



PFR SPTS No. 23030

Kānuka oil: current knowledge and potential markers

Perry N

October 2022

Report for:

Hikurangi Bioactives Limited Partnership

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PUBLICATION DATA

Perry N. October 2022. Kānuka oil: current knowledge and potential markers. A Plant & Food Research report prepared for: Hikurangi Bioactives Limited Partnership. Milestone No. 96314. Contract No. 40014. Job code: P/241003/01. PFR SPTS No. 23030.

Report prepared by:

Nigel Perry
Team Leader, Bioactives
October 2022

Report approved by:

Farhana Pinu
Science Group Leader, Biological Chemistry & Bioactives
October 2022

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Executive summary

Kānuka oil: current knowledge and potential markers

Perry N
Plant & Food Research Dunedin

October 2022

This report covers the current knowledge on kānuka oils, defined as steam-distilled oils from the foliage of *Kunzea* species native to Aotearoa/New Zealand. Papers were reviewed against the following criteria: defined collection/harvest site of kānuka with botanist identification and retained voucher specimens; defined steam distillation conditions; and stated compound identification methods.

No published evidence was found for any unique steam-volatile natural products that could be marker compounds for kānuka oils. However, kānuka leaves do contain unique **non-volatile** natural products, and these are discussed. Most of the published reports support the proposal by Hikurangi Bioactives Limited Partnership (HBLP) that α -pinene and viridiflorol are consistent kānuka oil markers. Of the other proposed HBLP markers, 1,8-cineole and *p*-cymene were much more variable, and limonene was found at lower concentrations or not identified. The most detailed and best documented studies did not show any distinct regional chemotypes for kānuka oils, by contrast to the very distinct regional chemotypes for mānuka oils. Furthermore, there was no evidence for volatile compositions differing consistently between the ten New Zealand *Kunzea* species defined by de Lange.

Published reports on the bioactivity of kānuka (and non-New Zealand *Kunzea*) oils and extracts are listed.

For further information please contact:

Nigel Perry
Plant & Food Research
Dept of Chemistry
University of Otago
Box 56
Dunedin 9054
NEW ZEALAND
DDI: +64 3 742 8354

Email: Nigel.Perry@plantandfood.co.nz

1 Introduction

Hikurangi Bioactives Limited Partnership (HBLP) is seeking a robust kānuka oil standard to support a flourishing kānuka oil industry with Māori partners around Aotearoa. HBLP's "Kānuka Handbook" is a compilation of what they have learned about kānuka, with contributions by various experts (Rolfe et al. 2020).

The purpose of this Plant & Food Research report is to cover the current knowledge on kānuka oils, defined as steam-distilled oils from the foliage of *Kunzea* species native to Aotearoa, and to identify possible marker compounds to distinguish kānuka oils from mānuka oils, and potentially other essential oils. The focus was on finding papers that: define the collection/harvest site of the kānuka with botanical identification and retained voucher specimens; define steam distillation conditions; and state how compounds were identified. The report includes consideration of five compounds noted by HBLP as potential kānuka oil markers: α -pinene, limonene, eucalyptol/1,8-cineole, *p*-cymene, and viridiflorol. The report also comments on chemical markers in kānuka oil for (1) geographic origin, and (2) speciation.

2 Standards for related oils

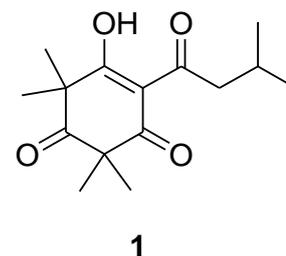
2.1 International Standards on essential oils

[ISO – International Organization for Standardization](#) – develops and publishes International Standards, including over 200 on individual oils and on characterization methods. They do not have any standards on kānuka or *Kunzea*, or on mānuka or *Leptospermum*. They do have several standards on eucalyptus oils, but probably the most relevant is on antimicrobial Australian tea tree oil. This is a terpinen-4-ol rich chemotype obtained by steam distillation of the leaves and terminal branchlets of *Melaleuca alternifolia* (Maiden et Betche) Cheel or of *M. linariifolia* Sm. (Myrtaceae) (ISO 2017). The standard defines general characteristics and chromatographic profiles of marker compounds, and gives example gas chromatography (GC)-flame ionisation detector (FID) analysis traces using different stationary phase columns.

As shown below, this level of information is already available for kānuka oils.

2.2 Mānuka oils

Plant & Food Research has studied in detail the variation of foliage essential oils of mānuka (*Leptospermum scoparium* J.R. et G. Forst., Myrtaceae), growing in Aotearoa (Douglas et al. 2004). Analyses of oils from 261 individual mānuka plants collected from 87 sites throughout New Zealand showed a high triketone (>20%) chemotype localised on the Tairāwhiti/East Cape, although oils with triketone content up to 20% were found in the Marlborough Sounds area. Cluster analysis defined other chemotypes localised in other areas. The Tairāwhiti chemotype has antimicrobial properties, and the volatile triketones (mainly leptospermone **1**) are both the antimicrobial bioactives and the marker compounds. These volatile triketones are rare, reported only from a few *Leptospermum* species and a few other Australian Myrtaceae (Hellyer 1968).



The Plant & Food Research mānuka variation study found a high α -pinene chemotype, especially from Te Tai Tokerau/Northland (Douglas et al. 2004). Oils from Australian *L. scoparium* had higher monoterpene concentrations (α -pinene and 1,8-cineole) than oils from most New Zealand *L. scoparium*, and low or no triketones (Perry et al. 1997a).

2.3 Australian *Kunzea* oils

In 2010 de Lange and colleagues wrote: “As currently circumscribed, *Kunzea* (Myrtaceae: Leptospermeae) is an Australasian genus of 38 formally recognised taxa and 21 informally recognised tag-named entities ... All but two species, *Kunzea ericoides* (A.Rich.) Joy Thomps. and *K. sinclairii* (Kirk) W.Harris, are endemic to Australia ...” (de Lange et al. 2010) (but de Lange has since suggested splitting the New Zealand *Kunzea* into ten species, see below). Of these many Australian *Kunzea* species, only the essential oil from *K. ambigua* (Smith) Druce is commercially important (Thomas 2012). This *Kunzea* oil has been listed as a therapeutic substance by the Therapeutic Goods Administration in Australia for topical application for the treatment of various dermatological ailments (AUSTL 72143; 1996, cited by Thomas 2012).

K. ambigua is endemic to northeast Tasmania as well as the Furneaux Islands and eastern coastal regions of Victoria and southern New South Wales (Thomas 2012). Thomas and colleagues have reported GC analyses of commercial kunzea oil (Ducane kunzea oil) and oils from four individual *K. ambigua* plants (Thomas et al. 2010). Ducane kunzea oil contained monoterpenes (70%) including α -pinene (48.3%), 1,8-cineole (14.5%) and α -terpineol (1.9%). Oils from individual *K. ambigua* plants varied greatly in their content of α -pinene (0.6–62.5%), 1,8-cineole (0–11.2%), bicyclogermacrene (0.4–14%), spathulenol (0.5–12.2%), globulol (0.5–22.6%) and viridiflorol (0.3–38%). One commercial website ([Kunzea Oil \(australiankunzea.com\)](http://australiankunzea.com)) states that a typical analysis of the major components in kunzea oil is: α -pinene 35–52%; 1,8 cineole 9–16%; viridiflorol 7–12%; globulol 7–11%; bicyclogermacrene 4–5%; and α -terpineol 2%. A recent paper reports anti-inflammatory and antimicrobial activities of a commercial *K. ambigua* oil, but fails to report the chemical composition of this oil (Barillot & Cock 2021).

3 Kānuka oils: current knowledge and potential marker compounds

3.1 Published research

Kānuka, kahikātoa and mānuka are the main Māori names for shrubs and trees in Aotearoa/New Zealand, now botanically classified as both *Kunzea* Rchb. and *Leptospermum* J.R.Forst. et G.Forst (Myrtaceae) (Kirk 1899; Riley 1994; de Lange 2014; Dawson & Lucas 2019). These taonga (treasured species (Anon 1998)) are listed together in the main written compilation of traditional uses (Riley 1994). For the purpose of this review, kānuka oils are defined as steam-distilled oils from foliage of *Kunzea* species native to Aotearoa.

Early European botanists classified kānuka as *Leptospermum ericoides* A. Rich. with several varieties, plus *L. sinclairii* Kirk on Aotea/Great Barrier Island (Allan 1961). These were then classified as *Kunzea* (Harris et al. 1992), then in 2014 a taxonomic revision by de Lange recognized ten *Kunzea* species, all endemic to New Zealand (de Lange 2014). These are well illustrated in the HBLP Kānuka Handbook (Rolfe et al. 2020). On the other hand, a 2021 paper on the genetic variation of New Zealand *Kunzea* stated: “The weak level of genetic support for the ten *Kunzea* species, lack of breeding barriers between them, and problems in recognising some of the species in the field, raises questions as to their validity. However, given the ecological, conservation and economic importance of *Kunzea*, the ten species currently circumscribed should be retained until decisions are made as to how to recognise the variation within the species complex.” (Heenan et al. 2021).

Kānuka chemistry, including essential oil composition, is covered in reviews by researchers from the University of Canterbury (Maddocks-Jennings et al. 2005; Maddocks 2019b, a); and the University of Auckland (Essien et al. 2019).

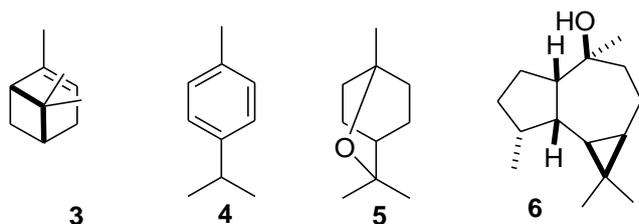
The main basis of this current systematic Plant & Food Research review was a search of the Chemical Abstracts Service database (searched using SciFinder 1 December 2021) for “leptospermum ericoides” or *Kunzea* or kānuka’. This search retrieved 229 references dating back to 1923, but only 26 of these were deemed relevant. Other sources were the Plant & Food Research literature database of natural products of taonga plants, plus a Google Scholar search. Reports about kānuka essential oils are summarised below (Table 1).

Table 1. Reports on kānuka essential oils composition (in chronological order). GC-MS = gas chromatography-mass spectrometry; NMR = nuclear magnetic resonance.

| Reference | Collection and identification | Distillation conditions | Analysis method and main components | Comments |
|---|--|--|---|---|
| Corbett RE, Gibson MGC 1959. Extractives from the New Zealand Myrtaceae. V. The volatile oil of <i>Leptospermum ericoides</i> . J Sci Food Agric 10: 198-200. (Corbett & Gibson 1959) | April, 1952, at Whare Flat, near Dunedin. No botanist or voucher code given. | Steam distilled in 250-lb batches, extraction being complete after 24 h. Yield 0.33% v/w. | Separated by fractional distillation, identified by chemical derivatisation: α -pinene (52.4%); 1,8-cineole (3.8%); aromadendrene (2.5%); unidentified sesquiterpene hydrocarbon (10.6%). | No GC or NMR at that time. Limonene (dipentene) 0.6%, trace of sesquiterpene alcohol, possibly viridiflorol. This paper states that Johnson & Short (1923) identified α -pinene (77%), α -terpineol (2%), citral (1%), aromadendrene (10%), and a phenol (25%) later identified as leptospermone from an Auckland kānuka oil. |
| Lis-Balchin M, Deans S, Hart S 1996. Bioactivity of New Zealand medicinal plant essential oils. Proc Int Symp Medicinal and Aromatic Plants/Acta Hort eds: L E Craker; L Nolan; K Shetty 426: 13-30. (Lis-Balchin et al. 1996) | Essential oils supplied by Absolute Essential Auckland, and Brooklyn Valley Essential oils, Motueka. | Not stated. | Capillary GC: about 70% α -pinene. | No other compounds noted. |
| Perry NB, van Klink JW, Brennan NJ, Harris W, Anderson RE, Douglas MH, Smallfield BM 1997. Essential oils from New Zealand manuka and kanuka: chemotaxonomy of <i>Kunzea</i> . Phytochemist 45: 1606-1612. (Perry et al. 1997b) | Two plants each of <i>Kunzea</i> plant populations growing at a single site (Lincoln Landcare), derived from seed collected from different sites around New Zealand and Australia. Identified by W. Harris, CHR vouchers. | Laboratory-scale steam distillation, 2 h (Perry et al. 1997a). | Capillary GC, identified by reference compounds and ^1H NMR: α -pinene (67.6 \pm 10.8%); p-cymene (5.8 \pm 8.3%); 1,8-cineole (4.3 \pm 2.5%); viridiflorol (2.8 \pm 1.7%). Detailed compositions below. | Most comprehensive study of variation to date. Limonene not identified. |
| Porter NG, Wilkins AL 1998. Chemical, physical and antimicrobial properties of essential oils of <i>Leptospermum scoparium</i> and <i>Kunzea ericoides</i> . Phytochemist 50: 407-415. (Porter & Wilkins 1998) | Kānuka oils were obtained from commercial production batches produced by Tairāwhiti Pharmaceuticals from steam distillation of freshly cut foliage from Te Araroa, East Cape. Samples of other commercial mānuka and kānuka oils (samples A–J) were obtained from the New Zealand Coromandel Mountains Whitianga, or retail outlets. | Tairāwhiti Pharmaceuticals steam distillation of freshly cut foliage: 3–4 tonnes of foliage distilled for 4–6 h. | Capillary GC-MS, identified by reference compounds and MS library matching: α -pinene (55.5%); p-cymene (3.4%); 1,8-cineole (3.9%); viridiflorol (7.2%). Detailed compositions below. | Good data on commercial East Cape “Kanex” oil. Limonene 3.94%. |
| Harkenthal M, Reichling J, Geiss H-K, Saller R 1999. Comparative study on the in vitro antibacterial activity of Australian tea tree oil, cajuput oil, niaouli oil, manuka oil, kanuka oil, and eucalyptus oil. Pharmazie 54: 460-463. (Harkenthal et al. 1999) | Commercial oil | Paper not read. | GC-MS. | Paper not read – not likely to be useful. |

| Reference | Collection and identification | Distillation conditions | Analysis method and main components | Comments |
|--|--|---|---|--|
| Christoph F, Kaulfers P-M, Stahl-Biskup E 2000. A comparative study of the in vitro antimicrobial activity of tea tree oils s.l. with special reference to the activity of b-triketones. <i>Planta Med</i> 66: 556-560. (Christoph et al. 2000) | Commercial oils provided by C. Melchers Essential Oils Handels-GmbH, Bremen: kanuka oil, origin New Zealand. | Not stated | GC-MS: α -pinene (61.6%); 1,8-cineole (6.0%); p-cymene (4.8%); E-calamenene (2.5%); viridiflorol (3.2%). | Limonene 1.4%. |
| Hethelyi E, Korany K, Domokos J 2001. Analysis of kanuka and manuka essential oils by using GC, and GC/MS analysis. <i>Olaj, Szappan, Kozmet</i> 50(4): 149-157. (Hethelyi et al. 2001) | Samples supplied by Coast Biologicals Ltd. | Paper not read | GC-MS: α -pinene main component (69–72%). | |
| Van Vuuren SF, Docrat Y, Kamatou GPP, Viljoen AM 2014. Essential oil composition and antimicrobial interactions of understudied tea tree species. <i>S Afr J Bot</i> 92: 7-14. (Van Vuuren et al. 2014) | Plant material consisting of the aerial parts of <i>K. ericoides</i> were sampled monthly from February 2007 to January 2008 from a cultivated site in Magoebaskloof, north of Polokwane, Limpopo Province, South Africa. No New Zealand source, botanist or voucher code given. | 500–1500 g fresh plant material packed into a Clevenger apparatus for 3-h hydrodistillation | GC-MS: for the monthly samples major compounds were α -pinene ($37.6 \pm 6.3\%$), p-cymene ($13.5 \pm 4.1\%$), and viridiflorol ($5.3 \pm 2.1\%$). | Detailed study, but no New Zealand source stated, not steam-distilled. 1,8-cineole not identified, limonene $0.1 \pm 0.04\%$. |
| Maddocks WA 2021. Diversity in the essential oil of New Zealand grown kānuka, <i>Kunzea ericoides</i> (A. Rich) Joy Thomps. <i>Am J Essent Oil Nat Prod</i> 9(1): 32-38. (Maddocks 2021) | Two Aotea/Great Barrier, one each East Coast North Island, Coromandel, Arapaoa Island; GPD locations given; no botanist, no voucher. | Steam- or vacuum-distilled, no details | GC-MS by Flinders Cook Technical Services (FCTS) (Auckland New Zealand): major compounds were α -pinene (60-74%), 1,8-cineole (4.3–6.6%), and viridiflorol (1.3–4.1%). | p-cymene 0%, limonene not identified. |
| Fuller ID, De Lange PJ, Burgess EJ, Sansom CE, van Klink JW, Pery NB 2021. Chemical diversity of kānuka in Aotearoa New Zealand: 5,7-dihydroxy-6,8-dimethyl flavanone as a chemical marker for <i>Kunzea sinclairii</i> (Myrtaceae) Photochemistry submitted. (Fuller et al. 2022) | Voucher specimens from across the geographic ranges of the ten New Zealand <i>Kunzea</i> species identified by de Lange. | Solvent extracts, included volatiles and non-volatiles | GC-MS for volatiles: α -pinene main in all; some also had high p-cymene; trans-calamenene main sesquiterpene hydrocarbon; viridiflorol main sesquiterpene alcohol. | Not quantitatively comparable with steam distillation results, but very comprehensive study of 39 foliage samples from around New Zealand. 1,8-cineole relatively high in some samples, not identified in others. Limonene traces in most samples. |

These reports all agree on α -pinene **3** as the predominant volatile in kānuka oils from a wide range of sources. The only report on the isolation of α -pinene is Corbett & Gibson (1959), who found a specific rotation $[\alpha]_D$ of +48, suggesting that it was predominantly the enantiomer **3** shown below. *p*-Cymene **4** and/or 1,8-cineole **5** were the other main monoterpenes. *trans*-Calamenene **2** and viridiflorol **6** were the main sesquiterpenes identified. Little or no leptospermone **1**, or other triketones, present in some mānuka oils were recorded (apart from in a 1923 paper that we could not access to check whether the botanical identification was rigorous).



These reports (Table 1) generally support the proposal by HBLP that α -pinene and viridiflorol are potential kānuka oil markers. 1,8-Cineole and *p*-cymene were more variable in concentrations, and limonene was found at lower concentrations or not identified.

α -Pinene **3** is perhaps the most common terpene in plants worldwide, found as a major component in a wide variety of essential oils (O'Neil 2001). Viridiflorol **6**, named after its first isolation from Queensland *Melaleuca viridiflora* (Myrtaceae) (Jones & Haenke 1938), has over 3300 references in the Chemical Abstracts. These include reports from several Australian *Kunzea* species e.g. (Thomas et al. 2010), and from mānuka and a wide range of Australian *Leptospermum* species e.g. (Brophy et al. 2000).

3.2 Quantitative kānuka oil compositions

Of the reports summarised in Table 1, only the Plant & Food Research study (Perry et al. 1997b) is judged to have used sufficiently well-defined plant materials and distillation conditions, combined with some replication, to justify detailed quantitative composition comparisons.

Fifty-three kānuka plants were examined, derived from seed collected from 27 different sites around Aotearoa/New Zealand. These were identified by botanist Warwick Harris, with CHR herbarium voucher specimens kept. They were classified as *K. sinclairii* (seed from Aotea/Great Barrier Island) and *K. ericoides* sensu lato (s.l., i.e. how this species was defined before the de Lange revision). The *K. ericoides* seed sources ranged from Kaipara Harbour to Otago Harbour. These plants were grown at a single site (Lincoln Manaaki Whenua Landcare Research) The parallel Plant & Food Research study on mānuka oils showed that oil composition is largely genetically rather than environmentally controlled: mānuka seed from Tairāwhiti but grown at Lincoln had the same high triketone chemotype as the plants in their own rohe (Perry et al. 1997a). Therefore we assume that this is also true for kānuka.

Foliage samples from two separate plants from each site's seed (except for only a single plant from one site) were extracted and analysed, to give replication of genetically distinct plants. Laboratory-scale steam distillation was done for 2 h, as this had been shown to optimise oil yields (B. Smallfield, unpublished results), as for the parallel mānuka study (Perry et al. 1997a), and the later mānuka national survey (Douglas et al. 2004). This 2-h distillation gives proportionally more of the volatile

α -pinene and less of the higher boiling point viridiflorol than the 4- to 6-h commercial-scale distillations analysed by Porter and Wilkins (Porter & Wilkins 1998).

All the kānuka oils in the Plant & Food Research study (Perry et al. 1997b) had α -pinene **3** as the main component, but two Okiwi Bay-sourced plants stood out for their high *p*-cymene **4** (mean 31%) and relatively low α -pinene **3** contents (mean 41%). A just-published Plant & Food Research study confirmed that there is a high *p*-cymene (> 5%) chemotype of kānuka, but suggested that this is not a regional chemotype and does not align with the de Lange species (Fuller et al. 2022).

The first Plant & Food Research study (Perry et al. 1997b) included *K. ericoides* s.l. from Australian seed sources. These were not strongly distinct in composition from kānuka oils, but five of these six Australian oils were high *p*-cymene types. Thomas and colleagues reported that oils from three individual Tasmanian *K. ambigua* plants varied widely in their compositions (Thomas et al. 2010), but none of these overlapped with kānuka oil compositions.

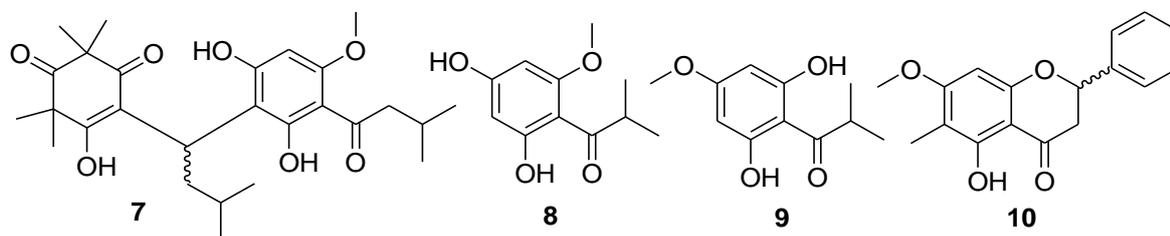
The published results show that kānuka oils (Perry et al. 1997b) were clearly distinct from mānuka oils in having higher α -pinene and lower sesquiterpene hydrocarbons and triketones (Perry et al. 1997a; Douglas et al. 2004). Porter and Wilkins reached the same conclusion, with fewer samples in their published data (Porter & Wilkins 1998) but augmented by years of experience analysing commercial oils. They also noted that kānuka oils generally had lower densities (i.e. in g/mL) than mānuka oils, especially high-triketone mānuka oils.

Overall, the Plant & Food Research studies did not show any distinct regional chemotypes for kānuka oils (Perry et al. 1997b; Fuller et al. 2022), by contrast to the very distinct regional chemotypes for mānuka oils (Perry et al. 1997a; Douglas et al. 2004). Furthermore, there was no evidence for volatile compositions differing consistently between the species defined by de Lange (Fuller et al. 2022).

3.3 Other potential kānuka marker compounds

As summarised above, there is no published evidence for any unique volatile natural products that could be marker compounds for kānuka oils. By contrast, there are New Zealand Myrtaceae oils for which this would be the case. Steam-distilled oils from leaves of ramarama and rōhutu, the two taonga species in the New Zealand-endemic genus *Lophomyrtus*, are the only known natural source of the volatile bioactive compounds bullatenone and 4-methyl-1-phenylpentane-1,3-dione (Woollard et al. 2008).

However, kānuka leaves do contain unique **non**-volatile natural products. Bloor reported phloroglucinol-triketone hybrid compound **7** in an ethanol extract from a single collection (from Southern Wairarapa) of *K. ericoides* s.l. (Bloor 1992). This compound **7** [CAS Registry Number 139979-85-4] and a homolog [RN 139955-98-9] have never been reported from any other sources, or chemically synthesised, even though they are antiviral. However, compound **7** is probably too polar and has too high a molecular weight to be steam distilled into kānuka oils. Bloor also reported the simpler isomeric acylated phloroglucinols **8** [RN 102092-19-3] and **9** [RN 42541-62-8] in the *K. ericoides* ethanol extract and in an extract from one collection of *K. sinclairii* (Bloor 1992). These compounds might be steam distilled into kānuka oils, being less polar and having lower molecular weight.



Compounds **8** and **9** have been reported only once each from other natural sources, both Myrtaceae: **8** from *Eucalyptus* (Bloor 1992) and **9** from *Callistemon* (Qin et al. 2017). So they might be good markers for kānuka oils if they were shown to be consistently present in *Kunzea* from around Aotearoa.

Flavanones, e.g. **10**, were found by researchers (including Plant & Food Research) in *K. robusta* de Lange et Toelken leaf ethanol extracts, active against the kauri dieback pathogen *Phytophthora agathidicida* (Lawrence et al. 2019). These flavanones are sufficiently volatile to be analysed by GC and might be extracted by prolonged steam distillation – the triketone grandiflorone has similar molecular weight and polarity, and is detectable in some mānuka oils (Douglas et al. 2004). On the other hand, these flavanones are found only in some kānuka (Lawrence et al. 2019), and they are also found in some mānuka (Killeen et al. 2015).

3.4 Kānuka and other *Kunzea* oil and extract bioactivities

Various reports on the bioactivity of kānuka (and non-New Zealand *Kunzea*) oils and extracts were noted that may be of assistance to HBLP and Māori partners. In view of HBLP's anti-inflammatory focus, some Australian *Kunzea* studies may also be relevant.

Kānuka (New Zealand *Kunzea*) oils and extracts:

- Anti-eczema activity in a clinical trial sponsored by HBLP (Shortt et al. 2022)
- Antimicrobial (Porter & Wilkins 1998; Harkenthal et al. 1999; Christoph et al. 2000; Lis-Balchin et al. 2000; Christoph 2001; Gniewosz et al. 2012; Van Vuuren et al. 2014; Chen et al. 2016; Essien et al. 2021; Maddocks et al. 2021)
- Antiviral (Bloor 1992)
- Other (Lis-Balchin & Hart 1998; Maddocks-Jennings et al. 2009; Mishra 2018; Lawrence et al. 2019; Majid & Silva 2021).

Australian *Kunzea* oils and extracts (see review by Thomas 2012):

- Anti-inflammatory (Armstrong 2004; Wyatt et al. 2005; Thomas et al. 2009a; Thomas et al. 2015; Barillot & Cock 2021)
- Antimicrobial (Florence 2012; Barillot & Cock 2021)
- Other (Khambay et al. 1999; Khambay et al. 2002; Khambay et al. 2003; Ito et al. 2004; Hood 2008; Schnitzler et al. 2008; Thomas et al. 2009b; Park et al. 2017).

4 Acknowledgements

We thank Damian Skinner (HBLP), and John van Klink and Bruce Smallfield (Plant & Food Research) for discussions and suggestions.

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