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Letters to the Editor

Antibacterial activity of honey from the Australian stingless bee *Trigona carbonaria*

Sir,

Honey produced by European honeybees (*Apis mellifera*) has been well studied for its antimicrobial properties, and selected honeys display broad-spectrum antimicrobial activity against a range of pathogens [1]. This activity stems primarily from the production of hydrogen peroxide by glucose oxidase, a bee-derived enzyme. Some honeys also possess non-hydrogen peroxide-dependent antimicrobial activity that is linked to phytochemical factors derived from the floral source and is particularly potent in honeys from certain *Leptospermum* species such as manuka (*Leptospermum scoparium*) [1,2]. However, few studies have examined honey from stingless bees, a diverse group of the highly eusocial bees. *Trigona carbonaria* is a stingless bee of the east coast of Australia and is the country's most commonly domesticated stingless bee species. The flavour and aroma of *Trigona* honey is distinct from the honey of *A. mellifera* and little is known about its antimicrobial properties. Therefore, the aim of this study was to investigate the antibacterial activity of Australian *T. carbonaria* honey.

Twenty-two *Trigona* honey samples were collected from separate hives in various locations around Brisbane (Queensland, Australia) between July and November 2006. One sample (Sample 14) was collected from a hive of an unknown species of *Trigona*; the remaining 21 samples were from hives of *T. carbonaria*. Honey samples were assayed for antibacterial activity against *Staphylococcus aureus* ATCC 9144 using an agar well diffusion method described by Allen et al. [2]. Briefly, 25% (w/v) solutions of honey were prepared in sterile deionised water to test total activity (including hydrogen peroxide-dependent activity) and in 2800 U/mL catalase solution to test non-peroxide activity. Solutions of 2–7% (w/v) phenol were prepared in sterile deionised water and zones of inhibition produced by these solutions were used to generate a standard curve. A commercially available manuka honey (Comvita, Te Puke, New Zealand) was included in each plate as a positive control. All solutions were tested in duplicate during each assay and each honey sample was tested on at least five separate occasions over a period of up to 30 weeks.

The antibacterial activities of the 22 samples of *Trigona* honey are shown in Table 1. The initial total antibacterial activity of the *T. carbonaria* honey samples was high (mean 26.3% (w/v) phenol equivalent) compared with the manuka honey control (mean total activity $18.0 \pm 0.9\%$ phenol equivalent; mean non-peroxide activity $17.5 \pm 1.0\%$ phenol equivalent) and other *A. mellifera* honeys (mean 13.4% (w/v) phenol equivalent) [2]. Previous studies on the antibacterial activity of Meliponini honeys using various methods have found a range of activities from non-inhibitory [3] to effectively inhibiting both Gram-positive and Gram-negative organisms [3–6].

The antibacterial activity of *A. mellifera* honey is stable over time [2], whereas the hydrogen peroxide-dependent activity of 15 of the *T. carbonaria* honey samples (Samples 6, 7, 9–13 and 15–22) decreased by at least 4% (w/v) phenol equivalent over the experimental period. This may be due to the intrinsically higher water content of stingless bee honey; however, the activity of seven samples (Samples 1–5, 8 and 14) was stable across all replicates. Little is known about the biochemistry of *T. carbonaria* and it is likely that the enzymes added during the conversion of nectar to honey are different to those of *A. mellifera*. This may result in varying stability of either the enzyme producing hydrogen peroxide or the hydrogen peroxide itself. The non-peroxide activity present in all 22 samples was largely stable throughout the experimental period.

Temaru et al. [6] identified non-peroxide activity in honey samples from nine species of stingless bees from various geographic regions and suggested that this was due to phytochemical factors. Non-peroxide activity in *A. mellifera* honeys is rare and is most commonly reported in honeys derived from *Leptospermum* species. However, this is unlikely to be the case for these *Trigona* honeys as the hives were situated in suburban areas with a low abundance of *Leptospermum* and in some cases the flowering period was outside the 6-month foraging period prior to honey extraction. This, together with the fact that all the honey samples possessed non-peroxide activity, indicates an entomological rather than a phytochemical source of the activity. Cuticular secretions from *T. carbonaria* have been reported to inhibit bacterial growth [7] and may be linked to this activity in *T. carbonaria* honey.

Table 1
Antibacterial activity of *Trigona* honey expressed as mean % (w/v) phenol equivalent

Sample number	Initial antibacterial activity[a]			Final antibacterial activity[b]		
	Sample age (weeks)	Total activity	Non-peroxide activity	Sample age (weeks)	Total activity	Non-peroxide activity
1	10	19.0 ± 1.13	18.4 ± 0.07	40	23.1 ± 0.21	21.7 ± 0.21
2	10	28.5 ± 0.78	17.3 ± 1.63	40	27.8 ± 0.28	19.6 ± 0.14
3	10	27.5 ± 0.07	18.0 ± 1.91	40	26.0 ± 0.35	21.0 ± 0.99
4	10	25.3 ± 1.56	19.4 ± 1.41	40	26.8 ± 0.07	21.6 ± 0.07
5	6	17.5 ± 0.92	13.6 ± 0.92	28	16.3 ± 0.14	14.9 ± 0.57
6	6	25.6 ± 0.28	12.6 ± 0.21	28	17.4 ± 0.71	14.0 ± 0.49
7	6	28.9 ± 0.64	14.9 ± 0.07	28	20.4 ± 0.64	18.1 ± 0.35
8	3	26.4 ± 0.07	23.7 ± 0.14	24	24.3 ± 0.42	21.5 ± 0.00
9	10	23.8 ± 0.00	16.4 ± 0.14	30	18.2 ± 1.56	17.8 ± 0.42
10	7	23.7 ± 0.57	15.1 ± 1.06	28	18.5 ± 0.28	16.7 ± 0.07
11	3	25.0 ± 0.57	13.2 ± 0.14	25	22.0 ± 0.07	14.6 ± 0.49
12	6	27.0 ± 0.57	16.2 ± 0.57	28	21.4 ± 0.35	18.1 ± 0.35
13	6	25.6 ± 1.84	13.7 ± 0.42	28	19.4 ± 0.14	16.2 ± 0.28
14	6	20.4 ± 0.07	16.1 ± 0.57	28	19.5 ± 0.85	18.3 ± 0.49
15	9	27.5 ± 0.57	13.4 ± 0.64	23	14.2 ± 0.00	12.3 ± 0.00
16	9	29.6 ± 0.28	11.5 ± 0.35	23	11.5 ± 0.07	11.1 ± 0.21
17	9	28.6 ± 0.14	13.3 ± 0.42	23	17.1 ± 2.33	13.4 ± 0.57
18	7	27.6 ± 0.49	16.5 ± 0.28	20	19.3 ± 0.71	16.5 ± 0.64
19	7	27.4 ± 0.64	17.3 ± 0.14	20	18.4 ± 0.00	18.0 ± 0.49
20	7	30.8 ± 0.71	14.7 ± 0.14	20	16.1 ± 2.12	13.9 ± 0.14
21	7	31.5 ± 1.13	13.8 ± 0.35	20	25.2 ± 0.57	13.9 ± 0.49
22	7	32.1 ± 0.78	13.4 ± 0.28	20	16.1 ± 0.42	14.7 ± 0.21

[a] Based on initial four replicates.
[b] Based on final two replicates.

This is the first comprehensive study of the antimicrobial activity of native Australian *Trigona* honey. All of the honey samples possessed a high level of antibacterial activity and a substantial proportion of this was stable in the presence of catalase and over time. Non-peroxide activity is particularly advantageous when using honey in clinical situations as it is not destroyed by catalase present in serum. Whilst further studies are required to establish the spectrum of activity of *Trigona* honey against other pathogens, the stability and potency of this activity in *T. carbonaria* honey indicates its potential use as a therapeutic agent.

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