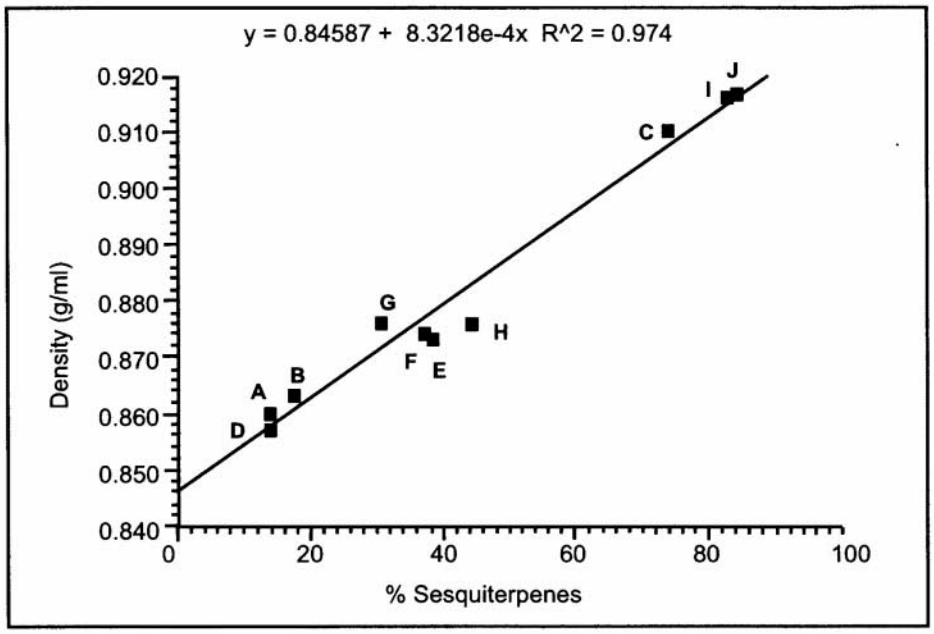


Fig. 1. The relationship between density and total sesquiterpene content in North Island manuka and kanuka oils.

the oil. The advantages of this approach are that valuable information can be obtained with basic laboratory equipment. In contrast with GC-FID or GC-MS methods, there is no requirement for the prior determination of response factors for the different classes of compounds (monoterpenes, sesquiterpenes, triketones, esters, etc.) present in manuka and kanuka oils.

The more extensive identification of oil components in a range of oil types clarifies the relationships between geographical variation of oil composition, especially in levels of triketones, and antimicrobial activity. These results should correct a common consumer misconception that all manuka oils have equivalent antimicrobial activity. They also provide the basis of simple measurements to predict antimicrobial activity and to discriminate between pure and mixed oils. These results illustrate the wide variation in oil composition. The variation between sites and between individual plants (Perry et al., 1997a,b; Porter et al., 1998) is a commercially valuable resource for selection and establishment of improved clonal lines with specific composition and functional properties. The same variation also illustrates the need for further work to clarify chemical and taxonomic relationships between and within manuka and kanuka.

3. Experimental

3.1. Essential oils

Samples of manuka and kanuka oils were obtained from commercial production batches produced by Tairawhiti Pharmaceuticals from steam distillation of freshly-cut foliage (3–4 tonnes of foliage distilled for 4–6 h) from Te Araroa, East Cape. Samples of other commercial manuka and kanuka oils (samples A–J) were obtained from the New Zealand Coromandel Mountains Whitianga, or retail outlets. Samples of oils were obtained from wild stands of manuka at Kaiteriteri, Nelson, using a mobile pilot scale steam distillation plant (70–80 kg, 3 h). Smaller samples of manuka oils from wild stands at different sites in New Zealand were obtained from bench-scale distillations (3 kg, 3 h). All oils were obtained from freshly harvested foliage and were filtered, dried and stored at 4°C until analyzed.

3.2. Fractionation of manuka oils

For tests of antimicrobial activity, a sample of oil (5 g) was loaded onto a 2×18 cm column of activated silica gel and eluted with 250 ml of petrol (b.p. 30/40°C) and then with 15% EtOAc in petrol to afford non-polar and polar fractions. Solvent was removed in vacuo at 40°C and the fractions dried and stored at 4°C before analysis and testing.

3.3. Antimicrobial activity

The activity of oils and oil fractions was determined as the minimum bactericidal concentration (MBC) required to inhibit growth of a range of bacteria and fungi in liquid culture. Samples of oils were solubilized in Tween 80 and EtOH and incorporated in a decreasing two-fold dilution series starting at 10% oil in

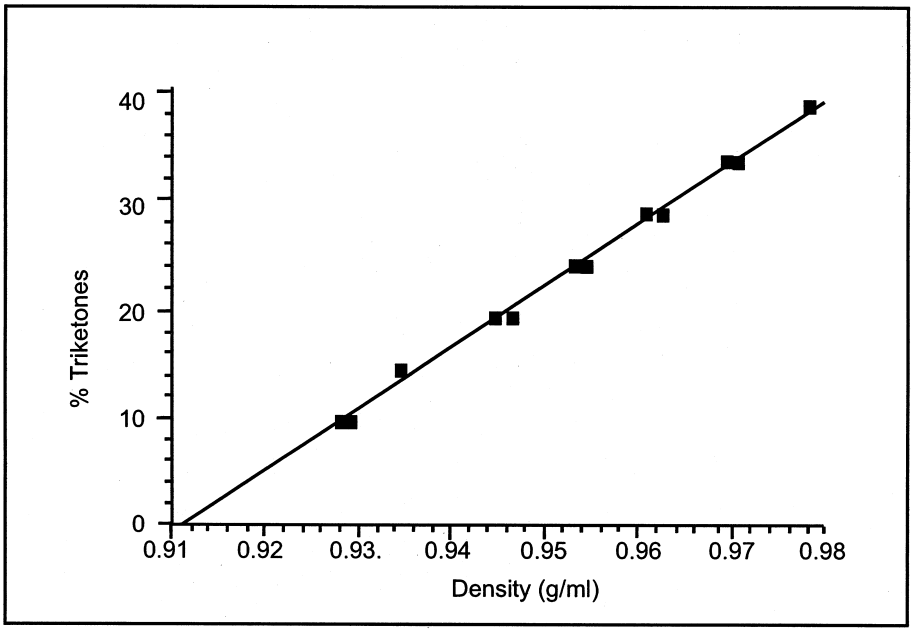


Fig. 2. The relationship between density and % total triketones in Manex oil blends.

appropriate liquid culture media in tubes. The MBC for each oil sample was determined visually after five days' incubation. The MBC was confirmed by plating out the liquid cultures in which growth appeared to be inhibited.

3.4. GC-FID

Samples were analyzed on a 30 m × 0.33 mm id (0.25 m film thickness) BP1 bonded phase fused silica capillary column installed in a HP5840 GC. Hydrogen was employed as the carrier gas at an inlet head pressure of 0.4 kPa. Injector (splitless mode) and FID detector temperatures were maintained at 240 and 300°C, respectively, and the oven was programmed from 80 to 240°C at 2°C/min. A 1 μl sample of 0.5% oil in petrol (b.p. 30/40°C) was manually injected.

3.5. GC-MS

Samples were analyzed using a 25 m × 0.22 mm id (0.22 m film thickness) HP1 bonded phase WCOT fused silica capillary installed in a HP5890 GC interfaced to a HP5970B mass selective detector (MSD) using EIMS (70 eV) and operated in total ion current mode, m/z 40 to 400 mass range. Helium was employed as the carrier gas with an inlet head pressure of 1.8 kPa. Injector and GC/MSD interface temperatures were maintained at 250 and 285°C respectively, and the column was programmed from 60 to 240°C at 4°C/min. 1 μl (split injection) of 0.5% oil in dichloromethane was injected using an HP5973A autoinjector.

3.6. 2(1-Oxopropyl)-4,4,6,6-tetramethylcyclohexan-1,3,5-trione (*4*)

GC-MS m/z (rel. int.): 238 $[M]^+$, 41 (40), 42 (73), 43 (88), 57 (88), 69 (47), 70 (73), 81 (42), 96 (46), 125 (49), 150 (56), 168 (86), 238 (96).

3.7. 2-(1-Oxobutyl)-4,4,6,6-tetramethylcyclohexan-1,3,5-trione (*5*)

GC-MS m/z (rel. int.): 252 $[M]^+$, 41 (96), 42 (42), 43 (100), 69 (34), 70 (41), 71 (42), 81 (29), 96 (44), 111 (25), 164 (27), 182 (69), 252 (53).

3.8. Peak identification

Identifications were based on a combination of authentic standards available in our laboratories (α-pinene, β-pinene, camphene, myrcene, cineole, *p*-cymene, humulene, β-caryophyllene, and calamenene), matching against NBS and NIST library mass spectra, published tabulations of Kovats indices (Davies, 1990) and mass spectra (Jennings & Shibamoto, 1980) and GC-MS matching of components of established structure present in related oils (Brophy et al., 1989; Melching et al., 1997).

Acknowledgements

The authors gratefully acknowledge Mr M. Kerr of Tairawhiti Pharmaceuticals Ltd. for samples of Manex and Kanex oils and information on commercial pro-

duction; the New Zealand Coromandel Mountains Co. Ltd. Whitianga, for oil samples; Amanda Hay and Cameron Marrett for technical assistance; Drs A. and M. Cooke of the Cawthron Institute, Nelson, for the antimicrobial activity tests and the Foundation for Research, Science and Technology for Maori Business Development Scheme and Public Good Science Funding.

References

Allan, H. H. (1961). *Flora of New Zealand*, *vol. 1*. Wellington: DSIR.
Atkinson, N., & Brice, H. E. (1955). *Australian Journal of Experimental Biology and Medical Science, 33,* 547.
Best, E. (1905). *Polynesian Society Journal, 13,* 213.
Briggs, L. H. (1947). *Journal and Proceedings of the Royal Society of New South Wales, 80,* 151.
Briggs, L. H., Penfold, A. R., & Short, W. F. (1938). *Journal of the Chemical Society (London)*, 1193.
Briggs, L. H., Hassall, C. H., & Short, W. F. (1945). *Journal of the Chemical Society (London)*, 706.
Brooker, S. G., Cain, B. F., & Cambie, R. C. (1963). *Transactions of the Royal Society of New Zealand, 1,* 61.
Brooker, S. G., Cambie, R. C., & Cooper, R. C. (1981). *New Zealand medicinal plants* (2nd ed.). Auckland: Heinemann.
Brophy, J. J., Davies, N. W., Southwell, I. A., Stiff, I. A., & Williams, L. R. (1989). *Journal of Agricultural and Food Chemistry, 37,* 1330.
Brophy, J. J., Goldsack, R. J., Forster, P. I., Clarkson, J. R., & Fookes, C. J. R. (1996). *Journal of Essential Oil Research, 8,* 465.
Connor, H. E., & Edgar, E. E. (1985). *New Zealand Journal of Botany, 25,* 115.
Davies, N. W. (1990). *Journal of Chromatography, 503,* 1.
Flynn, T. M., Lassak, E. V., & Smyth, M. P. (1979). *Phytochemistry, 18,* 2030.
Gardner, R. (1924). *Journal of the Society of Chemical Industry, 43,* 34T.
Gardner, R. (1925). *Journal of the Society of Chemical Industry, 44,* 528T.
Harris, W., Porter, N. G., & Dawson, M. I. (1992). *New Zealand Journal of Botany, 30,* 213.
Hellyer, R. O. (1968). *Australian Journal of Chemistry, 21,* 2825.
Joulain, D. (1996). *Perfumer and Flavorist, 21*(2), 1.
Jennings, W., & Shibamoto, T. (1980). *Qualitative analysis of flavor and fragrance volatiles by glass capillary gas chromatography*. Academic Press.
Lis-Balchin, M., Deans, S., & Hart, S. (1996). *Acta Horticulturae, 426,* 13.
Melching, S., Bulow, N., Wihstutz, K., Jung, S., & Konig, W. (1997). *Phytochemistry, 44,* 1291.
Penfold, A. R. (1921). *Journal and Proceedings of the Royal Society of New South Wales, 49,* 170.
Perry, N. B., Brennan, N. J., Van Klink, J. W., Harris, W., Douglas, M. H., McGimpsey, J. A., Smallfield, B. M., & Anderson, R. E. (1997a). *Phytochemistry, 44,* 1485.
Perry, N. B., Van Klink, J. W., Brennan, N. J., Harris, W., Anderson, R. E., Douglas, M. H., & Smallfield, B. M. (1997b). *Phytochemistry, 45,* 1606.
Porter, N. G., Smale, P. E, Nelson, M. A., Hay, A. J., Van Klink, J. W., & Dean, C. M. (1998). *New Zealand Journal of Botany, 36*, 125.
Short, W. F. (1926). *Journal of the Society of Chemical Industry, 45,* 96T.
Wardle, P. (1991). *Vegetation of New Zealand.* Cambridge: Cambridge University Press.
Yin, R., Mark, A. F., & Wilson, J. (1984). *New Zealand Journal of Botany, 22,* 493.