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Evaluation of the antibacterial activity of honeys (including Manuka and Sedr honeys) against bacteria causing opportunist infections

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ABSTRACT

The antibacterial properties of the following honeys were studied against a range of bacteria capable of causing opportunistic infections: "standard" commercially available honeys, Manuka honey (Unique Manuka factor [UMF], 10, 15, 20) and Sedr honeys from Saudi Arabia. All honeys studies showed antibacterial activity which varied depending on the honey and the bacterium under test. Manuka 15 and 20 honeys were generally more antibacterial than the standard honeys, although the latter were generally equally active as Manuka 10 honey. Of the honeys tested, the Sedr varieties generally exhibited the most marked antibacterial activities. The activity of all honeys was reduced by heating and by treatment with catalase, the latter demonstrating that much of their antibacterial activity is due to hydrogen peroxide; residual activity in the presence of catalase demonstrated the likely presence of complex antibacterial phytochemicals. There appear to have been no previous reports on the antibacterial activity of Sedr honeys; the relatively marked antibacterial activity of these honeys, which was only partially reduced by catalase and heat treatment suggests that they should be fully evaluated in a clinical setting for use in would treatment.

Key words: Saudi honey, UK honey, opportunist infections, antibacterial activity, Manuka and Sedr honeys.

INTRODUCTION

Pathogenic bacteria are increasingly showing resistance to previously effective antibiotics, a fact which led to the search for alternative approaches to the management of bacterial infections (Lione, 1998; Wainwright,1994), including, for example bacteriophage (Stone,2002) and maggot therapy (Bonn, 2000) and the use of antibacterial honeys (White *et al.,*1963; Dustmann,1979; Molan and Russell, 1988; Molan, 1992a; Molan, 1992b; Fernandez, 1996; Molan, 1992; Molan,1999; Weston *et al.,*1999; Weston *et al.,*2000; Weston, 2000). In their raw form, all honeys exhibit some degree of antibiotic activity. This is largely due to the acidity and high osmolarity present which is typical of any concentrated sugar solution, which sequester free environmental water, thereby preventing bacterial growth. Honey also retains some activity when it is diluted and the osmolarity is reduced. Hydrogen peroxide is a second antibacterial component which is universally present in honey; this denatures bacterial DNA and interrupts membrane potential thereby causing cell lysis (Molan, 1992; Molan, 2001). Honeys may also contain antibacterial phytochemicals, which are a major component of, so-called, Manuka honey (MH); these are produced (mainly in New Zealand) by bees feeding on *Leptospermum scoparium*. Manuka honey has proved successful as a topical application for the treatment of ulcers, gangrene (Molan and Betts, 2004) and burns (Cooper *et al*., 2002). Although an active compound specific to Manuka honey has not yet been isolated, a range of benzoic and cinammic acid derivates have been isolated from MH, as well as flavonoids; such compounds often act synergy, thereby making it difficult to isolate the individual active compounds

(Western *et al.,* 1999). Manuka honeys are graded by their relative ability to inhibit the growth of *Staphylococcus* aureus. The so-called UMF (Unique Manuka Factor) scale compares the antibacterial action to that of phenol, with a honey graded 'Factor 10' inhibiting *S. aureus* growth as successfully as a 10% phenol solution (Bell, 2008). .

Here, the relative antibacterial activity of commercial or "standard "honeys were compared with Manuka and Sedr honeys and MH. A range of common bacteria, which have been shown to be opportunistic pathogens in humans and animals, were used as test organisms. The effect of heat and catalase treatment on the antimicrobial activity of the honeys was also determined One of our main aims was to compare the antibacterial effects of honeys (i.e." standard" and Sedr honeys) for which no claims for antibacterial activity is made by the producers with that of MH, for which relatively marked antibacterial activity is claimed.

The five test bacteria chosen for use in this the study were the gram positive species, *Staphylococcus epidermidis* and *Bacillus sphaericus*, *Bacillus subtilis.* and the gram negative species, *Serratia marcescens*, and *Escherichia coli.* These bacteria are all opportunistic pathogens, which commonly cause persistent wound infections particularly in immunocompromised patients.

MATERIAL AND METHODS

Types of honey tested

The following honeys were tests: a) commercially available UK- monofloral honeys (i.e. from one plant source: pasture, chestnut and lavender); b) New Zealand Manuka honeys (Unique Manuka factor (UMF 10, 15 and 20) and Sedr and Sedr Mountain honey form Saudi Arabia

Bacterial strains and growth medium

A culture (0.2ml) of the test bacterium was spread on the surface of Nutrient Agar (Oxoid) in petri dishes and three wells (1cm) were cut from the centre of the medium using a flame-sterilized cork borer). The honey under test was added to the wells, using a wide-tip pipette and the plates were incubated at 37°C for 48 h.

Honey dilution

Some samples of honey were diluted to 50% and 10% weight/volume with sterile distilled water, and mixed thoroughly. Diluted samples settled over time, and were resuspended prior to use.

Catalase treatment

The honey samples (diluted to 50%) were treated with lyophilised bovine liver catalase (Sigma) (1 ml of a 10% w/v solution), so that the final catalase concentration of 50%w/v was achieved.

Heat treatment of honey

The honeys were heated using direct heat to reach boiling point, then immediately removed from the heat source. The boiling point of honeys sampled varied. All samples were allowed to cool in airtight vessels for 24hrs to 25°C before use and further dilution to 50% and 10% w/v (as above).

RESULTS AND DISCUSSION

Antibacterial effects of "standard" and Manuka honeys

All of the honeys tested inhibited the growth of all of the bacteria used as test organisms. (Table1), thereby showing that honey has a broad spectrum of antibacterial activity against

Table 1: The antibacterial effect of "standard" and Manuka honeys

	Pasture	Chestnut	Lavender	Manuka 10+	Manuka 15+	Manuka 20+
B.subtilis	7.5 ± 2.5	7.5 ± 2	6.5 ± 1	5.5 ± 0.5	11± 1	8.5
B.sphaericus	2.5 ± 1	3 ± 0.5	4.5 ± 1	3.5 ± 3.5	5.5 ± 1.5	9.5± 1
E.coli	3 ± 1.5	4 ± 1	4 ± 0.5	3.5 ± 0.5	7 ± 1	0.5 ± 0.5
S.epidermidis	3.4± 1	5 ± 0,5	7.5 ± 2.5	4.5 ± 2.5	8 ± 2	10 ± 1.5
S.marcescens	2.5 ± 1.5	3.5 ± 1.5	2.5	2.5 ± 2	6 ± 1.5	8.5

opportunistic pathogenic bacteria. Each individual honey showed varying inhibitory effect on bacteria; for example the mixed pasture honey markedly inhibited *B.subtilis*, but had less inhibitory effect on *B.sphearicus*. This makes it difficult to generalize regarding the overall antibacterial properties of a given honey Similarly, the Chestnut and Lavender monofloral honeys inhibited *B.subtilis* but less so *S.marcescens*. As a generalization, the three pasture honeys showed broadly similar inhibitory effects as shown by the Manuka 10 honeys. The Manuka 15 and 20 honeys on the other hand generally inhibited bacterial growth more effectively than did the "standard " honeys. The largest inhibition zone seen in Table 1 was produced by Manuka 20 when tested against *B.sphaericus*. Manuka 20 did not overall outperform Manuka 15 and Manuka 20, unlike Manuka 15, did not inhibit the growth of *E.coli*. Table I shows that although Manuka 15 and 20 honeys generally have greater antibacterial activity than Manuka 10 and the "standard" honeys, bacterial inhibition is dependent on the bacteria in question.

Thus, Manuka 20 would probably be less effective than any of the "standard" honey and the other Manuka honeys for treating surface infections caused by *E.coli*, but would be the honey of choice for applying to infections caused by *S.epidermidis* and *B.sphearicus*. Table 1 also shows that there is generally little point in investing in relatively expensive Manuka 10 honeys when cheaper, more readily available "standard" honeys have similar antibacterial effects. Clearly, honeys have differing antibacterial properties and there is no honey which can be expected to be useful in the treatment of all opportunistic infections; even applying relatively expensive Manuka 20 honey would not necessarily be the most effective choice, both in terms of pathogen inhibition and cost effectiveness. The results shown in Table 1 suggests that in every case, the bacterium responsible for an opportunistic infection needs to isolated and then tested against a range of honeys in order to determine which is the likely t be the most effective honey type. Although generally more effective than standard honeys, and Manuka 10 honey, Manuka, 15 and 20 honeys cannot be used as a universal honey antibiotic for use in the treatment of all opportunistic infections. On the positive side, the result show that "standard

Table 2: The effect of heating and catalase treatment on diluted pasture and manuka honeys

	pasture honey			UMF "10+" Manuka honey			UMF "15+" Manuka honey		
	50%	50% heated	50% Catalase	50%	50% heated	50% Catalase	50%	50% heated	50% Catalase
B.subtilis	2 ± 0.5	1	2 ± 0.5	4 ± 1	3 ± 2	0	4 ± 1.5	2 ± 0.5	2 ± 1
B.sphaericus	3.5 ± 0.5	1 ± 0.5	0	0	1.5 ± 1.5	0	4.5 ± 1.5	3 ± 1.5	3 ± 0.5
E.coli	1	0	1.5 ± 0.5	1± 1	0	1	1.5 ± 1.5	2 ± 1	3.5
S.epidermidis	3 ± 0.5	1	1.5 ± 0.5	2.5 ± 1.5	1± 1	1	5.5 ± 0.5	3 ± 1.5	3 ± 0.5
S.marcescens	0	0	0	0	0	0	0	2.5 ± 1	2 ± 1

" honeys, obtained commercially in retail outlets are effective antibacterial agents, a fact that is particularly importance in developing countries, where local honeys are likely to be effective in treating opportunistic infections; in short, there is no *a priori* reason why inexpensive, local honeys(after sterilization) should not be evaluated in the treatment of opportunistic infections caused by the bacteria used here, and presumably other, similar infections.

Manuka honeys for medical use are supplied pre-sterilized (by filtration or by the use of ionizing radiation) in order to avoid the possibility of potentially pathogenic indigenous bacteria and fungi being transferred to the infection site. If cheaper, and more readily available a "standard" honeys are to be used as antibacterial agents it would be desirable to be able to sterilize them using a readily available method, i.e. heating. Table 2 however, shows that heat treatment generally markedly reduces the antibacterial effect of "standard "honeys although some activity is retained by Manuka 15 honey.

The results shown in Table 2 support claims made by producers of Manuka honey, that the antibacterial effect of these honeys is due to a factor other than hydrogen peroxide, since activity is retained (although diminished) by the application of catalase; the antibacterial effects of "standard" honeys in contrast is markedly reduce by catalase treatment showing that their effectiveness against bacteria is due mainly to hydrogen peroxide and not complex phytochemicals.

Evaluation of Sedr Honeys

The two Sedr honeys tested here generally showed marked antibacterial activity; for example both Sedr honey and the mountain variety showed marked activity against *S.marcescens* (Table 3). As a generalization, the Sedr honeys we showed greater antibacterial activity than the both the "standard" and Manuka honeys (Table 1 ad 3).

Heat treatment reduced the activity of both types of Sedr honeys, but did not destroyit; suggesting that heat treatment could be used as means of cheaply and effectively sterilizing these

Table 3: The antibacterial effects of heated and non-heated Sedr honeys

	Mountain Sedr honey		Sedr honey	
	Non heated	**Heated**	**Non heated**	**Heated**
B.subtilis	11 ± 0. 5	2.3 ± 0.7	11.6 ± 1.7	7 ± 0.6
B.sphaericus	12 ± 0.6	9.2 ± 0.8	12.3 ± 0.3	9.2 ± 0.8
E.coli	22.7 ± 0.6	6.6 ± 0.8	7.3 ± 1.3	5.3 ± 0.6
S.epidermidis	7.7 ± 0.3	5.3 ± 0.2	7.7 ± 0.3	6.1 ± 0.6
S.marcescens	19 ± 0.6	6.6 ± 0.8	21.3 ± 1.9	10.5 ± 0.2

Table 4: The effect of heat and catalase treatment on diluted Sedr honeys

	Mountain Sedr honey			Sedr honey		
	50%	**50% heated**	**50% Catalase**	**50%**	**50% heated**	**50% Catalase**
B.subtilis	9 ± 1	14 ± 0.5	12.3±0.6	7.4 ± 2.3	11.3±0.8	10
B.sphaericus	10	7.3 ± 0.2	10.6±0.6	11 ± 0.3	7.12±0.3	10.7±0.3
E.coli	18 ± 1.5	4.2 ± 0.8	10.3±0.3	0	4.3±0.9	9.3±0.3
S.epidermidis	1.7 ± 0.9	5	6±0.1	1.7 ± 0.9	4.6±0.3	7.3±0.6
S.marcescens	12.7 ± 0.7	4.2 ± 0.8	5.8±0.4	16.7 ± 2.3	7.3±0.3	5 ±0.5

honeys prior to use on wounds; a characteristic which would make these honeys particularly useful for use in treating wounds in low technology hospitals and field treatment centers. When diluted (50% w/v), the Sedr honeys retained considerable activity (increased in the case of *B.subtilis* for both Sedr honey types following heat treatment (Table 4).

The ability of a honey to retain activity following dilution is useful as a diluted honey is likely to better penetrate wounds and reach hidden bacteria than is a full strength honey. Catalase reduced the antibacterial effects of Sedr honeys, but again activity remained high; a result which shows that the antibacterial activity of Sedr honeys is not due solely to hydrogen peroxide, but also to complex phytochemicals (Table 4).

There appear to have been no previous published reports on the antibacterial effects of Sedr honeys. The relatively marked antibacterial activity of the two Sedr honeys tested here however, suggests that these products could find a place in would treatment and replace the Manuka honeys which currently the main honey-type used in medicine; as a result, we suggest that it would be worthwhile to further, fully, assess the medical potential of Sedr honeys.

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