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Identification and quantitative levels of antibacterial components of some New Zealand honeys☆

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Abstract

High performance liquid chromatograms of the phenolic fraction of 19 samples of New Zealand manuka honey, some with high levels of non-peroxide antibacterial activity and some with no such activity, were identical, which indicated that phenolic components of this honey are not responsible for the presence or absence of this activity in manuka honey. Similarly, the result showed that geography does not influence the phenolic composition of manuka honey. Antibacterial bee peptides and the antibacterial β-triketone leptospermone were not detected in manuka honey. Methyl syringate constituted approximately 70% w/w of the phenolic fraction of manuka honey and can be regarded as a floral marker for this honey. High performance liquid chromatographic profiles of the phenolic components of manuka, heather, clover and beech honeydew honeys were significantly different and could be used to differentiate honeys if they can be shown to be as consistent as those of manuka honey.

Keywords: Non-peroxide antibacterial activity; Manuka; Clover; Heather; Honeydew honey

1. Introduction

In a survey of unifloral New Zealand honeys, Allen et al. (1991) found several which exhibited non-peroxide antibacterial activity. The greatest activity was observed in manuka honey, a very popular and economically important product derived from the native New Zealand "manuka" tree *Leptospermum scoparium* (Myrtaceae). However, not all manuka honey exhibits non-peroxide antibacterial activity. Instead, the bioactivity is recorded in manuka honey only from specific localities (Molan, 1995), particularly the East Cape region of the North Island of New Zealand.

Manuka honey contains a number of aromatic acids (Russell, Molan, Wilkins & Holland, 1990) of which syringic acid and phenyllactic acid are the most abundant (Wilkins, Lu & Molan, 1993). Recently we described the identification of some phenolic acids and flavonoids in bioactive manuka honey (Weston, Mitchell & Allen, 1999). Phenolic acids and flavonoids, particularly those derived from propolis, exhibit weak antibacterial activity (Marcucci, 1995) and we believed that different absolute levels of these constituents might explain why some manuka honey, especially that from the East Cape region of New Zealand, exhibits non-peroxide antibacterial activity and why manuka honey from most other regions of the country, along with nearly all other honeys, does not possess this property (Allen, Molan & Reid, 1991). Another unique feature of Eastland manuka is discussed below in Section 3.2.

Much work has been published by a Spanish group on the use of chromatographic profiles of both flavonoids and phenolic acids to relate honeys to geographical origin and floral source (Andrade, Ferreres & Amaral, 1997; Ferreres, Ortiz, Silva, Garcia-Viguera, Tomás-Barberán & Tomás-Lorente, 1992; Tomás-Barberán, Ferreres, Garcia-Viguera & Tomás-Lorente, 1993). This approach appealed to us as a potential means of distinguishing manuka honey with non-peroxide antibacterial activity, from inactive manuka and other honeys in New Zealand and whether a unique floral marker was responsible for that activity. Preliminary results of this work (see title footnote) indicated that there were no differences between any manuka honeys using the profiles of phenolic

☆ Preliminary results of this work were presented in a poster (paper 65) at the Apimondia99 congress of the International Federation of Beekeepers Associations, Vancouver, September 1999.

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components and, in fact, manuka honey was homogeneous with respect to these constituents. More comprehensive results of that work are reported in this paper. A search for other products, which conceivably might contribute to the non-peroxide antibacterial activity of manuka honey is also described. HPLC profiles of the phenolic components of one sample each of heather, clover and beech honeydew honey indicated that different unifloral honeys can be successfully distinguished by this method.

2. Materials and methods

2.1. Materials

Samples of manuka honey were obtained from Professor P.C. Molan, Honey Research Unit, Waikato University, Hamilton; Mr. T. Harvey, Comvita (NZ) Ltd., Te Puke; and W.L. and M. Bennett, SummerGlow Apiaries Ltd., Hamilton. Samples are described as having high or low non-peroxide antibacterial activity, based on the diameter of the area of inhibition of growth in the standard agar well diffusion assay for non-peroxide antibacterial activity, which was carried out by the Honey Research Unit, Waikato University, Hamilton (Allen et al., 1991; Molan & Russell, 1988). Values <8, no detectable activity; 8–11, low; 12–15, medium; 16–19, high and 20+, exceptional activity.

2.2. Methods

2.2.1. Liquid–liquid extraction of honey

Manuka honey with a high level (18) of non-peroxide antibacterial activity (50 g) was dissolved in water, filtered through glass wool and made up to 230 ml in a liquid–liquid extractor. A solution (5 ml) of 2,4,6-tri-*tert*-butylphenol (8 mg) in ethanol (25 ml) was added as an internal standard and the mixture was extracted continuously with diethyl ether for 20 h. The ethereal solution was filtered and concentrated to dryness under vacuum at 40°C.

2.2.2. Extraction of honey on XAD-2 resin

Amberlite XAD-2 resin (60 g) was soaked overnight in a mixture of water (200 ml) and methanol (200 ml). It was then washed with water and packed into a column (25×2 cm). Manuka honey with a high level (19) of non-peroxide antibacterial activity (236 g) was dissolved in a mixture of water (1 l) and concentrated hydrochloric acid (37%, 1 ml) and the solution was filtered slowly through the column, followed by acidified water (pH 2, 100 ml), distilled water (300 ml) and methanol (300 ml). The methanol extract was concentrated under vacuum at 40°C and freeze-dried to afford an extract of 749 mg (0.32%). The recovery of flavonoids with this method is 95% (Ferreres, Tomás-Barberán, Soler, Garcia-Viguera, Ortiz & Tomás-Lorente, 1994).

2.2.3. Extraction of honey with 2-butanol

Manuka honey with a high level (19) of non-peroxide antibacterial activity (200 g) was dissolved in a mixture of water (1 l) and concentrated hydrochloric acid (37%, 1 ml) and the solution was extracted three times with 2-butanol (200 ml). The alcoholic extract was concentrated under vacuum at 40°C. It was then dissolved in a small volume of water and freeze-dried to leave an extract of 848 mg (0.42%) (Weston et al., 1999).

2.2.4. Methylation of honey extracts

The honey extracts were dissolved in methanol (2 ml). BF_3:MeOH (14% BF_3; 2 ml) was added and the mixture was heated under reflux for 2.5 h. The solutions were poured into saturated sodium bicarbonate solution and extracted three times with dichloromethane. The organic layer was dried, filtered, concentrated and examined by GC.

2.2.5. Isolation of leptospermone from manuka oil

A mixture of the cyclic β-triketones, leptospermone, isoleptospermone and flavesone was separated from the essential oil of manuka leaves by extraction of an ethereal solution of the oil with 5% aqueous sodium hydroxide (Briggs, Penfold & Short, 1938).

2.2.6. Gas chromatography and mass spectrometry

GC was carried out on a Hewlett-Packard 5890 Series II gas chromatograph with a Hewlett-Packard Ultra-2 glass capillary column containing 5% methylphenylsilicone (25 m×0.2 mm; film thickness, 0.33 μm) and a flame ionisation detector. Helium was used as a carrier gas at 50 ml/min. The column was programmed from 60°C (1 min) to 145°C at 7°C/min, then to 190°C at 2°C/min and finally to 300°C at 10°C/min. Combined GC–MS was performed as above, interfaced to a Hewlett-Packard mass selective detector (5970 Series) which was operated in the scan mode, at 70 eV.

2.2.7. High performance liquid chromatography

The phenolic extract (~40 mg from 20 g honey) was dissolved in MeOH (2 ml) for HPLC, which was carried out on an Alltech Econosil column (250×4.6 mm; C-18; 5 μm particle size) at 35°C with detection at 280 nm. The solvent programme was a combination of those of Ferreres et al. (1994) and Andrade et al. (1997) (Table 1). Phenolic compounds were identified by comparison of chromatographic retention times with those of authentic standards. Quantitative levels were determined from the UV absorption during HPLC and extinction coefficients, which were determined at 280 nm from Beer-Lambert graphs of the standards. The limit of detection was 0.1 μg/100 g honey.

2.2.8. Dialysis of honey

a. Manuka honey with a medium level (11.6) of non-peroxide antibacterial activity (100 g) was added

Table 1
Solvent programme for HPLC of phenolic components of honey[a]

Minutes	%A	%B
0	97	3
5	92	8
10	69	31
15	59	41
20	55	45
25	55	45
55	18	82
60	3	97
75	3	97
85	97	3

[a] Solvent A acetic acid:water (2:98); solvent B acetic acid:methanol (2.98).

to water (100 ml) and the mixture was warmed at 40°C to effect dissolution. It was filtered through glass wool to provide a solution of 160 ml.

b. 77 ml of this solution was dialysed in a Union Carbide membrane with molecular weight cut-off (MWCO) of 10 kDa.
c. 44 ml of the solution (a) was dialysed in a Spectra/Por membrane with MWCO of 1 kDa.

Dialysis was carried out at 4°C for 65 h. The retained solutions were concentrated under vacuum at 40°C on a rotary evaporator and then freeze-dried. Residues: (b) MWCO 10 kDa: 178 mg (0.37% of honey); (c) MWCO 1 kDa: 83 mg (0.30% of honey).

2.2.9. Antibacterial assays of dialysed honey

The honey solution above [(a) in Section 2.2.8] was diluted to afford a 25% aqueous solution of honey (250 g/l). The dialysis residues were dissolved in water to provide solutions with a concentration of 20.5 g/l. With catalase added, the honey solution yielded an antibacterial value of 11.6, while no antibacterial activity was detected for either of the dialysis residues.

3. Results and discussion

3.1. Manuka honey

Our earlier work demonstrated that manuka honey contained a range of benzoic and cinnamic acids and flavonoids (Weston et al., 1999) similar to those found in all other honeys (Andrade et al., 1997; Tomás-Barberán et al., 1993). Previous work by the Waikato group (Russell et al., 1990; Wilkins et al., 1993) had demonstrated that phenyllactic acid (2-hydroxy-3-phenylpropanoic acid) and methyl syringate (methyl 4-hydroxy-3,5-dimethoxybenzoate) were major components of manuka honey and were considered to be floral markers of this honey. However, other work showed that both of those substances occur in many honeys (Joerg & Sontag, 1993; Steeg & Montag, 1988) and neither can be described as unique to manuka honey. Our interest in defining the phenolic constituents of manuka honey arose from the belief that the profile of these constituents might differentiate manuka honey which had a high level of non-peroxide antibacterial activity (active manuka honey) from manuka honey which possessed no such activity (inactive manuka honey). These profiles were also expected to indicate which phenolic component(s) was primarily responsible for the observed non-peroxide antibacterial activity of the active manuka honey.

Aromatic acid and phenolic components are the only substances with antibacterial properties other than hydrogen peroxide and the enzyme lysozyme (Molan, 1992), which have been isolated or detected in any honey, but our previous work demonstrated that phenolic extracts from both active and inactive manuka honey had the same level of antibacterial activity, by using agar disc assays. Furthermore, these same extracts afforded similar liquid chromatograms of the phenolic components, contrary to what we expected. The present work extends that published previously (Weston et al., 1999) to a much larger number of samples, in order to corroborate that work and has used a solvent programme which is superior to that used earlier, to separate both the phenolic acids and the flavonoids.

Comparison of the levels of the phenolic components in the antibacterially active manuka honeys (group I, Table 2) with those in the inactive manuka honeys (group III), indicated that there was no difference qualitatively or quantitatively between these two types of manuka honey, by this criterion. Furthermore, the levels of cinnamic acid and the flavonoids in all the honeys (Table 2) were very similar and were also comparable with those in many European honeys (Ferreres et al., 1994) as were those of the phenolic acids (Table 3) (Andrade et al., 1997; Steeg & Montag, 1988). Many of the phenolics in honey are derived from propolis (Ferreres, Ortiz, Silva, Garcia-Viguera, Tomás-Barberán & Tomás-Lorente, 1992) and their levels in honey depend on the amount and distribution of propolis in a hive but variations in the levels of individual phenolic components of honey have been used to fingerprint a honey in relation to both its botanical and geographical origin (see Introduction). The 19 samples of manuka honey used for the present survey were obtained from various regions of the North Island (N.I.) of New Zealand. The 10 samples in group I, all possessed high levels of non-peroxide antibacterial activity and were obtained from central (Waikato, Bay of Plenty) and Eastland areas of the N.I. The remaining three manuka honeys in group II and the six manuka honeys in group III, respectively, possessed a low level or no non-peroxide antibacterial activity and were obtained from the remaining northern, western and

Table 2
Levels of some phenolic compounds in manuka and other New Zealand honeys (μg/100 g honey)

Group[a]	Methyl syringate	Cinnamic acid	Pinobanksin	Pinocembrin	Chrysin	Galangin
Group 1						
No. of samples	10	10	10	10	10	10
Mean	63	1.5	4.3	5.1	2.2	2.2
S.D.	25	0.675	2.0	2.8	0.8	1.4
Group 2						
No of samples	3	3	3	3	3	3
Mean	49	1.4	6.3	7.3	2.7	3.4
S.D.	9	0.23	2.8	3.6	0.8	1.5
Group 3						
No. of samples	6	6	6	6	6	6
Mean	76	1.7	5.0	4.5	2.2	1.9
S.D.	32	1.1	2.6	2.6	0.8	1.4
Group 1, 2, and 3 combined						
No. of samples	19	19	19	19	19	19
Mean	65	1.6	4.8	5.3	2.3	2.3
S.D.	27	0.8	2.3	2.8	0.8	1.4
CV[b]	41	49	48	54	34	61
Group 4						
No. of samples	3	3	3	3	3	3
Mean	1.5	0.8	2.0	1.9	1.3	2.2
S.D.	0.8	0.2	0.6	0.4	0.2	1.8

[a] Group 1, Manuka honey with a high level of non-peroxide antibacterial activity; group 2, Manuka honey with a low level of non-peroxide antibacterial activity; group 3, Manuka honey with no non-peroxide antibacterial activity; group 4, Clover (*Trifolium repens*), Heather (*Calluna vulgaris*) and Beech (*Nothofagus* spp) honeydew honey.
[b] Coefficient of variation (100×S.D./mean).

Table 3
Levels of phenolic acids in some New Zealands honeys (μg/100 g honey)

Honey[a]	4-Hydroxy-benzoic acid	Vanillic acid	Syringic acid	4-Hydroxy-cinnamic acid	Benzoic acid	Cinnamic acid
Honeydew	4.4	1.1	0.6	0.9	77	0.9
Heather	23	109	2.6	0	385	0.8
Clover	2.6	9.9	0.6	0.3	37	0.6
Manuka	0	0	2.0	0	0	1.6

[a] Honeydew, *Nothofagus spp*; heather, *Calluna vulgaris*; clover, *Trifolium repens*; manuka, *Leptospermum scoparium*.

southern regions of the N.I. However, all provided a consistent HPLC profile (Fig. 1) and the coefficient of variation for each phenolic component of all the manuka honeys (groups I, II, III combined, Table 2) fell between 34 and 61% (average 48%) which indicated a very homogeneous group of honeys (Gil, Ferreres, Ortiz, Subra & Tomás-Barberán, 1995). This similarity indicated that geographical origin did not determine the profile of the phenolic components of the manuka honeys (see Section 3.4 below).

3.2. Leptospermone

Following extensive assays of the antibacterial strength of honeys from many areas of New Zealand, Molan (1995) indicated that not all manuka honey exhibits non-peroxide antibacterial activity. However, manuka honey which originates from the Eastland region of the North Island of New Zealand consistently shows high levels of this activity.

In unrelated work, Perry et al. (1997) discovered that three chemotypes of manuka exist in New Zealand and these can be distinguished by the composition of the essential oil from the leaves. One type is characterised by an oil which contains a high proportion of pinenes, a second type by a high proportion of sesquiterpenes and the third type which grows in the Eastland region, by an oil which is characterised by a high proportion of a cyclic-triketone, leptospermone. This oil has the greatest anti-microbial activity of the three types, which is attributed to leptospermone (Harkenthal, Reichling, Geiss & Saller, 1999; Porter & Wilkins, 1998).

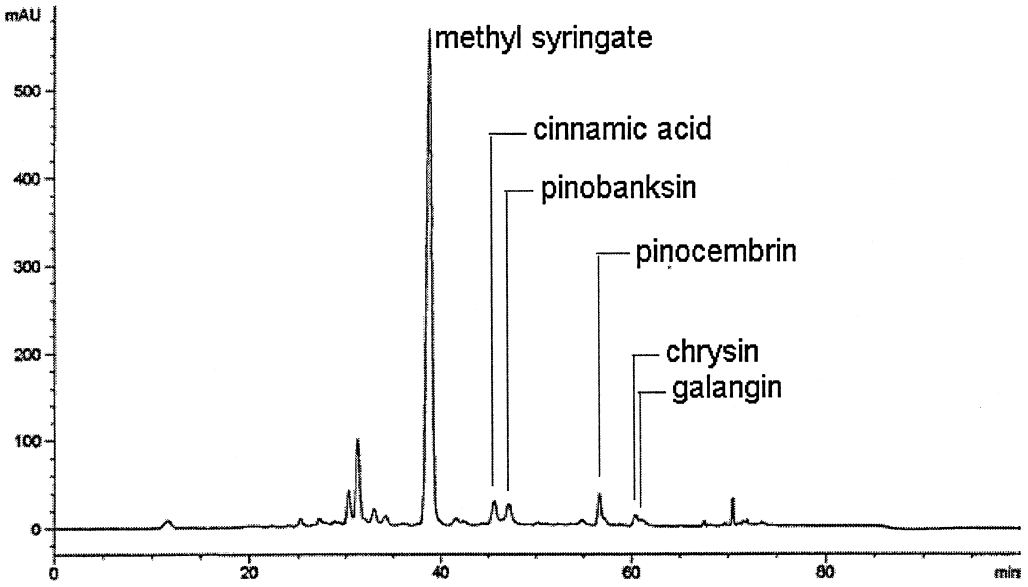

Fig. 1. High performance liquid chromatogram of manuka honey.

In an endeavour to link these two observations, manuka honey was extracted by three different methods and the extracts were analysed by GC–FID and HPLC for the presence of leptospermone. In two earlier publications, Tan, Holland, Wilkins and Molan (1988) and Wilkins et al. (1993) partitioned an aqueous solution of manuka honey of unknown geographical origin and antibacterial activity, with diethyl ether in a liquid–liquid extractor and analysed the methylated extracts by GC–FID. They did not detect leptospermone as a component of their samples of manuka honey.

In the present work, a sample of a manuka honey, which had the highest non-peroxide antibacterial activity (19) of any manuka honey commercially produced in New Zealand, was extracted by three different methods, to separate the phenolic components from the water-soluble carbohydrates and proteins. One method utilised adsorption of the phenolics on XAD-2 resin, another employed extraction of the honey with 2-butanol (Weston et al., 1999) and the third was the liquid–liquid extraction, which was employed by Wilkins et al. (1993). Extracts of honey, obtained by the above methods, in dichloromethane, were co-chromatographed with the mixture of β-triketones (Section 2.2.3). Analyses of the underivatised and methylated extracts by GC and GC–MS failed to detect the triketones in any of the extracts of manuka honey. This result was consistent with those of HPLC analyses, which had a limit of detection of 1 μg/kg honey. Recovery of flavonoids from XAD-2 resin was shown to be 95% (Ferreres et al., 1994) and a similar result was assumed here for leptospermone.

Because leptospermone is insoluble in water, it is unlikely to be present in nectar and consequently in honey and the results above were rational. If leptospermone does occur in manuka nectar, it is possible, as with flavonoid glycosides, that it could be modified or destroyed by bee enzymes before the honey is ripe. Although leptospermone occurs characteristically in the leaves and twigs of manuka, it does not follow that the triketone will also occur in the nectar. Indeed, we showed earlier (Weston et al., 1999) that the uncommon flavonoids which were identified in the leaves of manuka by Mayer (1990) and Haberlein and Tschiersch (1994), do not occur in the honey and by inference, in manuka nectar.

3.3. Peptides and proteins

Other products which conceivably could contribute to the non-peroxide antibacterial activity of manuka honey, are some antibiotic peptides which, over the past decade, have been characterised from the body fluid of bees which had been injured and infected by bacteria, including abaecin (Casteels et al., 1990), apidaecin (Casteels, Ampe, Jacobs, Vaeck & Tempst, 1989), hymenoptaecin (Casteels, Ampe, Jacobs & Tempst, 1993), royalisin (Fujiwara, Imai, Fujiwara, Yaeshima, Kawashima & Kobayashi, 1990) and lysozymes (Table 4). Lysozymes are enzymes which are considered to be ubiquitous components of the antibacterial defences of insects (Hultmark, 1996). The enzymes are present in the blood and like the peptides above, are strongly induced when the insect is infected. Molan (1992) considered that lysozymes were unlikely to contribute to the non-peroxide antibacterial activity of honey since they were detected in honey by the use of a species of bacteria which is very sensitive to lysozyme.

The peptides possess strong antibiotic activity and, if they were present in honey, could contribute significantly to the non-peroxide antibacterial activity of manuka

Table 4
Molecular weights of antibacterial insect peptides

Peptide	Molecular mass (kDa)
Lysozyme	14–23
Defensin	4
Royalisin	5.5
Hymenoptaecin	10.5
Apidaecin	2.1
Abaecin	3.9

honey. However, these peptides occur only in infected bees and therefore it would be surprising to find these products in honey.

A solution of manuka honey with a medium level (11.6) of non-peroxide antibacterial activity was dialysed within two different membranes, one which retains molecules with a mass greater than 1 kDa and one which retains molecular mass greater than 10 kDa. White (1978) indicated that roughly half the nitrogen content of honey was attributable to proteins. From a nitrogen content of 0.046%, the protein content of honey can therefore be calculated to be approximately 0.13%. Using this information and the fact that about half the dialysable material from honey is protein, it can be shown that the protein concentrations of the solutions of dialysis residues, which were submitted for antibacterial assay, were 30 times greater than the concentration of protein in the assayed honey solution. The antibacterial assay of the dialysed honey fractions demonstrated that these were devoid of detectable activity from which it is possible to conclude that antibacterial proteins were unlikely to be present in this honey. Table 4 lists the molecular weight of several antibacterial bee peptides, from which it can be seen that a 1 kDa membrane should retain all these products while a 10 kDa membrane should retain only the lysozymes. Since the membranes probably have a range of MWCO and since only one sample of manuka honey was dialysed in this work, the result should be regarded as indicative rather than conclusive that antibacterial proteins do not occur in manuka honey.

White (1978) pointed out, in his review, that honey proteins are derived from both bees and plants. The experimental work described here implies that peptides, proteins and polysaccharides with molecular weights greater than 1 kDa, which occur in manuka honey, have no antibacterial properties, or occur at such low levels that no such properties are observed.

3.4. Floral markers

The chromatograms of the four types of honey derived from manuka *(Leptospermum scoparium)* (Fig. 1), heather *(Calluna vulgaris)* (Fig. 2), clover *(Trifolium repens)* (Fig. 3) and beech *(Nothofagus spp)* honeydew (Fig. 4) shown in this paper, all had quite different phenolic profiles. The phenolic components of honey originate from nectar, pollen and propolis and the botanical source of these materials will vary with the geographical location of the beehives. The HPLC profile of the phenolic components of honey should therefore reflect the floral source of nectar and be modified by the source of pollen and propolis. These ideas are the basis of an attempt to fingerprint unifloral honeys by the HPLC profile of their phenolic components. Iberian groups have published several papers of their work on this topic (Andrade et al., 1997; Ferreres et al., 1992; Ferreres, Andrade, Gil & Tomás-Barberán, 1996; Tomás-Barberán et al., 1993).

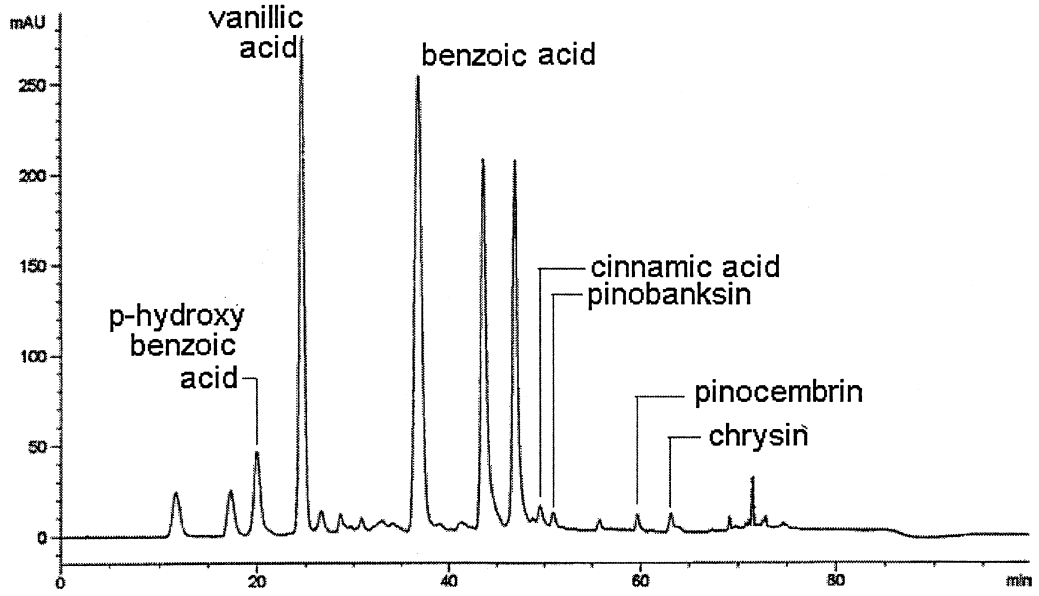

Fig. 2. High performance liquid chromatogram of heather honey.

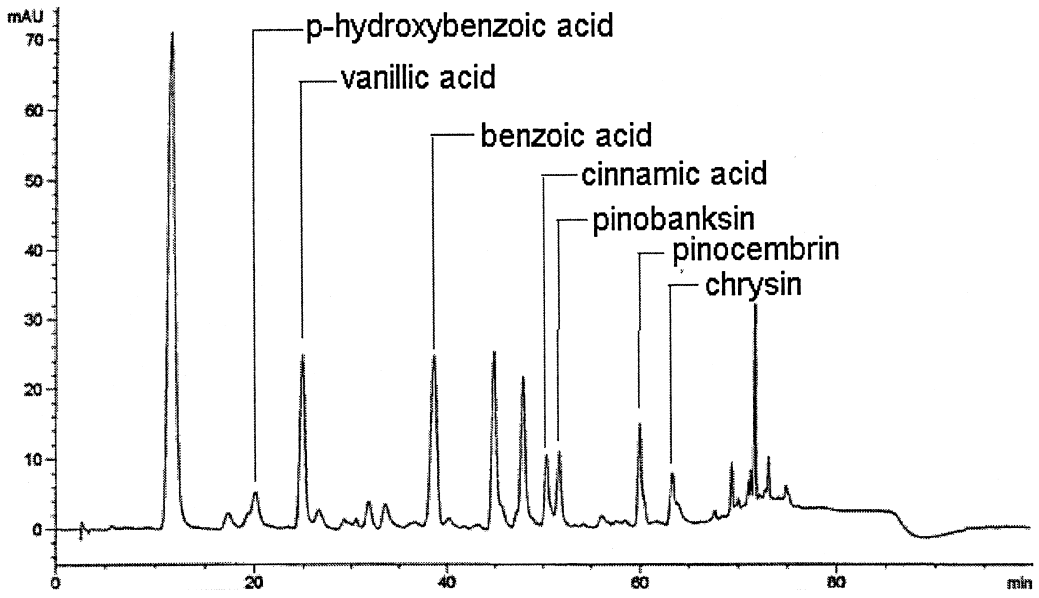

Fig. 3. High performance liquid chromatogram of clover honey.

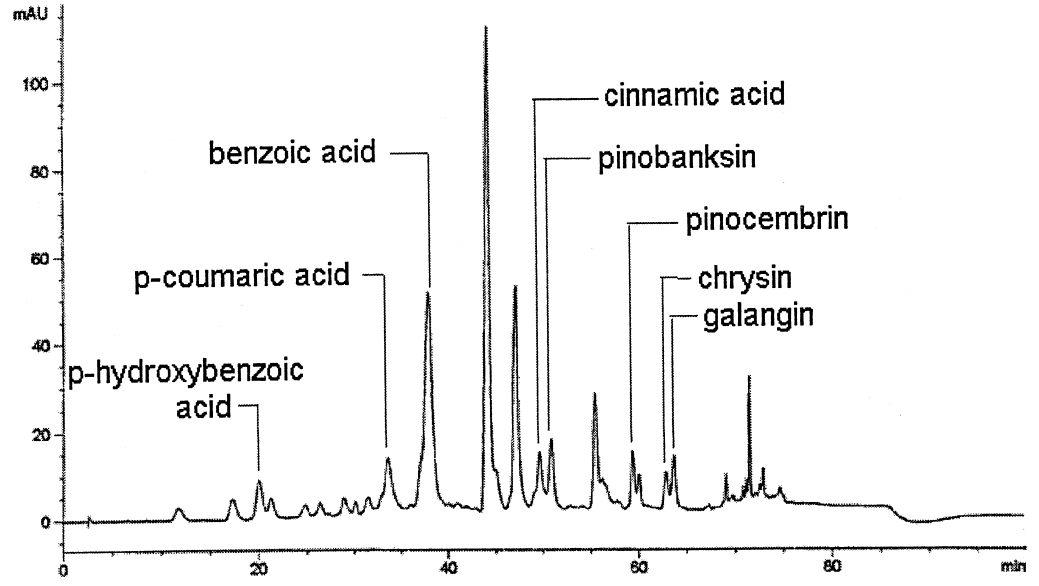

Fig. 4. High performance liquid chromatogram of beech honeydew honey.

In this work, 19 samples of manuka honey were collected from various areas of the North Island of New Zealand (see Section 3.1) and, without exception, all provided an identical HPLC profile of phenolic components (Fig. 1). The coefficient of variation in the levels of all the phenolic components ranged from 34 to 61% (average 48%, Table 2), indicating the homogeneity of this group of honeys. From this result it is clear that geography does not influence the phenolic profile of manuka honey. The same cannot be deduced for the clover, heather and honeydew honeys sampled here as only one sample of each honey was analysed.

The component of manuka honey with a retention time of 31 min was identified in our earlier paper as phenyllactic acid, but that was incorrect since phenyllactic acid does not absorb UV light at 280 nm and the identity of that substance remains unknown. The outstanding feature of the HPLC phenolic profile of manuka honey is the abundance (approximately 70% w/w of the phenolic fraction) of methyl syringate, which can be regarded as a floral marker for this honey, although it is not unique to this honey (see Section 3.1). Wilkins et al. (1993) arrived at a similar conclusion from their work. While syringic acid is a common plant constituent, its

methyl ester is rare. It was first isolated from grape vines (Spencer, Tanaka & Towers, 1990) and was subsequently found to be the major phenolic component of rape honey (Joerg & Sontag, 1993).

Benzoic and vanillic acids were abundant in clover and particularly heather honey. All honeys, but especially clover honey, contained a very polar component at 11 min which could not be identified. Clover, heather and honeydew honey all contained the same two components at 44 and 47 min which similarly were not identified. Their UV spectra suggested that they were not flavonoids.

Unlike the manuka honeys, only one sample each of clover, heather and honeydew honey was analysed in this study and chromatographic uniformity of the phenolic components was not established. Therefore conclusions regarding the occurrence and abundance of phenolics in these honeys are only tentative. Benzoic and cinnamic acid derivatives, along with the flavonoids, pinobanksin, pinocembrin, chrysin and galangin are all common constituents of propolis and therefore the clover, heather and honeydew honeys do not appear from this study to possess useful phenolic components, capable of "fingerprinting" the honeys.

4. Conclusions

The results of this current work confirmed and extended the findings of our earlier work (Weston et al., 1999) that:

- The HPLC profile of the phenolic components of manuka honey, which has a high level of non-peroxide antibacterial activity (NPABA) is identical to that of manuka honey which has no NPABA.
- Phenolic components of manuka honey might contribute to, but do not account for the observed NPABA of manuka honey.
- Leptospermone, an antimicrobial β-triketone which characteristically occurs in the essential oil of some manuka varieties, does not occur in manuka honey which has a high level of NPABA.
- Antibacterial peptides such as lysozyme and royalisin were not detected in manuka honey which had a medium level of NPABA.
- Methyl syringate is a floral marker of manuka honey and constitutes more than 70% w/w of the phenolic fraction from manuka honey.
- Levels of phenolic components of manuka honey are similar to those recorded in European honeys.

We have recently shown that oligosaccharides do not contribute to the observed NPABA of manuka honey (Weston & Brocklebank, 1999). Honey monosaccharides do not possess any such activity and to date no chemical entity has been detected or isolated which can account for the observed NPABA of manuka honey. However, a hypothesis for this activity and its variability has been developed and is the subject of a forthcoming paper (Weston, Brocklebank & Lu, 2000).

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