Review Paper

HONEY: A REALISTIC ANTIMICROBIAL FOR DISORDERS OF THE SKIN

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ABSTRACT

Resistance of pathogenic micro-organisms to antibiotics is a serious global health concern. In this review, research investigating the antimicrobial properties of honeys from around the world, against skin relevant microbes is evaluated. A plethora of *in vitro* studies have revealed that honeys from all over the world have potent microbicidal activity against dermatologically important microbes. Moreover, *in vitro* studies have shown that honey can reduce microbial pathogenicity as well as reverse anti-microbial resistance. Studies investigating the antimicrobial properties of honey *in vivo* have been more controversial. It is evident that innovative research is required to exploit the anti-microbial properties of honey for clinical use and to determine the efficacy of honey in the treatment of a range of skin disorders with a microbiological aetiology.

Key Words: Anti-microbial, dermatology, honey, wound infections.

INTRODUCTION

In traditional medicine, honey has been recognised around the world for its skin healing properties. The ancient Greeks and Egyptians, for example, used topical application of honey to treat skin wounds and burns and Persian traditional medicine documented honey as effective in the treatment of wounds, eczema and inflammation [1, 2].

Micro-organisms have been associated with the pathophysiology of a range of dermatological disorders. Wound infections, for example, are commonly caused by the micro-organisms *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* and infection with *S. aureus* is common in atopic dermatitis [3, 4]. Other examples are Malassezia yeasts which have been associated with the skin conditions pityriasis versicolor, seborrheic dermatitis, atopic dermatitis and psoriasis [5]. Conventional treatments for some of these conditions are

unsatisfactory e.g. corticosteroids cause skin thinning and ultraviolet radiation therapy has been associated with the development of skin cancer [6].

Scientists first reported the ability of honey to kill disease causing microbes in the late 1800s but with the advent of antibiotics in the early 1900s scientific interest in honey waned (cited in Molan, 2001 [7]). Today, with the emergence of antibiotic resistant microbial strains, such as Methicillin resistant *Staphylococcus aureus* (MRSA); a cause of difficult to treat wound infections and a global health concern, honey has again caught the attention of medical researchers [7, 8].

In clinical practice today, Manuka Honey produced by honey bees (*Apis mellifera*) collecting nectar from the Manuka tree (*Leptospermum scoparium*) is used topically in the management of wound infections [9]. Products include γ irradiated honey in gels, ointments and impregnated dressings. Revamil honey is another medical grade honey commonly used in clinical practice for wound care [10]. It is produced in greenhouses by manufacturers in the Netherlands but further details about the origin of the honey have not been disclosed.

In this review, research findings for the antimicrobial activities of honeys from around the world, against skin relevant microbes, are evaluated. Furthermore, the mechanisms of the antimicrobial properties of honey are explored. The principle aim was to understand more about the therapeutic potential of honey as a treatment for skin diseases with a microbiological aetiology.

ANTIMICROBIAL PROPERTIES OF MANUKA HONEY AGAINST SKIN RELEVANT MICROBES: *IN VITRO* STUDIES

The most widely researched honey, to date, is Manuka honey from New Zealand. Studies have shown that Manuka honey has anti-microbial activity *in vitro* against the most common wound infecting micro-organisms, including MRSA, *S. aureus*, *P. aeruginosa* and *E. coli* [11, 12]. Manuka honey can also inhibit the growth of *Streptococcus pyogenes*, a cause of cellulitis, impetigo and necrotising fasciitis, and the dermatophyte *Trichophyton mentagrophyte*; a cause of ringworm [11, 13]. Indeed, Manuka Honey has been shown to inhibit the growth of a range of dermatophytes including *Epidermophyton floccosum*, *Microsporum canis*, *Microsporum gypseum*, *Trichophyton rubrum* and *Trichophyton tonsurans;* indicating that honey may be therapeutic in the treatment of Dermatophytosis (Tinea infections) [13]. Studies have reported that *Candida albicans* is more resistant to Manuka honey than many other microbial species [14, 15]. Manuka honey has also been shown to have anti-viral activity *in vitro* against varicella zoster virus suggesting that honey may be therapeutic for viral skin rashes [16]. The antiviral properties of honey against other skin relevant viruses such as human papilloma virus (HPV) may be worth investigating.

As the antimicrobial activity of honey varies not only between different types of honey but also between batches of the same type of honey, Manuka honey is often ascribed a Unique Manuka Factor (UMF). The UMF is a measure of the strength of the antibacterial activity of

the honey against *S. aureus* and is calculated based on the concentration of a phenol solution that gives a similar zone of growth inhibition in a radial diffusion assay as the honey being tested. A criticism of the UMF classification is that it is only a measure of activity against *S. aureus* and not other relevant microbes.

ANTIMICROBIAL ACTIVITY OF HONEYS FROM AROUND THE WORLD AGAINST SKIN RELEVANT MICROBES: *IN VITRO* STUDIES

A plethora of scientific papers have reported *in vitro* anti-microbial activity of honeys from all over the world; some examples are described in this section.

Honey produced in South Gondar, Ethiopia, by the bee *Apis mellipodae*, a stingless bee, is used in traditional medicine in Ethiopia to treat a variety of diseases including skin infections [17]. Using the method of agar well diffusion Andualem (2013) demonstrated that this honey inhibited the growth of the wound infecting microbes *E. coli* and *S. aureus* with minimal inhibitory concentrations (MICs) of 12.5% and 6.25% respectively.

In a study by Pimentel (2013), honey samples collected from the stingless bee *Melipona compressipes manaosensis* in Manaus, Amazonas, Brazil, were active against *E. coli, S. aureus, Proteus vulgaris and Klebsiella species* [18]. Using agar well diffusion assays it was demonstrated that honey collected during the rainy season inhibited the growth of *E. coli* only in undiluted forms, whilst, honey collected during the dry season inhibited the growth of *E. coli, S. aureus* and a range of other microbes at much more diluted concentrations. These results clearly show the influence of seasonality on the anti-bacterial activity of honey. Plant derived factors or entomological factors such as the health of the bee colonies could be affected by season with consequences for the antimicrobial activity of the honey produced. The researchers also compared the ability of honey to inhibit microbial growth by agar well diffusion with a broth dilution assay and found that the broth dilution assay was a more sensitive method, most likely, due to better movement of the antimicrobial components of honey in liquid broth than in agar. Rutin, a flavonoid previously shown to have antibacterial activity was identified in the honey by high powered liquid chromatography (HPLC).

Kuncic *et al* (2012) reported that Slovenian honeys from diverse floral origins had antibacterial activity against *E. coli*, *P. aeruginosa* and *S. aureus* [19]. Slovenian chestnut and pasture honeys were found to be the most active, for example, the MIC of the chestnut honey against *S. aureus* was found to be 2.5%. *C. albicans* was not inhibited by any of the Slovenian honeys tested and *Candida parapsilosis* and *Candida tropicalis* were inhibited only by honey concentrations higher than 50%.

In other studies, the growth of *C. albicans* was inhibited by Jujube honey, a honey obtained from bee keepers in Al-baha, Saudi Arabia from bees feeding on the plant *Ziziphus jujuba* and by a mixture of honey, olive oil and beeswax containing multifloral honey from the United Arab Emirates [20, 21]. Such findings indicate the potential for some honeys being used in the treatment of skin disorders caused by *C. albicans* such as cutaneous candidiasis.

Tualang honey, obtained from bees (*Apis dorsata*) feeding on Tualang trees (*Koompassia excelsa*) in the jungles of Malaysia, was found to inhibit the growth of MRSA, *S. aureus*, *S. pyogenes*, *P. aeruginosa* and *E. coli* in a broth dilution assay, with MICs comparable with Manuka honey [11].

In 2013, researchers at Queen Margaret University, Edinburgh, Scotland reported the antimicrobial activity of a Scottish Honey called Portobello Honey [22]. Portobello Honey was produced by honey bees in an apple orchard in Portobello, Edinburgh, Scotland. Five concentrations of the Portobello Honey and medical grade Manuka Honey (0, 1, 10, 50 and 70%) were tested against *S. aureus*, *P. aeuginosa* and *E. Coli* using agar disc diffusion and a broth dilution assay. The agar disc diffusion method did not demonstrate any antimicrobial activity of the honeys tested however it was reported that the honey remained on the surface of the disc and did not diffuse into the agar. The broth dilution assay, on the other hand, demonstrated antimicrobial activity of Portobello Honey and Manuka Honey at concentrations of 50 and 70% which were found to inhibit the majority of all of the bacterial species tested. The MIC of Portobello Honey was not calculated but the authors concluded that honey is a superior antibacterial agent.

In a study by Carnwath et al (2014), the anti-microbial activities of a selection of 10 honeys against 10 microorganisms were tested at the department of Veterinary Medicine, University of Glasgow, Scotland [23]. The honeys tested included medical grade and shop bought Manuka honeys, Scottish Heather Honey (from a local bee keeper), Blossom Honey, Vipers Bugloss Honey, Inverness Floral Honey and Glasgow Floral Honey. The microorganisms tested included MRSA, *S. aureus*, *E. coli*, *P. aeruginosa and Acinetobacter baumannii*. Serial dilutions of the honeys were prepared in distilled water and mixed with equal volumes of nutrient agar to give final honey concentrations ranging from 2-16%. Plates were inoculated with the appropriate microorganism and incubated aerobically overnight. All the honeys tested demonstrated antimicrobial activity but the most active was found to be the Scottish Heather Honey which inhibited the growth of all the micro-organisms tested with MICs ranging from <2% to 6%. The Scottish Heather Honey was even more active than all of the Manuka honeys used in the study.

Remarkably, *in vitro* research has also shown that honey can actually reverse antibiotic resistance, suggesting that honey used in combination with antibiotics may have additional therapeutic effects [24]. A suggested mechanism is via honey induced down-regulation of mecR1 gene product, a transducer associated with antibiotic resistance in MRSA. Indeed, Muller et al (2013) reported that Manuka honey worked synergistically with the antibiotic Rifampicin to inhibit the growth of MRSA and clinical isolates of *S. aureus* [25].

The evidence is clear that, in a laboratory setting, honeys from all over the world have potent antimicrobial activity against skin relevant microbes. Indeed, the antimicrobial activity of honey from Iran has been shown to be comparable with the sulphonamide family of antibiotics [26]. The micro-organism *S. aureus* is clearly inhibited by honeys of different floral origins. As well as wound infections, *S. aureus* is an important cause of boils, furuncles, styes and impetigo. Honeys have broad spectrum anti-microbial properties and it may be that

honey has therapeutic value in the treatment of other skin disorders in which microbes have been associated with the aetiology of the disease, as well as those disorders that are commonly treated with topical antibiotics e.g. acne. Analysis of the antimicrobial activity of different types of honey against other dermatologically relevant microbes should be encouraged.

Table 1. *Activity of some honeys from around the world against common skin relevant microbes.*

Type of Honey	**MRSA**	***S. aureus***	***P. aeruginosa***	***E. coli***	***C. albicans***	***Dermatophytes***	***Malassezia species***	**HPV**
Manuka Honey [1]	+ (12)	+ (11)	+ (11,12)	+ (11,12)	- (14,15)	+ (13)	☨	☨
Scottish Heather Honey [2]	+ (23)	+ (23)	+ (23)	+ (23)	☨	☨	☨	☨
Portobello Honey [2]	☨	+ (22)	+ (22)	+ (22)	☨	☨	☨	☨
Tualang Honey [3]	+ (11)	+ (11, 27)	+ (11, 27)	+ (11, 27)	☨	☨	☨	☨

Key: + active, - not active or low activity, ☨ unknown. 1 New Zealand, 2 Scotland, 3 Malaysia. (Numbers in brackets are references)

ANTIMICROBIAL PROPERTIES OF HONEY: *IN VIVO* HUMAN STUDIES

The majority of studies to date have demonstrated the antimicrobial activity of honey against a range of microbial strains including clinical isolates, using *in vitro* antimicrobial assays. Fewer studies have demonstrated the antimicrobial activity of honey *in vivo*; those studies that have been carried out have mainly investigated the antimicrobial activity of honey in relation to wound infections. In the first decade of the 21st Century, several case studies involving wound patients produced optimistic findings. A brief report by Cooper et al (2001) described how treatment of a *S. aureus* infected, recalcitrant surgical wound in a 38 year old female with Manuka honey impregnated dressings and oral co-amoxiclav resulted in significant healing of the wound and bacterial clearance 7 days after commencing treatment [28]. The wound was 3 years old and had failed to respond to other conventional wound treatments and antibiotics during the 3 year period prior to commencing the honey/antibiotic combination therapy. Natarajan et al (2001) treated an MRSA infected leg ulcer of an immunosuppressed patient with topical application of Manuka honey, consequently, MRSA was eradicated and the wound successfully healed [29]. Chambers (2006) reported bacterial

clearance in three cases of MRSA infected leg ulcers following treatment with topical Manuka honey whilst Visavadia et al, (2008) reported that Manuka honey, based on clinical experience, was now one of their first line treatments for infected wounds at the Maxillofacial Unit at Royal Surrey County Hospital [30, 31].

Larger clinical studies have produced more controversial findings. Gethin and Cowman (2008) recruited 108 patients with venous leg ulcers and treated them with either Manuka honey or hydrogel [32]. In their study, Manuka honey successfully eliminated MRSA from 70% of MRSA infected wounds, in comparison hydrogel eradicated MRSA in only 16% of infected wounds. For *P. aeruginosa* infected wounds, Manuka honey cleared infection in just 33% of wounds and hydrogel cleared infection in 50% of wounds. Jull et al (2008) in a randomised clinical trial of 368 participants reported no significant difference in occurrence of infection in venous leg ulcers treated with either Manuka honey impregnated dressings or usual care [33]. Another clinical study showed no significant difference, in terms of development of peritoneal dialysis related infections when patients undergoing peritoneal dialysis were treated with either Medihoney antibacterial wound gel (containing honey from Leptospermum species) or the topical antibiotic Mupirocin applied to catheter exit sites [34].

ANTIMICROBIAL PROPERTIES OF HONEY: *IN VIVO* ANIMAL STUDIES

Antimicrobial effects of honey have been observed in animal studies *in vivo*. Al-Waili (2004) reported that application of a natural honey from the UAE to *S. aureus* or *Klebsiella* species inoculated surgical wounds induced in mice, reduced the time for bacterial elimination to occur [35]. Khoo et al (2010) reported that Tualang honey was superior to hydrofibre and hydrofibre silver dressing in reducing the growth of bacteria in *P. aeruginosa* inoculated burn wounds induced in *Sprague Dawley* rats [36]. Conversely, hydrofibre and hydrofibre silver dressings were superior to Tualang honey in reducing bacterial growth in *A. baumannii* inoculated wounds, while, there was no significant difference between the three treatments in inhibiting the growth of bacteria in *Klebsiella pneumonia* inoculated wounds. Gunaldi et al, (2013) investigated the antimicrobial activity of Manuka honey in clearing MRSA infection in MRSA inoculated spinal implants inserted in rats [37]. The results showed that whilst Manuka honey significantly reduced MRSA growth on the implants it did not eradicate the MRSA entirely. In the vertebral column of the rats, MRSA growth was also reduced more in the Manuka honey treated group compared to the control group but this was not statistically significant.

It could be said that the research findings for the antimicrobial activity of honey *in vivo* have not been as 'outstanding' as those observed *in vitro* and the reasons for this require investigation. Human and animal cells are known to contain the enzyme catalase, an enzyme that breaks down hydrogen peroxide (an important antimicrobial component of some honeys) into hydrogen and oxygen. If the anti-microbial properties of honey are due to hydrogen peroxide it may be that the anti-microbial activity is reduced when honey comes into contact with live cells [38]. Innovative research that can overcome obstacles associated with *in vivo* use of honey is urgently required.

It is also important to consider that some honeys have been shown to be contaminated with bacteria and fungi and therefore non-γ-irradiated honeys may not be suitable for application on damaged skin [23]. The production of local honeys into medical grade honeys suitable for use in clinical practice would be economically advantageous and beneficial to local communities.

THE EFFECTS OF HONEY ON MICROBIAL PATHOGENICITY OF SKIN RELEVANT MICROBES: *IN VITRO* STUDIES

Incredibly, recent research has shown that the antimicrobial properties of honey *in vitro* are more than bactericidal because honey has also been shown to reduce bacterial pathogenicity. The ability of pathogenic microbes to cause disease is partly caused by the production of pathogenicity factors. *S. aureus*, for example, produces a range of disease causing proteins including; catalase, haemolysin (α, β, γ and δ), epidermolytic toxins and enterotoxins. Alpha-toxin (α-haemolysin) causes tissue damage during wound infections by creating pores in host cell membranes; allowing the discharge of low molecular weight compounds and by inducing cytokine production and apoptosis.

Recently, Jenkins et al (2014) reported that Manuka honey reduced expression of α-toxin in MRSA [39]. Expression of other virulence genes, quorum sensing genes and genes associated with cell division were also reduced. Lee at al (2011) reported that three types of honey (Korean Acacia, Korean Polyfloral and American Clover honey) at concentrations as low as 0.5% significantly inhibited pathogenic *E. coli* O157:HA biofilm formation *in vitro* [40]. Furthermore, low concentrations of the Korean acacia honey reduced the expression of curli genes (csgBAC), quorum sensing genes (AI-2 importer, indole biosynthesis) and virulence genes (LEE genes) in the bacterial strain. Kronda et al (2013) reported that sublethal concentrations of Manuka honey reduced siderophore production, a virulence factor that scavenges iron for bacterial growth, in clinical and non-clinical strains of *P. aeruginosa* [41]. Manuka honey has also been shown to alter the structure of *P. aeruginosa*; scanning and transmission electron microscopy revealed changes in cell shape and cell lysis following incubation with honey [42]. A honey flavonoid extract was also found to alter membrane integrity and branching processes associated with virulence in *C. albicans* [43].

As well as the more commonly investigated wound pathogens, sub-inhibitory concentrations of Manuka Honey and Slovakian honeys (Hawthorn, Honeydew and Acacia) significantly inhibited *Proteus mirabilis* and *Enterobacter cloacae* biofilm formation *in vitro* [44].

In vivo studies investigating the efficacy of sub-lethal concentrations of honeys against biofilms would advance our knowledge of the ability of honey to modulate bacterial pathogenicity.

ANTIMICROBIAL MODE OF ACTION OF HONEY

The anti-microbial properties of honey have been attributed to multiple components including high sugar concentration, low pH, hydrogen peroxide (H_2O_2), methylglyoxal (MGO), anti-

microbial peptide bee defensin-1 and other compounds such as polyphenols that have not been fully elucidated.

The high sugar concentration and low moisture content of honey causes osmotic stress to microbial cells and low pH is unfavourable for the growth of many micro-organisms. However, if a sugar solution with identical sugar components and pH to that of honey is prepared the antimicrobial activity of the sugar solution is often considerably lower than that of honey suggesting that other factors in the honey are responsible for its antimicrobial activity [23].

Honey bees add an enzyme called glucose oxidase to the collected nectar during the honey making process which converts the glucose in the honey into hydrogen peroxide (H_2O_2) and gluconic acid. H_2O_2 is toxic to many microbes. During the ripening of honey glucose oxidase is inactivated but regains its activity if the honey is diluted. In a study by Kwakman et al (2011) it was found that Revamil honey produced 3.47± 0.25 mM H_2O_2 in 40% (v/v) honey after 24 hours but no H_2O_2 was detectable in the Manuka honey they tested suggesting that non-peroxide factors are responsible for the antimicrobial activity of Manuka honey [10].

Manuka honey has been shown to contain high levels of methylglyoxal (MGO); 44 fold higher than Revamil. MGO in Manuka honey is produced by the non-enzymatic conversion of dihydroxyacetone (DHA) present at high concentrations in the nectar of *L. scoparium* flowers. The change occurs slowly during honey storage. Kwakman et al (2011) reported that neutralisation of MGO in Manuka honey abolished the antimicrobial activity of the honey against *S. aureus* but did not abolish the antimicrobial activity against *E. coli* and *P. aeruginosa* [10]. The authors concluded that MGO is not fully responsible for Manuka honeys non peroxide antimicrobial activity and that other components possibly polyphenols may be responsible.

Polyphenols derived from plant nectar are natural organic chemicals characterised by the presence of multiple phenol structural units. Many are antioxidants e.g. flavonoids. The antibacterial properties of flavonoids have been attributed to inhibition of bacterial energy metabolism, bacterial DNA gyrase and cytoplasmic membrane function [45]. Researchers in New Zealand identified the polyphenol methyl syringate as the major component of the phenolic fraction of Manuka Honey [46]. A novel glycoside of methyl syringate, named leptosin, was recently identified in Manuka honey and levels were found to correlate positively with the UMF [47]. Identification of phenolic compounds in honey may be important for the production of new antimicrobials and therefore the analysis of the phenolic profile of active honeys should be encouraged. Combinations of polyphenols may be more effective as they may act synergistically to inhibit microbial growth or structural alteration of individual polyphenols could be employed to enhance antimicrobial activity.

Bee defensin-1 is an anti-microbial peptide that is part of the honey bee innate immune system. It is secreted by the honey bee hypopharyngeal gland and can enter honey via bee saliva during the regurgitation process of honey making. Bee defensin-1 has strong activity

against gram positive bacteria including *S. aureus*. Kwakman and Zaat (2010) identified Bee defensin-1 in Revamil honey but not Manuka honey [48].

Raw honey may also contain propolis; a substance composed of plant resins and used by bees to seal the hive. Scientific research has shown that propolis has antimicrobial properties [49].

The research of Kwakman et al demonstrates the diversity and complexity of the antimicrobial components of different types of honey. Analysis of the antimicrobial components of other active honeys will be important for a fuller understanding of their applicability to medicine.

CONCLUSIONS

It can be concluded from *in vitro* studies that honey has powerful antimicrobial activity against dermatologically relevant microbes. These findings are particularly promising in current times when the problem of antimicrobial drug resistance is considered a global crisis and the World Health Organisation (2014) has acknowledged the possibility of a post antibiotic era where common infections kill. Even more exciting are the *in vitro* findings that honey can reverse antimicrobial resistance and reduce microbial pathogenicity. Despite these optimistic findings *in vitro*, the use of honey in clinical practice today as an antimicrobial agent does not appear to have yet reached its potential. Innovative research that can maximally exploit the antimicrobial properties of this natural substance and overcome obstacles associated with *in vivo* use may in the future lead to the production of an antimicrobial agent that is highly valued in clinical practice. Interestingly, no honey resistant microbial strains have emerged to date and this may be unlikely because of the multifactorial nature of the antimicrobial properties of honey. As honeys from diverse floral origins have been shown to have antimicrobial activity against a range of skin relevant microbes research should continue to investigate the efficacy of honey in the treatment of other types of skin disorders where microbes have been implicated in the pathophysiology of the disease. There are countless varieties of honey being produced worldwide and some may have superior anti-microbial activities that are yet to be discovered.

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