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ORIGINAL ARTICLE

Bactericidal Activity of Different Honeys against Pathogenic Bacteria

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Background. Renewed interest in honey for various therapeutic purposes including treatment of infected wounds has led to the search for new antibacterial honeys. In this study we have assessed the antibacterial activity of three locally produced honeys and compared them to three commercial therapeutic honeys (including Medihoney® and manuka honey).

Methods. An agar dilution method was used to assess the activity of honeys against 13 bacteria and one yeast. The honeys were tested at five concentrations ranging from 0.1 to 20%.

Results. Twelve of the 13 bacteria were inhibited by all honeys used in this study with only *Serratia marcescens* and the yeast *Candida albicans* not inhibited by the honeys. Little or no antibacterial activity was seen at honey concentrations $<$1%, with minimal inhibition at 5%. No honey was able to produce complete inhibition of bacterial growth. Although Medihoney® and manuka had the overall best activity, the locally produced honeys had equivalent inhibitory activity for some, but not all, bacteria.

Conclusions. Honeys other than those commercially available as antibacterial honeys can have equivalent antibacterial activity. These newly identified antibacterial honeys may prove to be a valuable source of future therapeutic honeys.

Key Words: Antibacterial, Honey, Medihoney, Lavender, Maunka.

Introduction

The increase in consumer use of complementary medicines has prompted an increasing interest in traditional and non-conventional medical treatments. One treatment that has received much interest is honey. Honey has a long tradition of use within various medical systems (1,2) and over the past decade several research groups have focused their attention to this product (3–6). Whereas honey has a number of uses therapeutically and as a food preservation agent, it is most well known for its beneficial actions within the wound environment. Honey maintains a moist wound environment that promotes healing, and its high viscosity helps to provide a protective barrier to prevent infection. In addition, the mild acidity and low-level hydrogen peroxide release assists both tissue repair and contributes to the antibacterial activity of honey. This antibacterial activity is a major factor in promoting wound healing where infection is present (7,8).

In Australia, two honeys, Medihoney® (Capillano, Australia) and manuka (various brands), are sold as therapeutic honeys suitable for use in ulcers, infected wounds and burns. Indeed, the majority of the available research in this area has been done using either Medihoney® or manuka honey (4,9). Both these honeys are derived from *Leptospermum* spp. and for this reason the similarity in their activity is not surprising. Previous research has suggested that these honeys have specific antibacterial activity due to a non-hydrogen peroxide mechanism (10,11)—the so-called unique manuka factor (UMF). However, the difference in minimum inhibitory concentration for antibacterial activity between UMF honeys and other honeys are often small ($<$5%) (12,13), and the significance of this in a clinical environment is unclear. Nevertheless, honey does have significant potential to assist with wound healing and this has been demonstrated repeatedly (3,7,14).

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As the potential for honey as a topical wound dressing is further recognized by the health care community, there continues to be a search for other honeys that can be used in this way. These newly identified honeys may be advantageous due to enhanced antibacterial activity, local production (and therefore ready availability), greater selectivity or broad spectrum of activity against medically important organisms. In this study we investigate the activity of three locally produced honeys against a range of medically important bacteria, including those found in wound environments, and compare them to three commercial, antibacterial honeys.

Materials and Methods

Honeys used in this study were obtained from either commercial producers of therapeutic honeys or local beekeepers. Locally produced honeys used were lavender (*Lavanadula* x *allardii*), red stringy bark (*Eucalyptus macrorrhyncha*) and Patersons curse (*Echium plantagineum*). Each honey was gamma irradiated (10 kGy; Steritech Pty Ltd) prior to use in this study. Gamma irradiation does not affect antibacterial activity (15). Three commercial honeys, maunka (*Leptospermum scoparium*), rewa rewa (*Knightia excelsia*) and Medihoney® were provided by commercial suppliers. These three honeys are marketed as therapeutic honeys with antibacterial activity.

Microorganisms used in this study were originally purchased from the University of New South Wales culture collection and maintained at the David Morell Laboratory, Charles Sturt University. Organisms were grown on nutrient broth (10 g tryptone, 5 g yeast extract, 10 g sodium chloride, water to 1 L) and/or nutrient agar (as for nutrient broth plus 15 g agar/L of media).

An agar dilution method was used to assess the antibacterial activity of the selected honeys against the yeast *Candida albicans* and 13 bacteria: *Alcaligenes faecalis, Citrobacter freundii, Escherichia coli, Enterobacter aerogenes, Klebsiella pneumoniae, Mycobacterium phlei*, *Salmonella california, Salmonella enteritidis*, *Salmonella typhimurium, Serratia marcescens*, *Shigella sonnei*, *Staphylococcus aureus* and *Staphylococcus epidermidis*. Petri dishes containing a nutrient agar/honey mix were prepared for each honey at concentrations of 0.1% (w/v), 1% (w/v), 5% (w/v), 10% (w/v) and 20% (w/v) and a grid containing sixteen 15 × 15-mm squares was drawn on the underside of the dish. Each of the 14 bacterial strains was inoculated into nutrient broth and incubated overnight (37°C) until growth was 0.5 optical density (450 nm). A loop of each bacterial broth was then streaked onto the honey agar, one bacterial strain per square. The growth after 24 h incubation at 37°C was then compared to a control plate that contained no honey. Bacterial growth was rated using an inhibition score. A score of 0 indicated growth equivalent to the control with growth inhibition scored from 1–4 (e.g., 25% inhibition = 1; 50% inhibition = 2, etc.); growth greater than the control was scored in the same way and given a negative value (e.g., 25% increase = −1; 50% increase = −2, etc.). All assays were repeated in triplicate for each of the honey concentrations.

Results

The results of the assays of antibacterial activity of the six honeys used in this study are shown in Figure 1. The growth of all except one bacterium (*S. marcescens*) and the yeast, *C. albicans*, were inhibited by the honeys. In the case of *C. albicans* and *S. marcescens* there was little or no evidence of growth inhibition at concentrations up to and including 20% honey. Of the remaining honeys no inhibition of bacterial growth was observed at 0.1% honey. At 1% some inhibition was observed with *C. freundii*, *E. coli*, *M. phlei* and the three Salmonella species. At higher concentrations there was a progressive increase in inhibition as honey concentration increased with most honeys having an inhibition score of at least 3 (i.e., 75% inhibition) at 20%. The exception was *Klebsiella pneumoniae* with no honey producing an inhibition score >2 (i.e., 50% inhibition) at 20% honey concentration. No honey used in this assay produced complete inhibition at any of the concentrations tested.

There was some variation in the inhibition score for the six honeys with the Paterson's curse, rewa rewa and lavender honey generally having the poorest antibacterial activity (Figure 1). Some selectivity of honey against the bacteria was observed, particularly with *A. faecalis*, *E. aerogenes* and *S. aureus* where Medihoney®, manuka honey and red stringy bark honey produced greater inhibition that the other honeys.

Discussion

In this study we have demonstrated that both locally obtained unprocessed honeys and commercial antibacterial honeys have activity against a range of bacteria. Our data show that all honeys tested have some antibacterial action from concentrations as low as 5%; however, the greatest inhibition is seen at 20%. The growth of only two organisms (*S. marcescens* and *C. albicans*) was not inhibited by the honeys. Although these data are generally consistent with other reports showing that manuka honey has good antibacterial activity, we have also shown that other honeys have equivalent activity for some, but not all, bacteria. Those bacteria that appear to be more sensitive to Medihoney® and manuka are *A. faecalis*, *E. aerogenes* and *S. aureus*. Red stringy bark honey was also found to have a similar activity profile to the manuka honey. Although antibacterial activity was noted for several honeys at concentrations as low as 1%, the level of inhibition was low and it is doubtful whether the activity at

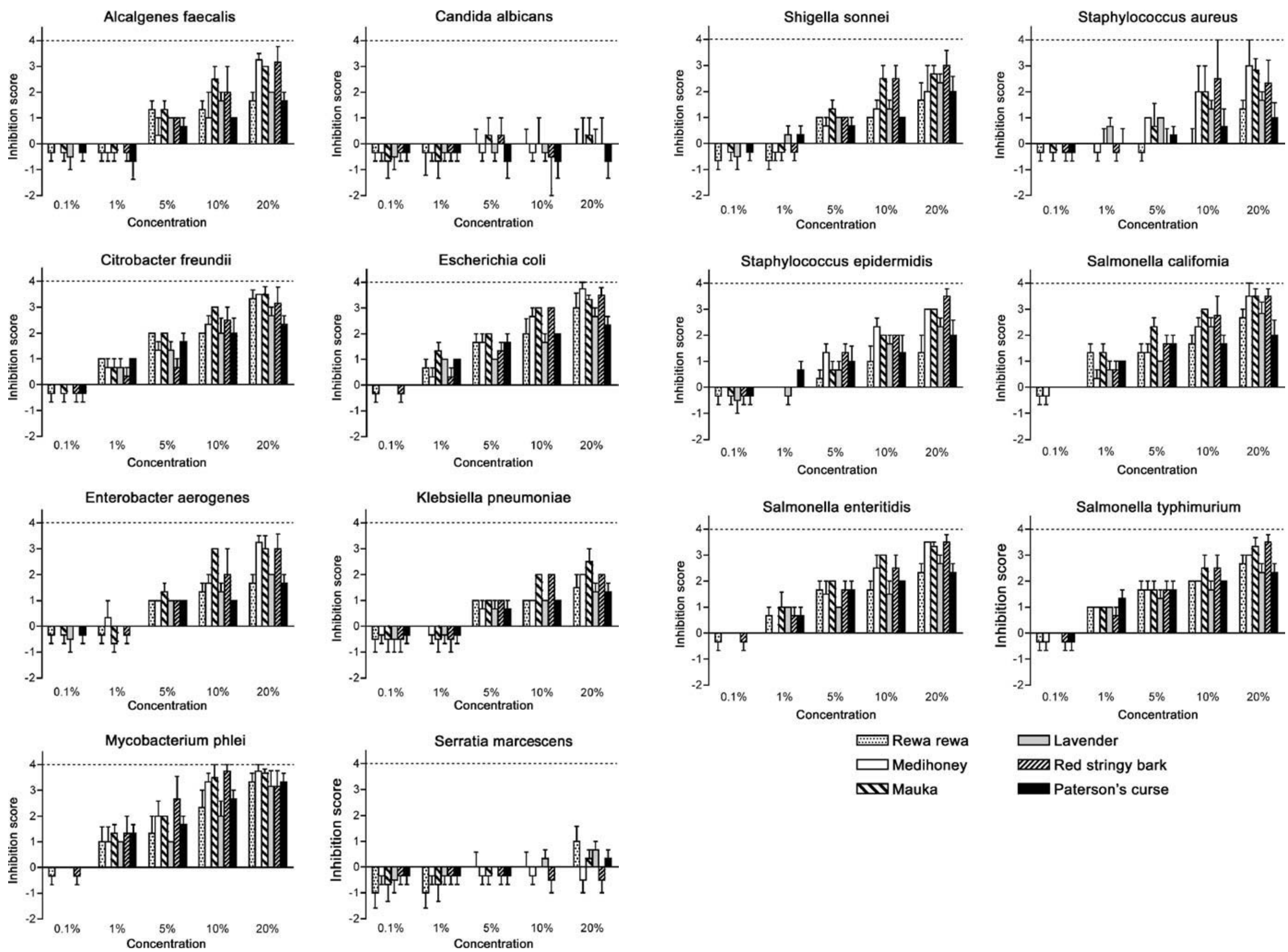

Figure 1. Inhibition of bacterial growth by honey. Graphs show the inhibition score for each honey against the 13 bacteria and *Candida albicans* used in the study. Data are presented as mean ± SEM with inhibition scored between 0 and 4 with 4 being complete inhibition and 0 growth equivalent to controls.

this concentration would be clinically significant. Similarly, the low-level inhibition of *K. pneumoniae* suggests that honey would not be a practical means of controlling the growth of this microorganism. However, it is also clear that at concentrations of 10–20%, depending on both honey and bacteria, there is significant inhibition of bacterial growth. For example, all honeys at 20% produced >75% inhibition of *M. phlei* growth.

The overall poor activity of the honeys against *S. aureus* was unexpected as previous reports have shown that maunka has an excellent activity against this organism. For example, Cooper et al. (16), who also used an agar dilution method, demonstrated that the minimum inhibitory concentration for maunka honey against 58 strains of Staphylococcus was 2–3% (v/v) and for pasture honey 3–4% (v/v). Part of the explanation for the difference in results may be due to methodological differences between studies because the agar dilution method used by Cooper et al. was slightly different from that used in this study. However, it is also likely to be due to variation in the composition of the honey being used.

In this study we have used an agar dilution method to assess antibacterial activity rather than well diffusion that is the most common method for testing honeys. In agar dilution methods the honey is incorporated directly into the growth media; hence, the bacteria are brought into direct contact with all honey components immediately on application to the agar rather than relying on diffusion of constituents through the agar. Whether there is a contribution to the size of zones of inhibition in well-diffusion assays of honey due to differential diffusion of activity components is unknown; however, this differential diffusion has been noted in assays of other natural products (17). It might also be suggested that agar diffusion better mimics the situation within a wound treated with honey where the honey is packed into a deep wound or applied as part of a dressing directly in contact with infected tissue. Further research is required to assess the correlation between antibacterial activity in vitro, as assessed by either well diffusion or agar dilution, and the actions *in vivo*.

A number of previous studies have demonstrated that various honeys, both commercially and locally produced, have antibacterial activity. Nzeako and Hamdi (18) in their study of six commercial honeys found that inhibition of *S. aureus*, *E. coli* and *P. aeruginosa* did not occur at honey concentrations $<$40%. In contrast to the current study, these authors also found that honey inhibited *C. albicans*, although the zone of inhibition was small compared with other organisms. Ceyhan and Ugar (19) tested 84 honeys against eight bacteria and two fungi showing that honey has broad-spectrum activity. In addition, these authors found that the antibacterial activity of honey was greater than that which could be attributed to the sugar content of the honey. The antibacterial activity of honey has also been investigated for its potential use in reducing food-borne pathogens (20), preventing catheter exit/entry site infection (21), for the treatment of colitis (22) or even to protect the gastric mucous in *H. pylori*-induced inflammation (23,24). The application of honey to wounds to animals in veterinary environments has also been noted (25).

In light of the enormous potential for application of honey within a clinical environment, it is important that research continues not only into those honeys recognized as antibacterial, but also into other locally produced, as yet untested, honeys. In this study we have demonstrated that three locally produced honeys have good antibacterial activity against a wide range of potential human pathogens. In addition, for many bacteria the antibacterial activity of the commercial antibacterial and locally produced honeys could not be distinguished. Further research is required to determine whether these honeys will also be useful as wound healing agents or for inclusion in food products to limit the spread of food-borne pathogens.

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References

1. Zumla A, Lulat A. Honey—a remedy rediscovered. J R Soc Med 1989;82:384–385.
2. Majno G. The Healing Hand. Man and Wound in the Ancient World. Cambridge, MA: Harvard University Press;1975.
3. Molan PC. The evidence for honey promoting wound healing. Aust J Wound Manag 1998;6:148–158.
4. Moore OA, Smith LA, Campbell F, Seers K, McQuay HJ, Moore RA. Systematic review of the use of honey as wound dressing. BMC Complementary and Alternative Medicine. 2001; http://www.biomedcentral.com/1472-6882/1/2.
5. Greenwood D. Wound healing: honey for superficial wounds and ulcers. Lancet 1993;341:90–91.
6. Postmes T, van den Bogaard AE, Hazen M. Honey for wounds, ulcers, and skin graft preservation. Lancet 1993;341:756–757.
7. Dunford C, Cooper R, Molan P, White R. The use of honey in wound management. Nurs Stand 2000;15:63–68.
8. Lusby PE, Coombes A, Wilkinson JM. Honey—a potent agent for wound healing? J Wound Ostomy Continence Nurs 2002;29:295–300.
9. Office of Complementary Medicines TGA. Honey. Scientific report. Canberra, Australia;1998.
10. Molan PC, Russell KM. Non-peroxide antibacterial activity in some New Zealand honeys. J Apicult Res 1988;27:62–67.
11. Weston RJ. The contribution of catalase and other natural products to the antibacterial activity of honey: a review. Food Chem 2000;71:235–239.
12. Willix DJ, Molam PC, Harfoot CJ. A comparison of the sensitivity of wound-infecting species of bacteria to the antibacterial activity of manuka honey and other honey. J Appl Bacteriol 1992;73:388–394.
13. Molan P, Brett M. Honey has potential as a dressing for wounds infected with MRSA. The Second Australian Wound Management Association Conference. Brisbane, Australia;1998.
14. Natarajan S, Williamson D, Grey J, Harding KG, Cooper RA. Healing of an MRSA-colonized, hydroxyurea-induced leg ulcer with honey. J Dermatol Treat 2001;12:13–36.
15. Molan PC, Allen KL. The effect of gamma-irradiation on the antibacterial activity of honey. J Pharm Pharmacol 1996;48:1206–1209.
16. Cooper RA, Molan PC, Harding KG. Antibacterial activity of honey against strains of *Staphylococcus aureus* from infected wounds. J R Soc Med 1999;92:283–285.
17. Southwell IA, Hayes AJ, Markham J, Leach DN. The search for optimally bioactive Australian tea tree oil. Acta Horticult 1993;344: 256–265.
18. Nzeako BC, Hamdi J. Antimicrobial potential of honey on some microbial isolates. SQU J Sci Res Med Sci 2000;2:75–79.
19. Ceyhan N, Ugar A. Investigation of in vitro antimicrobial activity of honey. Riv Biol/Biol Forum 2001;94:363–372.
20. Taormina PJ, Niemira BA, Beuchat LR. Inhibitory activity of honey against foodborne pathogens as influenced by the presence of hydrogen peroxide and level of antioxidant power. Int J Food Microbiol 2001; 69:217–225.
21. Quadri KH, Huraib SO. Manuka honey for central vein catheter exit site care. Semin Dial 1999;12:397–398.
22. Bilsel Y, Bugra D, Yamaner S, Bulut T, Cevikbas U, Turkoglu U. Could honey have a place in colitis therapy? Effects of honey, prednisolone, and disulfiram on inflammation, nitric oxide, and free radical formation. Dig Surg 2002;19:306–311.
23. Ali MATM. Prevention of ammonia-induced gastric lesions in rats by natural honey. J Nutr Environ Med 2003;13:239–246.
24. Osata MS, Reddy SG, Graham DY. Osmotic effect of honey on growth and viability of *Helicobacter pylori*. Dig Dis Sci 1999;44:462–464.
25. Mathews KA, Binnington AG. Management of wounds using honey. Compend Contin Educ Vet Pract 2002;24:53–61.