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Diversity in the essential oil of New Zealand grown Kānuka, *Kunzea ericoides* (A. Rich) Joy Thomps

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Abstract
Kunzea ericoides (Kānuka) is a New Zealand (NZ) native tree. The essential oil, obtained from leaves and branches, has been commercially produced in New Zealand since the early 1990's. Whilst there has been extensive work correctly categorising the tree, its habitat and its chemical composition little research has been undertaken on commercially produced samples of essential oil to identify chemical variations and the potential impact on commercial use. Currently most retail outlets, either within NZ or internationally, provide minimal or absent detail as to the location or recognition that geographical diversity can exist. The purpose of this study is to analyse commercially produced samples of essential oil as sold to consumers, produced indifferent geographical locations around New Zealand. These analyses, along with earlier research undertaken have identified some key differences in chemical composition, which have the potential to influence clinical applications. Kānuka oil is clearly differentiated from its relative Mānuka oil (*Leptospermum scoparium*) due to the high percentage of α-pinene and absence of β-triketones.

Keywords Kānuka, essential oil, *Kunzea ericoides*, chemical composition, α-pinene

1. Introduction
Kānuka, *Kunzea ericoides* (A. Rich) Joy Thomps. is a common scrub tree native to New Zealand (NZ). Its normal habitat spreads from the Far North down to most of the South Island, however, it is not generally found on the West Coast of North Island or the lower South Island [1]. Whilst the species is endemic to New Zealand it is also naturally found in Australia [2], South America and South Africa [3]. Extensive work has been undertaken to clearly identify the morphological characteristics of Kānuka and the ten different endemic *Kunzea* spp., with an emphasis on differentiating them from the closely related mānuka (*Leptospermum scoparium*) [4]. There is increasing recognition of geographical and seasonal variations in the chemical composition of the essential oil, which is obtained by steam distillation [5-7]. This author has been trying to raise awareness in the aromatic community about the potential benefits of Kānuka oil and the presence of geographical variations since the early 2000's [8] conducting clinical research based on these differences [9] and building on earlier extensive work completed by others [6, 10].
Now with at least eight known distillers producing oil on a regular commercial scale around NZ it is timely to investigate this variability further. The purpose of this study is to analyse commercially available Kānuka essential oil from different locations in New Zealand to see if there are significant variations. Further study is underway to assess if any variations result in different practical applications.

2. Material and Methods
2.1 Kānuka History
Kānuka, *Kunzea ericoides*, was previously named *Leptospermum ericoides* [4] and *Leptospermum phylicoides* [11], with the present name being used since 1983, after reclassification [4]. Various traditional and Māori names are also recorded and these vary depending on the location; white manuka, white tea tree, kōpuka, mānuka-rauriki, mārū(East Coast. 19thC); rawiri (NgaPuhi name documented in 19thC) and variants rauwiri, rauiri; toa mānuka (early 20thC); kahikātoa, manuea, manuoea(Nelson area 1832); and makahikatoa (white kahikatoa) [12]. It is possible that early references to Kānuka are actually referring to a different tree, *Kunzea robusta* [12], and detailed genetic marking and chromosomal mapping has been undertaken to confirm they are in fact a different species [13].

Two sub-varieties, *K. ericoides* var. *linearis* (Kirk) and *K. ericoides* var. *microflora* (G.Simpson) W. Harris, are known to exist, however for essential oil production neither are used for commercial distilling [13]. The habitat is mainly coastal to low alpine and grows up to 1600m above sea level. It has been found to be a valuable coloniser after deforestation and trees can live for up to 150 years [1, 7]. Its height is up to 10m and the trunk diameter is around 1m. This means mature trees are easy to distinguish from mānuka, which grows to around 4m in the same habitat. The common name 'white manuka' relates to the whitish colour of the bark again used to differentiate from mānuka, which has a reddish colour. The flowering season is November to March (later than mānuka), which is also when the oil is usually extracted. The trees are a vital habitat for native birds and now for honey production [7]. It is also of value for riparian planting and previously was considered a waste scrub and cleared off land for agricultural use [1, 4, 14-16].

2.2 Kānuka Oil Production

Kānuka oil has been commercially available since 1990's with the first distillery set up on the East Coast ofthe North Island [17]. Harvesting is either by hand with petrol powered scrub cutters of the young growth or using larger hedge trimmers attached to a tractor. Access could be hampered by steep or remote habitats so most commercial producers obtain kānuka from regenerated pastureland or from smaller tress up to 3m high. Kānuka is not plantation grown and harvesting does not damage the ecosystem in any way as the tree regenerates quickly [16]. When harvesting it is important to both differentiate the plant from mānuka, and to focus on obtaining maximum foliage rather than wood [16] to enable rapid regeneration. Distillers do this by careful identification of the raw material through touch, location, aroma of leaves, using experience gained over a number of years or involving local plant experts such as beekeepers. The physical appearance of the scrub and trees is confirmed before any harvesting [18-20]. If older trees are being harvested they are easily identified by the white flaky bark and the height, which again differentiates from any mānuka growing in the same location [20]. Commercial producers also regularly have their oil analysed to ensure it is consistent with kānuka oil.

The essential oil is produced by steam distillation of the freshly harvested plant material, whichis usually wilted beforehand and chopped into smaller pieces containing both stem and leaves and flowers. Oil production occurs more quickly than mānuka with the bulk of oil extracted within 40 minutes, compared to up to six hours for manuka. This speed is attributed to the high number of monoterpenes such as α-pinene present in kānuka oil [12]. The yield of essential oil can vary from 3-5 litres per tonne of foliage, which againis a higher yield than mānuka. The highest yield (0.6%) is Autumn (April) just as flowering ends and lowest (0.2%) in spring (October) [7]. The oil has a distinctive woody, green aroma, with a bitter medicinal taste. There can be noticeable differences in colour from clear golden to a greenish colour. This varies from both geographical location and age of plant when distilled, which is visible in Figure1 of five oil samples.

Fig 1: Image of the five Kānuka oil samples. Photo taken with iPhone 7+ using a flash [no filter or colour adjustment]

2.3 Experimental Method

An online search using the search term 'Kānuka/kanuka oil distillers' was conducted and arequest was placed on a Facebook group called "Aromatic Botanical Distillers", which primarily has a NZ membership. The initial planwas to obtain oil from at least seven different geographical locations in New Zealand, which would be representative of the main commercial locations where Kānuka was gown. The researcher rejected small-scale hobbyist distillers and those who were not able supply sufficient oil to purchase in larger quantities (>100 mL). Five growers were identified, from four of New Zealand's islands; Great Barrier Island; North Island, Arapaoa (previously Arapawa) Island and South Island (Figure 2). To be included in this project the grower/distiller needed to meet the following criteria; be selling their own oil in a retail capacity; have 100mLimmediately available for purchase; regularly undertake in-house quality measures to ensure correct identification of the raw material; and have previously had their oil independently analysed. Of these five, four met all inclusion criteria at the time of the study. The fifth distiller, from Central Otago in the South Island, was not distilling until later in the year and had no previously distilled stock available. A recent analysis of this oil, conducted by the same laboratory has been included for comparison in Table 2 [21]. This distiller however, has a mobile vacuum distillation unit and he travelled to the K4 location and undertook their distillation on site of harvest.

Fig 2: Google Earth Location of kānuka oil locations + K6 Analysis

Oil was obtained from the four growers after an explanation of the research project and the oil was either donated or supplied at a reduced price to assist the researcher (even though this was not asked for). A total of five samples of pure undiluted oil were obtained (N=5) as one grower, from Great Barrier Island (GB), had two distillations available, each undertaken around the same time, one from older trees and one from younger trees. As he observed different morphological characteristics between the oils (distinctive colour and aroma variations), he felt it was worthwhile to supply both for study. Table 1 presents the individual oils and details of their harvest, and production. All oils were steam distilled in commercial quantities, with one sample (K4) produced via vacuum steam distillation.

Table 1: Summary of Key Features of Kānuka Oil Samples

Source	Distillation Notes	GPS location	Notes	Specific Gravity [SG] @15/15ºC	Refractive Index [RI] @ 20ºC	Total # constituents
Great Barrier Island [K1]	Steam Distilled 04 AUG 20 Fine weather	S 36"12.972" E 175"20.305"	Distilled from plants last harvested 1 year ago, Lighter colour oil, lighter, fresher aroma	0.8833	1.4712	42
Great Barrier island [K2]	Steam distilled 01AUG 20 Fine weather	S 36"12.800" E 175"20.112	Distilled from plants last harvested 4 years ago Darker colour	0.8777	1.4701	59
East Coast North Island [K3]	Steam distilled 15/12/2019	37°34'51"S 178°11'45"E	Mature tress 760 kg =4.1 litres Hot, dry day, late afternoon	0.8838	1.4728	54
Coromandel North Island [K 4]	Vacuum distilled 30 AUG 20	36°44'56.4"S 175°43'42.6"E	Windy, rainy then sunny, juvenile trees	0.8741	1.4683	73
Arapaoa Island [Arapawa Island] K5	N/A	N/A	N/A	0.8725	1.4682	46

Once all oil samples had been obtained, they were decanted into individual amber dropper bottles, sealed and labelled K1, K2, K3, K4 and K5 and couriered to an independent laboratory to conduct the individual analyses. Flinders Cook Technical Services (FCTS) (Auckland NZ) undertake regular analyses of Kānuka oil from growers, along with other essential oils so had the expertise to conduct the analysis and had also conducted the 2018 analysis of K6 included for comparison. Therefore, consistency in lab processes and equipment was maintained. Whilst FCTS knew the samples were kānuka oil, they were not aware of each individual oil supplier. Before the oils were analysed one grower (K4) revealed they had just had their freshly distilled oil as supplied to the researcher analysed by FCTS and supplied this full data to the researcher. Flinders Cook confirmed they had carried out that analysis and it was agreed there was no need for a new analysis to be conducted on the same batch of oil just a week later.

All samples were tested on the same day (23 September 2020) and involved the following; refractive index (RI) @20°C (determined by Abbe Refractometer according to ASTM D1218 [22]), specific gravity (SG) (determined by Digital Density Meter according to ASTM D4052 @ 15/15°C [23]); and Gas Chromatography-Mass Spectrometry (GC-MS). The GC-MS analysis was carried out by direct injection into a Shimadzu GCMS QP2010SE. The column used was a 30-m Rxi-XLB using a temperature program from 40°C up to 300° C. Identification of the components was conducted through the spectral search software, comparing the mass spectra to the NIST14 library [24]. Where identified, the CAS numbers for each component were provided [25]. Figure3 shows an example of the retention times for sample K1

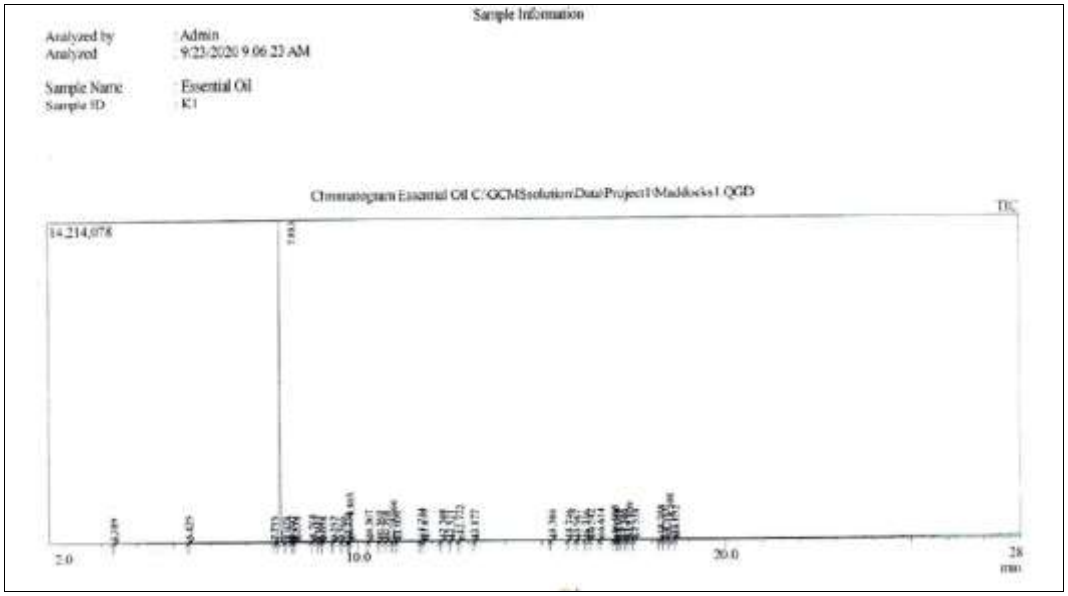

Fig 3: Example of retention times for sample K1

Flinders Cook Lab provided further validation of their results by running tests of reference samples using the following constituents, all sourced from Merck:

- (1*R*)-(+)-α-Pinene (Code no. 8.18632.0100): Lot No. S21251 246 ≥97%
- (1*S*)-(–)-β-Pinene (Code no. 8.41132.0100): Lot No. S6572632 243 ≥98%
- (+)-Camphene (Code no. 8.20254.0100): Lot No. S6181454 236 ~90%
- (*S*)-(–)-α-Terpineol (Code No. 8.21078.0250): Lot No. S6309978 714 ≥98%

A 0.15% (of each component) solution was prepared in acetone (BDH Chemicals Lot 19I054022) and injected into our GC-MS using the same program as used for the kānuka samples. It shows that the compounds elute at the following retention times under the conditions to which the samples were analysed. The retention times match fairly well with those seen in the kānuka samples – within 0.03-0.04 minutes (see Table 2and Figure 4). This variation (which is consistent) over the four compounds is likely to be due to slight differences in the GC-MS conditions (column head pressure, vacuum, or similar) [25]. As RI values are independent of GC-MS conditions these can be compared to other samples for identification purposes.

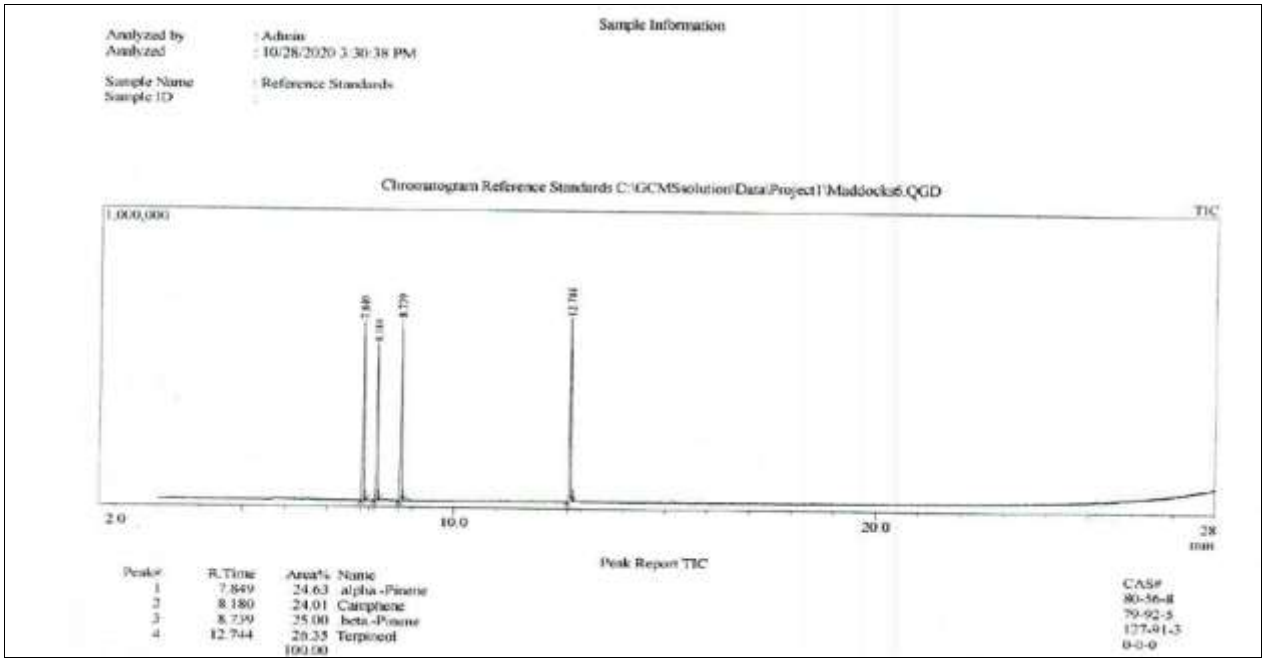

Fig 4: Retention times Reference Chemicals

Table 2: Retention times for reference samples

Compound	Retention Time, mins
α-Pinene	7.85
β-Pinene	8.74
Camphene	8.18
α-Terpineol	12.74

3. Results and Discussion

3.1 Results

A total of four oils, (K1, 2, 3, 5), were analysed by Flinders Cook Technical services with the analysis of the 5th oil, K4, supplied by the grower [26] (n=5). The analysis for K6 was a summary of an historical analysis conducted by the same

laboratory in 2018, from oil grown in Otago, South Island [21]. Coincidently, this historical sample had also been produced by vacuum distillation by the same distiller as K4. Table 3presents the analyses of samples >0.03% of each sample and the summary of K6 as publicly available. If a constituent is present at >0.3% in one sample the % in the others is given even if it is <0.3% to allow comparison.

Table 3: Main constituents present >0.3% for Kānuka oil samples

	CAS No.	K1	K2	K3	K4	K5	K6
Specific gravity [SG]		0.8833	0.8779	0.8838	0.8741	0.8725	n/a
Refractive index [RI]		1.4712	1.4701	1.4728	1.4683	1.4682	n/a
Constituents >0.3%							
α-pinene	80-56-8	71.68	74.34	60.12	74.08	70.64	61.39
1,8-cineole	4470-82-6	5.95	5.58	5.51	6.6	4.34	8.99
linalool	78-70-6	2.49	2.57	1.81	4.45	1.93	2.62
α-terpineol	98-55-5	1.39	0.94	1.06	1	0.8	n/a
viridiflorene	21747-46-6	1.39	0.15	2.95	0.61	0.95	n/a
cis-calamenene	72937-55-4	1.72	1.38	1.9	0.92	1.21	1.04
spathulenol	1139-30-6	1.35	0.64	0.94	0.75	0.5	1.32
viridiflorol	552-2-3	4.11	1.06	3.5	2.32	1.3	n/a
ledol	577-27-5	0.99	0.35	0.87	0.64	0.34	0.3
o-cymene	527-84-	1.14	1.27	2.99	0.44	3.95	n/a
β-selinene	17066-67-0	0.25	1.62	0	0	0	3.41
γ-terpinene	99-85-4	0.3	0.53	3.63	0.28	3.72	5.73
trans-sesquisabinene hydrate	145512-84-1	0	0	1.61	0	0	1.50
p-cymene	99-87-6	0	0	0	0	0	5.82
α-selinene	473-13-2	0.2	0.7	0	0	0	4.13
cadina-3,5-diene	NFO383	0	0	0	0	0	2.74
(*E*)-β-ocimene	3338-55-4	0	0	0	0.28	0	2.61
β-myrcene	123-35-3	0.06	0	0.11	0	0.08	2.19
methyl geranate	1189-09-9	0	0	0	0	0	2.12
cadinene isomer	483-749	0	0	0	0	0	1.47
germacrene D	37839-7	0	0	0	0	0	1.66
citronellyl acetate	67650-82-2	0	0	0	0	0	1.33
α-farnesene	28973-99-1	0	0	0	0	0	1.03
β-pinene	127-91-3	0.63	0.58	0.42	0.62	0.48	0.98
α-cubebene	16728-997-7	0.35	0.33	0	0	0	1.15
α-campholenal	4501-58-0	0.77	0.58	0.34	0.23	0	n/a
l-pinocarveol	547-61-5	0.49	0.4	0.24	0	0.3	n/a
pinocarvone	30460-92-5	0.52	0.4	0	0.22	0.08	n/a
terpinen-4-ol	562-74-3	0.24	0.21	0.4	0.4	0.27	n/a
α-caryophyllene	87-44-5	0.09	0.22	0.31	0	0.21	0.09
aromadendrene	489-39-4	0.25	0.41	0.8	0.3	0.23	n/a
isobutyrone	565-80-8	0.48	0.5	0	0	0	n/a
camphene	79-92-5	0.42	0.27	0	0.62	0.53	n/a
(*E*)-β-caryophyllene	68832-35-9	0.37	0	0.75	0	0.29	n/a
Napthalenes various	[mixed]	0.19	0.72	0.9	0.48	0	n/a
α-amorphene	20085-19-2	0	0.3	0	0.43	0	n/a
α-thujene	2867-5-2	0.18	0.3	0	0	0.76	n/a
terpinolene	586-62-9	0	0	0.82	0	0	n/a
α-copaene	3856-25-5	0	0	0.5	0	0	n/a
α-gurjunene	489-40-7	0	0.15	0.56	0.04	0.39	n/a
azulene	275-51-4	0	0	0.3	0.61	0	n/a
cycloheptane[methyl]	4126-78-7	0	0	0.68	0	0	n/a
β-cadinene	523-47-7	0	0	0.39	0	0	n/a
isovaleraldehyde	590-86-3	0	0	0	0	0.77	n/a
di-isopropyl ketone	565-80-0	0	0	0	0	0.56	n/a
isoterpinolene	586-63-0	0		0	0	0.91	n/a
neryl acetate	141-1208	0	0	0	0.43	0	n/a
alloaromadendrene	25246-27-9	0	0	0	0.49		
Total of main constituents		92.76	90.43	86.89	92.09	89.68	n/a

Previous analyses have identified at least 52 individual constituents present in kānuka oil [27]. In this study the total number of constituents present, range from 42-73 as per Table 1 with the largest number of constituents (73), observed in the vacuum distilled sample from Coromandel(K4). This is more than a 50% increase from previous non-vacuum distillations in 2015 & 2017, which averaged 41-42 constituents [28]. The additional constituents seem to be present in trace amounts and include constituents such as alloaromdendrene and (*Z*)-β-caryophyllene, which are not present in other steam-distilled samples. The main constituent in each oil is α-pinene, a monoterpene common across many pines and Myrtaceae oils. This ranged from 60.12% through 74.34%. Constituents were crossed checked with their CAS number using two data bases

[29, 30]. In each sample, more than 80% of the total volume was obtained from the top ten constituents with variations between the individual samples. Eucalyptol (1,8-cineol) was the second highest constituent across all oils, however at <7% in all cases it trailed well behind α-pinene. For the analysis sourced of K6, there appears to be quite significant differences in the type of constituents present compared to the other five oils. Four constituents present in K6 at >2% were not in any of the other samples. These are *p*-cymene (5.82%), α-selinene (4.13%), cadina-3,5-diene (2.74%) and methyl geranate (2.12%). Viridiflorol, an alcohol was also present in each sample, is also considered a useful anti-inflammatory and antioxidant constituent, which is present in a range of essential oils. γ-Terpinene, a monoterpene, has been of interest in several clinical areas for its anti-inflammatory and antioxidant properties and antifungal properties. It is found in a wide range of plants from *Citrus*, spice and *Cannabis* strains [31]. It is present in two of the sampled oils (K3 and K5) (East Coast and Arapaoa Island) at >3%, and >5% in the Otago-grown Kānuka oil. The other main constituent, which has significant variations between the samples, is linalool, with the highest being present in K4 at 4.45% and the lowest in K3 at 1.81%. Of note is that the oils with higher α-pinene also had higher linalool.

3.2 Discussion

It is clear that all samples of Kānuka oil are distinctly different from mānuka oil, which contain β-triketones at between 8-15%, (–)-*trans*-calamenene at between 9-18.5% and low α-pinene usually at below 8% [32]. One location in the Far North of New Zealand has produced mānuka oil with an α-pinene content of 22.5%, which differentiates it from the rest of the country [10]. However, as no kānuka oil was from this location, and the α-pinene content less than 2/3 lower than the lowest kānuka (K3), there is no chance of any contamination. This indicates correct harvesting techniques with no contamination of mānuka plant material. Based on the results given there does not appear to be a correlation between the amount of α-terpinene and either the refractive index or specific gravity so relying on either of these tests to determine cheaply whether the α-terpinen econtent was over a certain level is not possible. Therapeutically, kānuka essential oil is cited as having the following effects; antibacterial, antifungal and spasmolytic [33]. Some of these claims are supported by a limited amount of in-vivo and in-vitro studies summarised elsewhere [34]. High α-pinene oil from the East Coast has been found to be effective against the common skin bacterial organism, *Staphylococcus aureus* (but less so than mānuka and tea tree oil, *M. alternifolia*) [35].

The colour and aroma were discernibly different in each of the samples, and whist personal selection may in part be due wanting a particular perceived effect, it may also be due to personal preference. This is especially important for aesthetic perfumery blending as some oils have a greener fresher aroma, whereas others have a woodier aroma. In this sense, oils from different geographical locations are not interchangeable. The darkest oil was from K2, the oldest wood, which also had the highest α-pinene. However, the next highest α-pinene was from young wood distilled in the Coromandel so no definitive conclusion can be made about α-pinene and age of wood. Two other constituents were present in the darker K2 oil and were not present elsewhere. Possibly these contributed both to the distinctive colour and aroma (isobuytrone –a ketone, and (+)-α-amorphene, a sesquiterpene). Both these constituents are found in a range of other plant species where essential oil is extracted [29]. *p*-Cymene is considered to have analgesic and antinociceptive properties [36], and would be considered a valuable constituent by clinicians. Cadina-3,5-diene is considered a novel sesquiterpene which is also found in mānuka oil and Vetiver oil [37] and is considered anti-inflammatory. Methyl geranate is an ester which is also present in some other trees from the Myrtaceae family, particularly some Australian natives [38, 39] and hop oil [40].

4. Conclusion

This study has presented the analyses of five commercially-grown essential oils obtained from Kānuka, *K. ericoides*, grown in different locations in New Zealand. These were compared with an earlier analysis obtained from oil vacuum distilled in Otago, South Island. All oils contained α-pinene as the main component, counting for at least 60% of the total volume of oils. This is consistent with other analyses conducted elsewhere. The α-pinene content varies depending on the geographical location with avariance of 13.42% from lowest to highest. The highest α-pinene was from oil distilled on Great Barrier Island, from trees thathad not been harvested in the last four years. The total amount of constituents also varied from 42-73. However, whether this is due to geography or the mode of distillation (vacuum vs. steam) is too early to say, as there has been limited runs of vacuum distilled oils to compare with. None of the samples contained any ingredients that could be considered toxic for external use on humans. Within the minor constituents at <1%, there were variations between each of the samples, with each region containing some constituents that were not identified elsewhere. This study supports the concept that there are regional differences in kānuka oil grown in different locations around New Zealand, which ideally should be highlighted at the point of consumer sale. There is still more to be learnt about kānuka oil and its contribution to aromatic medicine and other ecological benefits, putting to rest the indiscriminate slashing and burning of scrubby land that occurred until the 1990s.

5. Acknowledgements

The author is grateful to the essential oil suppliers who provided samples and information in a timely manner, and either did not charge or offered a substantial discount on the purchase price. FlindersCook Technical services were able to complete the tests in a tight frame and provided additional quality information. This study is funded by the researchers' allocated research funds whilst employed at the University of Canterbury (Christchurch NZ)

6. Conflict of interest statement

The author does not have a commercial interest with any of the companies who supplied oils. Due to financial limitations, it was not possible to analyse every single kānuka oil supplier in NZ. Any errors or omissions with regards other producers are the author's alone and is unintentional.

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