Bee-guided Pharmacognosy?

By David Heaf, www.bee-friendly.co.uk

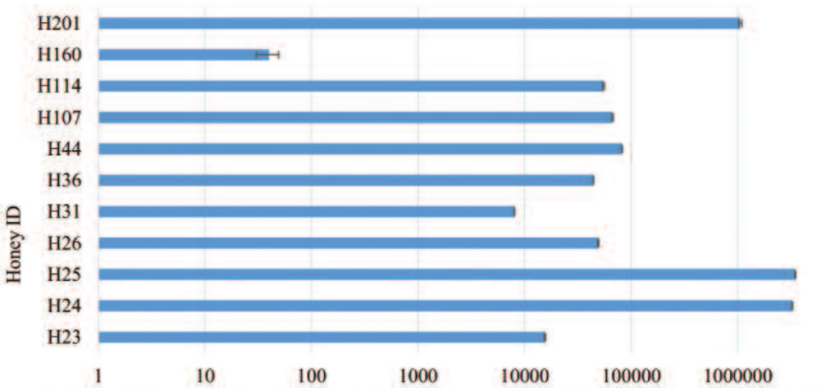
Manuka honey is a much-prized hive product, but might other honeys contain antibacterial activity? David Heaf looks at recent research and makes some interesting observations and suggestions.

In December 2015, several media reports announced that honey 'as potent as manuka' had been found in Wales.[1] They referred to a project at Cardiff University School of Pharmacy and Pharmaceutical Sciences under the supervision of Prof Les Baillie. The project concept was that diverse honeys were sampled, tested for antibacterial activity and the likely plants contributing to the more potent of the honeys identified by palynology – the whole process is a kind of bee-guided pharmacognosy. The research results, involving 220 honeys, were published in the PhD thesis of Dr Jennifer Hawkins.[2]

I read the thesis and was particularly struck by its figure 4.3 (reproduced, with permission, below) which illustrates the amounts of pollen in several of the honey samples.

What stands out in the figure is that three of the honeys, H24, H25 and H201 have pollen counts at least an order of magnitude higher than the rest. It made me wonder if what was giving rise to high pollen counts could also be in some way connected with the high antibacterial activities. Fortunately, the thesis gives the postcode provenance of these honeys and I was able to trace them to Chris Hickman of Aberdovey (H24 & H25) and Tim Moore of Hythe, near Southampton (H201). Hickman had also supplied another relatively potent honey sample, namely H180. Honeys H24, 25 and 201 happened to have high antibacterial activity against MRSA (methicillin resistant *Staphylococcus aureus*) and *Bacillus subtilis,* comparable to that of manuka honey, and most of this activity was retained after neutralisation of hydrogen peroxide, a known antibacterial constituent of honey, whereas, with all the other honeys tested, more than half their activity was lost after neutralisation of hydrogen peroxide.

Hawkins deduced from this that the honeys 'contain additional antibacterial compounds' (Hawkins, 2015; p66).[2] As methylglyoxal is responsible for the antimicrobial activity of manuka honey, she tested antibacterial activity after methylglyoxal neutralisation and found that whereas the manuka honeys lost all their activity, the honeys H24, 25, 180 and 201 retained it. Furthermore, having also ruled out bee-derived defensin as the antibacterial agent, Hawkins concluded that phytochemicals in the honey may be responsible for the observed activity.
A search for the likely plants contributing the phytochemicals to the samples of interest was pursued via microscopy and DNA analysis of pollen with the help of the DNA barcode reference library for 98% of the native Welsh flora as part of the Barcode Wales project.[3] From the analytical results woodruff, dandelion and bluebell were selected for further investigation for the presence of antimicrobial phytochemicals by standard biochemical extraction and separation techniques, guided by the bacterial growth inhibition assays. Pinobanksin was identified as a compound common to both the honey samples and the plants. Other, as yet unidentified, antibacterial compounds were also found in the plants.



	H23	H24	H25	H26	H31	H36	H44	H107	H114	H160	H201
■	15550	3254667	3467333	49300	8050	44633	82400	66733	55333	40	1058667

No. of pollen grains/ml (log scale)

The number of pollen grains per millilitre of 11 honeys. Taken from Hawkins (2015), fig. 4.3, page 89, with kind permission from the author.

Continued on page 260

Continued from page 258

The study apparently did not consider what the samples had in common apart from their palynology profiles, and in particular, why samples H24, 25 and 201 had such massive pollen counts. By asking the two beekeepers who supplied the samples, I ascertained that all had come from Warré-type hives. The Warré hive is extended as the colony grows by nadiring fresh boxes, which involves inserting them at the bottom of the hive under the brood nest, whereas most beekeepers extend their hives by supering. As the brood nest extends downwards in a Warré, cells at the top of the nest are filled with honey. Honey is then harvested by removing one or more boxes from the top. This means that some of the honey is harvested from comb that formerly had brood and/or pollen in it. Whereas, although supers can sometimes contain pollen cells, by far the most honey in them comes from pollen-free comb. Even so, some commercial honeys are warmed and microfiltered to remove particulates including pollen, or even pasteurised to improve shelf-life. Such processed honeys might have reduced antibacterial activities.

Another difference lies in the fact that whereas Warré honey is usually extracted by crush and strain, which is more likely to flush pollen grains out of any residual bee bread present in the comb (e.g. under honey and capping), supers combs are usually centrifuged, leaving any cells filled with pollen largely intact. So it appears very likely that the big differences in hive management and honey extraction contributed to the massive pollen counts of the Warré honeys in this study. I have mostly Warré hives myself, and notice that my Warré honey is somewhat more turbid than my honey from National supers. I have always put this down to a higher pollen content in the Warré honey.

Now let us examine the project's main aim, namely to search, with the help of the bees, for forage-derived (nectar or pollen) phytochemicals possessing antibacterial activity. It seems too much of a coincidence to me that the four Warré honeys out of 220 honey samples of very diverse provenance are the ones that contain enough forage-derived phytochemicals with a durable antibacterial activity to set them apart from all the rest. I therefore considered what other factors could be contributing to the antibacterial activity of these honeys. It seems likely that it is connected with their provenance from brood comb. Although honey harvested from supers can spend some of its time as nectar or partly processed nectar in brood comb before it is moved to the supers, this is likely to be of short duration compared with Warré honey, which may be stored for months in former brood comb. Furthermore, Warré combs are fragmented during extraction, thus very likely to release more substances than comb from supers that is spun and remains intact.

What relevant substances could be involved? The most obvious one is propolis. The regular propolising of brood cells during polishing after cleaning following each eclosion probably contributes to a higher, multi-layered propolis content in brood comb compared with comb from supers which may receive propolis treatment only once annually. Brood comb gradually becomes a complex composite comprising mainly wax, cocoon silk and propolis. Propolis is a well-known mixture of antibacterial phytochemicals among which can be pinobanksin. So, it is possible that the antibacterial activity of the investigated honeys was derived from propolis. If propolis is the key substance in the quest for antibacterials, a more direct approach via propolis may be more likely to succeed than the indirect approach being taken via honey and pollen.

Another possible source of antibacterial activity to consider is the presence of residues from both brood food and larval faeces in brood comb. These could, of course, be phytochemical in origin, but we should also consider that the microbiota of brood comb could play a part. For example, the lactic acid bacteria found in the bee gut and honey have been shown to inhibit the growth of bacteria such as *Paenibacillus larvae*. And other beneficial microbial symbionts might secrete substances, e.g. fungi secreting antibiotics. Beekeepers using Warré hives are less likely to put chemicals in the hive to kill varroa. As acaricides disrupt colony microbiota, those of Warré hives may be closer to those in natural honey bee nests.

I look forward with interest to developments in the Cardiff project, in particular to any focus on whether honey from more natural beekeeping methods in general has more antibacterial activity.