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SHORT COMUNICATION

ANTIBACTERIAL POTENTIAL OF HONEY FROM DIFFERENT ORIGINS: A COMPARSION WITH MANUKA HONEY

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ABSTRACT

The antibacterial activity of honey is well documented, this activity is mainly due to its low pH, osmolarity and hydrogen peroxide accumulation. Recently, more attention has been given to the importance of a unique extra antimicrobial activity, termed as a non-peroxide activity. The aim of this work was to investigate the antimicrobial activity of selected honeys from different origins; specifically to evaluate their non-hydrogen peroxide derived activity, against *Staphylococcus epidermidis, Bacillus sphaericus*, *Bacillus subtilis*, *Serratia marcescens*, *Escherichia coli* and *S. epidermidis*; manuka honey was used as the control. Antibacterial activity of the honeys was assayed using standard well diffusion methods. noticeable variations in the antibacterial activity of the different honey samples were observed. Most of tested honeys had broad-spectrum antibacterial activity, particularly Greek Pine, Scottish Heather, Chilean Ulmo, New Zealand Beech and Jarrah Honey. Unfortunately, none of the tested honey had a detectable non-peroxide activity.

Keywords: Antibacterial activity, manuka honey, Non-peroxide activity, MRSA

INTRODUCTION

Honey was used in the medicine of many ancient communities **(Molan, 2006)**, including the ancient Egyptians. The ancient Chinese and Sumerians provided the first written prescriptions relating to the medical use of honey, found as clay tablets, dating back to 2000 B.C.

The antibacterial potency of honey has been attributed to its strong osmotic effect, naturally low pH **(Kwakman and Zaat, 2012)**, the ability to produced hydrogen peroxide which plays a key role in the antimicrobial activity of honey **(Kačániová *et al*., 2011; Wahdan, 1998)** and phytochemical factors . Numerous reports and clinical studies have demonstrated the antimicrobial activity of honey against a broad range of microorganisms, including multi-antibiotic resistant strains. Honey samples collected from Northern Ireland and France showed a significant ability to inhibit the growth of community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) **(Maeda *et al*., 2008)**. Other studies demonstrated the antibacterial activity of honey against: *Escherichia coli, Campylobacter jejuni, Salmonella enterocolitis, Shigella dysenteriae* **(Adebolu, 2005; Voidaou *et al*., 2011)**, *Mycobacterium* **(Asadi-Pooya *et al*., 2003)**, methicillin-resistant *Staphylococcus aureus* and, Vancomycin –resistant enterococci **(Cooper *et al.*, 1999; Cooper *et al.*, 2002; Al-Waili *et al.*, 2005)**, common gastrointestinal pathogenic bacteria **(Lin *et al*., 2011),** and the development of *Streptococcus pyogenes* biofilms **(Maddocks *et al*., 2012).** The antifungal activity of the honey, especially anti-*Candida* activity **(Irish *et al.*, 2006; Koc *et al.*, 2008; Ahmad *et al*., 2012)** has also been reported.

Manuka honey which is derived from the Manuka tree (*Leptospermum scoparium*) a native of New Zealand. manuka honey is known to have a unique extra antimicrobial activity which is not related to its low pH, osmolarity or hydrogen peroxide accumulation, termed as a non-peroxide activity **(Windsor *et al*., 2012)**.

Much research effort has been made to identify the active component responsible for this non-peroxide antibacterial activity, although it was thought that it may be due to plant derived components such as flavonoids and phenolic compounds **(Weston *et al*., 2000)**, recent studies have successfully concluded that this component is methylglyoxal (MG), a highly reactive precursor in the formation of advanced glycation end products (AGEs) **(Adams *et al*., 2008; Mavric *et al*., 2008; Stephens *et al*., 2010)**.

The objective of our study was to investigate the antimicrobial activity of selected honeys from different origins; specifically to evaluate their non-hydrogen peroxide derived activity.

MATERIAL AND METHODS

Honey samples

Eighteen Honey samples from different origins were obtained from Rowse Honey Ltd, Wallingford, UK. Graded New Zealand manuka honey samples were obtained from Comvita UK Limited, Berkshire, UK or from Littleover Apiaries Ltd, Derby, UK.

Determination of pH

The pH of the honey was determined as described by the International Honey Commission **(Bogdanov *et al*., 2002).**

Test Organisms

The following test organisms (bacteria) were used: *Escherichia coli, Staphylococcus epidermidis, Serratia marcescens, Bacillus sphaericus,* methicillin-sensitive *Staphylococcus aureus* (MSSA), methicillin-resistant *Staphylococcus aureus* (MRSA) and *Bacillus subtilis*. the organisms were obtained from the Departmental Culture Collection (Department of Molecular Biology and Biotechnology, University of Sheffield, Sheffield).

Phenol calibration

Different phenol concentrations ranging from 1% to 14 % (w/v) were prepared and their antibacterial activities against *Staphylococcus aureus* were measured using the well diffusion method .The diameter of the clear zone around each well of the phenol references was measured, and plotted against the phenol concentration used, and the standard calibration curve equation shown in Figure 1 was used to compare phenol inhibitions with that of the various honey samples **(Baltrusaityte *et al*., 2007).**

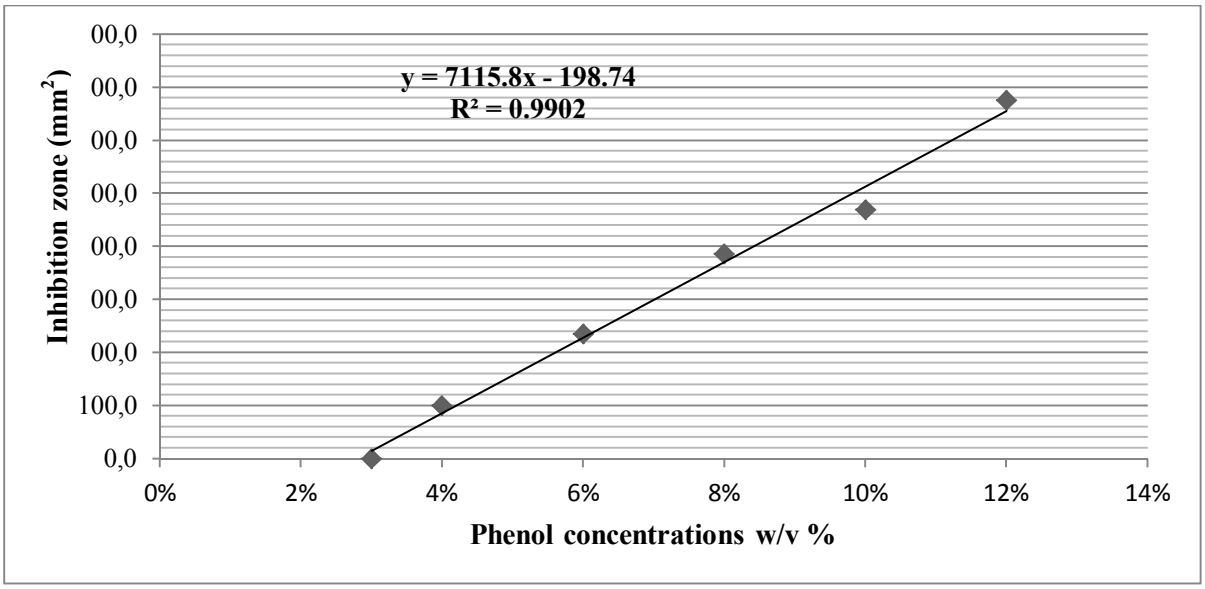

Figure 1 Calibration curves for the phenol reference solutions used in the agar well diffusion assay of antibacterial activity against *S. aureus*

Agar diffusion assay

The plates were prepared using 20 ml of sterile Nutrient Agar. The surface of the plates was inoculated using a 100 µL of 0.5 McFarland standardized inoculum suspension of bacteria and allowed to dry. Wells, 8.0 mm in diameter, were cut from the culture media using a sterile metal cylinder, and then filled with the test honey. The plates were incubated at 37ºC and observed after 24 hours for clear, circular inhibition zones around the wells were measured.

Catalase treatment

Honey samples were tested at a concentration of 50 % (w/v) for antibacterial activity. Catalase solution was made by dissolving 2 mg of catalase (Sigma, 1850 units/mg), in ultrapure distilled water (10 ml). The honey (2.00 g) was dissolved either in distilled water (2.00 ml) or in 2 ml of catalase solution (giving non-peroxide activity).

Statistical Analysis

All observations were presented as Mean ± SD. (Standard deviation). The data was analyzed by SigmaPlot© 11.0. $P<0.05$ was considered as significant.

RESULTS AND DISCUSSION

A total of eighteen honey samples from different origins were evaluated for their antibacterial activity against selected bacteria species representing the Gram-positive species, *Staphylococcus epidermidis Bacillus sphaericus*, and *Bacillus subtilis,* and the Gram negative species, *Serratia marcescens*, and *Escherichia coli. Bacillus subtilis, S. epidermidis*, *B. sphaericus* and *S. marcescens*. In general, as shown in (Table 1) all tested honeys, except Kent and Gain Japan honeys, showed a measurable antibacterial activity against all of the tested bacteria with different values. Four of the tested bacteria were most sensitive to Greek pine honey comparable to other tested honeys showed a significant inhibition zone against Gram-negative bacteria, S. *marcescens*, and *E. coli,* 17.0±1.0 and 18.3±1.2 respectively. Kent honey and Gain Japan honey either showed no or limited inhibition to the tested bacteria, especially Gram-negative bacteria. Scottish heather honey displayed a potent activity against *S. epidermidis*, 23.7±1.2 mm, and moderate activity against other bacteria. New Zealand beech honey displayed a potent activity against only *B. subtilis,* 20.7±0.6 mm, and moderate activity against other bacteria. *S. marcescens* displayed the highest resistance for 61% (11 out of 18) of tested honeys, whereas *B. subtilis* was the most sensitive bacteria for 56% (10 out of 18) of tested honeys. These data do not agree with the results reported by **Mohapatra *et al*. (2011)** who showed that the Gram-negative bacteria are more susceptible to the inhibitory action of honey than are Gram-positive bacteria .

Table 1 shows that the majority of tested honeys have broad-spectrum antibacterial activity, particularly Greek Pine, Scottish Heather, Chilean Ulmo, New Zealand Beech and Jarrah Honey**.**

In further attempts to determine if this broad-spectrum antibacterial activity was due to the activity of hydrogen peroxide or due to another factor, comparable to different medical grade manuka honeys, eighteen different origin honeys (Table 2) were evaluated for their total antibacterial activity and non-peroxide activity against methicillin-sensitive *S. aureus* (MSSA), expressed as equivalent phenol concentration (% w/v).

All of the investigated different origin honeys exhibited some antibacterial activity (total activity, peroxide + non-peroxide activity) as shown in (Table 2) but levels were lower than the most of the medical grade manuka honeys and ranged from 4.4% (w/v) to 8.8% (w/v).

Table 1 Antibacterial activity of selected honeys from different origins against five different bacterial species, determined by agar diffusion.

	Inhibition zone (mm)±SD				
Honey	*E. coli*	*S. marcescens*	*B. sphaericus*	*S. epidermidis*	*B. subtilis*
Greek Pine	17.0 ±1.0	18.3 ±1.2	16.3 ±1.5	17.3 ±2.1	20.3 ±1.2
Yorkshire	15.8 ±2.2	12.6 ±1.1	13.8 ±1.5	13.2 ±1.3	16.2 ±0.8
Chilean ulmo	15.7 ±0.6	15.3 ±0.5	15.3 ±0.5	19.0±0.0	16.0 ±1.0
Australian Eucalyptus	14.3 ±0.5	12.3 ±0.5	14.3 ±0.5	13.6 ±0.5	16.6 ±1.2
Himalayan wild flower	13.8 ±0.3	11.2 ±0.3	14.0 ±1.0	16.2 ±0.8	13.0 ±1.0
Scottish heather	13.7 ±1.2	12.7 ±0.6	15.7±0.6	23.7 ±1.2	17.0 ±0.0
Chilean	13.6 ±0.6	13.5 ±0.7	14.3 ±0.6	14.0 ±0.0	14.3 ± 0.6
New Zealand Clover	13.0 ± 0.6	12.3 ±0.5	16.2 ±0.8	15.2 ± 0.0	14.3 ±0.6
Cuban Comparitan	11.7 ±0.6	12.8 ±1.1	11.7 ±0.6	12.3 ±0.6	13.0 ±1.0
Acacia Hungarian	11.3 ±0.6	12.0 ±0.0	12.0 ±0.0	12.7 ±1.2	11.3 ±0.6
Spanish blossom	11.0 ± 0.5	13.2 ±0.6	11.3 ± 0.6	11.8 ±0.3	10.7 ±0.3
Tasmanian Leatherwood	13.2 ±1.3	12.7 ±0.6	13.7 ± 0.6	13.3 ±0.6	15.7 ±2.5
Organic honey	12.3 ±0.6	11.2 ±0.3	12.3 ±1.2	13.0 ±2.0	15.3 ±0.5
New Zealand beech	12.3 ±0.6	14.3 ±0.5	16.0 ± 1.0	15.7 ±2.5	20.7 ±0.6
Jarrah honey	13.0 ± 1.0	14.0 ±2.0	15.7 ±1.5	18.3±1.2	17.0 ±1.0
Kent honey	0	11.3 ±0.6	11.3 ±0.6	11.7 ±1.2	14.0 ±2.6
Gaint Japan	0	0	11.0 ±0.0	11.3 ±1.5	NT
Troway Hall	13.8 ±0.3	14.3 ±1.5	NT	16.3 ±2.1	16.7 ±1.5

The values are means of replicates (well (8.0mm)) ± Standard deviation. NT: not tested.

Table 2 The total antibacterial activity and the non-peroxide activity of selected honeys from different origin against methicillin-sensitive *S. aureus* (MSSA)

Honey	pH	Total antibacterial activity as phenol equivalent (w/v) %	Non-peroxide activity as phenol equivalent (w/v) %
Comvita® UMF® +25 manuka	3.6	11.9	10.7
manuka 250MGO	3.8	10.6	10.3
Comvita® UMF® +15 manuka	3.8	9.8	8.9
Littleover manuka active +10	4.1	9.3	8.7
Greek Pine	3.4	8.8	< 3.0
Comvita® UMF® +20 manuka	3.2	8.3	8.1
Scottish heather honey	4.5	7.5	< 3.0
Organic honey	NT	7.3	< 3.0
Australian Eucalyptus	3.7	7.2	< 3.0
Yorkshire Honey	3.5	6.9	< 3.0
New Zealand Beech	4.6	6.7	< 3.0
Chilean Honey	4.1	6.5	< 3.0
Himalayan Wild Flower	NT	6.5	< 3.0
Tasmanian Leatherwood	3.8	6.3	< 3.0
Spanish Orange Blossom	3.5	6.1	< 3.0
Chilean Ulmo	4.2	5.9	< 3.0
Troway Hall	NT	5.6	< 3.0
Jarrah Honey	4.5	5.6	NT
New Zealand Cclover	3.4	4.8	< 3.0
Kent Honey	3.5	4.8	NT
Cuban Comparitan	3.5	4.4	< 3.0
Hungarian Acacia	3.3	4.4	< 3.0
Gaint Japan	3.1	< 3.0	NT

Expressed as equivalent phenol concentration (% w/v), determined by agar diffusion, and the pH of these honeys. (Only the honeys with the highest activity were investigated to determine their non-peroxide activity). NT: not tested

Except for medical grade manuka honeys that are well known to have unique non-peroxide activity, none of the tested honey had a detectable non-peroxide activity, more than 3.0 w/v phenol. As expected Comvita UMF +25 manuka had the largest non-peroxide activity equivalent to 10.7% (w/v) phenol while Comvita UMF +20 manuka had the lowest non-peroxide activity equivalent to 8.1% (w/v) phenol among referenced medical grade manuka honeys.

All tested honeys showed a detectable activity except Gaint Japan Honey which did not exhibit any antibacterial activity against methicillin-sensitive *S. aureus* (MSSA). Ten of these honeys were equivalent to more than 6.0 w/v % phenol; eight types were equivalent to 4.4-5.9 w/v % phenol.

The variation in the honey antibacterial potency has been well reported; it can be vary much as 100-fold **(Molan, 2001)**. Peter Molan, a pioneer researcher in the Waikato Honey Research Unit, concluded that not all honeys can be used for therapeutic purposes, and he recommended that some care must be taken before a honey is chosen as a wound dressing, such honeys should have a high level, and a wide spectrum of antibacterial activity, particularly against bacteria commonly associated with wound infections, and should also have a marked non-peroxide activity.

The results shown in Table 2, show that none of tested honeys exhibited an exceptionally high non-peroxide activity, thereby suggesting that none of tested honeys could achieve medical grade status and act as an alternative to currently used manuka medical grade honeys.

Conclusion

The majority of the tested honeys exhibited inhibitory effects against different microorganisms, but none of them had an exceptionally non-peroxide activity. These results suggest that they might be used in treating a wide range of pathogenic Gram-positive and Gram-negative bacteria.

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