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Background

Honey has been used as a traditional folk medicine since ancient times, both externally on wounds to the epidermis and internally for respiratory and gastrointestinal tract infections (Mandal et al., 2010; Lusby et al., 2005; Kwakman et al., 2010; Ayaad et al., 2010). Honey has a proven anti-microbial effect, and due to an increasing level of bacterial resistance to antibiotics, new research is looking into the clinical application of honey as an alternative to conventional antibiotics. Most research analyses honey for application as a wound dressing, as it has several mechanisms that aid in the healing process other than an anti-bacterial activity. For example honey maintains a moist wound environment for healing, whilst offering a protective barrier via its high osmolarity; in addition the mild acidity and hydrogen peroxide release support tissue repair (Mandal et al., 2010; Lusby et al., 2005).

Whilst honey has proven efficient as a bactericidal and bacteriostatic agent in vitro and in case studies, the mechanism by which honey exerts this activity, against a broad spectrum of organisms, is still under debate. The high osmolarity and hydrogen peroxide content are often cited as likely contributors; however, when a sugar solution is made to the same osmolarity as the honey in question, it has a notably smaller effect on bacteria. In addition some honeys have been found to have a non-peroxide affect, the most well-known case being Manuka honey (Kwakman et al., 2010; Snow and Manley-Harris, 2004). Other components of interest are phytochemicals, pH, methyglyoxal (MGO) and bee defensin-1(Kwakman et al., 2010).

As honey is a natural product, its content is highly variable and will change with floral source, location and bee species, therefore it is difficult to standardise honeys and assess their usefulness in a medical setting. Honey can be used clinically if it is a medical grade product, i.e. a sterilised product that is licensed for use (Molan, 2006). Two medical grade honeys are Revamil source (RS) honey, and Manuka, i.e. Medihoney (Majtan, 2011); RS honey is produced under controlled conditions in greenhouses (thus having a reproducible anti-bacterial activity), and Manuka is assessed for a Unique Manuka Factor (UMF), in which the batch is given a number based on its bactericidal activity (Kwakman et al., 2010; Kwakman et al., 2011). The use of honey in a medical setting would not only be helpful in combating bacterial

resistance (there is no evidence to suggest bacteria develop resistance to honey), but aid the treatment of infections in developing countries, as honey is cheap, easily available and may avert the need for other more expensive medical treatments. Moghazy et al. (2010) also suggested after their in vivo experiment in Egypt that due to its long standing use as a remedy in certain countries, it may have a psychological benefit over other treatments.

Components of interest in honey
Hydrogen peroxide (H_2O_2) is one of the main elements of honey considered when looking into antibacterial activity. Its production is the result of the enzyme glucose oxidase produced by bees; this enzyme uses glucose and oxygen to create gluconic acid and H_2O_2, and is activated when honey is diluted (Tonks et al., 2001). Glucose oxidase is affected by heat and light and is inhibited by catalase. Catalase can be present in flower pollen and in the tissues of the body (Mandal and Mandal, 2011). Sherlock et al. (2010) have speculated that the presence of catalase in wound tissue may have an inhibitory effect on hydrogen peroxide production by honey, making non-peroxide activity an important factor in a clinical setting. Some honeys exhibit activity in the presence of catalase that cannot be explained solely by their high sugar content. Many honeys are kept in the dark and at room temperature prior to experimentation to prevent the degradation of glucose oxidase and the loss of hydrogen peroxide (Sherlock et al., 2010).

High osmolarity is also considered an important factor as it inhibits bacterial growth by drawing moisture from the environment and dehydrating the micro-organisms (Mandal and Mandal, 2011). This means that an artificial honey made to the same concentration of sugar (~80% w/v [Kwakman et al., 2010]) as real honey does exhibit some antibacterial activity, but this is often lower than that of the honeys it is being compared to.

pH is a likely factor in undiluted honey, as most honeys are naturally acidic (pH3.2-4.5) and this will inhibit the activity of many micro-organisms (Mandal and Mandal, 2011). When honey is diluted this pH changes and may become more neutral, but activity is still observed, so it is not thought to be a central factor.

Methylglyoxal (MGO) is a protein-glycating agent and has been found in medical honeys and is thought to be responsible for the non-peroxide activity observed in some honeys (Badet and Quero, 2011; Majtan, 2011). Its activity in diabetic ulcers has been questioned on the grounds of safety issues, due to the production of advanced glycation end products; which cause health complications in diabetes (Majtan, 2011).

Bee Defensin-1 was found in Revamil honey by Kwakman et al., (2010) and was shown to contribute towards the anti-bacterial activity against some bacterial species.

Propolis is a resinous structural component of the hive composed of floral elements, such as tree sap. It can have anti-microbial activity which some honeys may be exposed to it (Boorn et al., 2009).

The honey's micro flora has been implied in the activity of honey; Lee et al., (2008) tested bacterial isolates from American honeys and manuka to determine if they produced anti-microbial agents, and found that a majority did, with one of the manuka samples having the highest isolate activity.

Kwakman et al., (2010) gradually removed/inhibited the MGO, H_2O_2, bee defensin-1and altered pH to see what was responsible for the broad spectrum antibacterial activity, in Revamil. They found that different bacteria were sensitive to different components, e.g. the neutralisation of MGO alone reduced the activity against *Escherichia coli* and *Pseudomonas aeruginosa,* and the neutralisation of H_2O_2 reduced activity against all tested organisms accept *Bacillus subtilis.* When MGO, H_2O_2, and Bee defensin-1 activity were removed, and the pH was adjusted to pH7, the honey had the same activity as the artificial honey. The concentration of each of these components and any other factors involved will vary with each honey due to different floral sources, bee colonies, season, etc. (Sherlock et al., 2010)

Types of honey
Manuka is perhaps the most well characterised honey in anti-microbial exeriments, with many papers using it as a postive control based on its proven activity. Produced in New Zealand, Manuka honey is formed from nectar of the manuka bush (*Leptopsermum scoparium*) and is well known for its non-peroxide effect, as well as its anti-microbial potency. A UMF is given to each batch of Manuka honey to rate its anti-bacterial nature. This number is based on the comparison of the honey's activity, in a standard laboratory test, against that of phenol (a potent anti-septic); based on this a UMF of 10 would be equivalent to 10% phenol activity (Badet and Quero, 2011). Weston (2000) suggested that the experiments which had shown non-peroxide activity in manuka had not used sufficient catalase to destroy its activity, as it is continuously created during the testing process and is the only anti-bacterial compound of significance in honey. Snow and Manley-Harris (2003) tested this theory by performing a well diffusion assay with a 10-fold excess of catalase, and disovered that the activity of the honey treated with catalase was equal to that of un-treated manuka, strongly suggesting that manuka does it indeed act via a hydrogen peroxide independent mechanism. Other studies have tried to determine what then is responsible for manuka's activity and the question still remains open, though several options have been put forward. Bogdanov (1997) discovered when filtering the honey on an anion exchange column at pH 11 that all activity was irreversibly lost, and therefore concluded the activity was in the acidic fraction. Snow and Manley-Harris (2003) also tested this by altering the honey's pH (without chromatography) and found that at pH 9 activity became un-stable, and at pH11 the activity was lost immediatly and did not return when the pH was adjusted back to pH 7. Methylglyoxal (MGO) is derived from dihydroxyacetone, which can be found at high levels in nectar from the Manuka bush (Irish et al., 2011). MGO has been found at high levels in Manuka honey and is thought to be the cause of its non-peroxide activity; anti-bacterial activty is reduced in RS honey when MGO is neutralised (Kwakman et al., 2010), showing that MGO does have anti-bacterial activity. The non-peroxide activity of Manuka is important clinically,firstly because it is not affected by the body's own catalase, but also because Manuka can be sterilised by γ-irraditation without losing its activity (Irish et al., 2011).

Ulmo honey is produced in Chile and was investigated by Sherlock et al. (2010) to determine if its activity was comparable to the of Manuka honey; this was done on the grounds that local honeys should be assessed, as there are more easily available. Manuka UMF25+ was used in comparison to Ulmo 90, with the Ulmo tree (*Eucryphia cordifolia*) being the source of nectar for 90% of the Ulmo honey. The test bacteria used were *E.coli, P.aeruginosa* and 5 strains of *methicillin-resistant Staphylococcus aureus* (MRSA). Using agar well diffusion and two-fold dilutions from

50% down to 0.02%, the minimum inhibitory concentration (MIC) for each bacteria/honey combination was assessed. The results showed that Ulmo had results that were indistinguishable from Manuka, for *E.coli* and *P.aeruginosa*, however MRSA was found to be more (or equally) sensitive to Ulmo 90 than Manuka; with the Ulmo 90 MIC being 3.1-6.3% v/v compared to Manuka's 12.5%, while artificial honey had a 50% MIC for all bacteria. However, when catalase treatment was used, Ulmo 90 lost its activity at 25%, but Manuka maintained its activity. They therefore concluded that Ulmo 90 has peroxide dependent anti-bacterial activity that is greater than Manuka against 5 strains of MRSA. They do however point out that catalase may be present in the wound environment, as it is endogenous to tissue; also the bacterial composition of a wound is different to that seen in tests on planktonic bacteria, as the wound will contain a biofilm of several bacteria which may affect the ability of honey to kill or inhibit the organisms present.

Tualang Honey is local to Malaysia and its floral origin isn't yet known; its antibacterial activity was tested by Tze Tan et al. (2009) against wound and enteric micro-organisms (5 Gram-positive bacteria and 8 Gram-negative). Again Manuka was used as a comparison, this time with a UMF10+. Both honeys were γ-irradiated to kill any micro-organisms present in the honey; this treatment has been shown not to affect the activities of these honeys. MIC was determined by broth dilution and measuring optical density at 620nm, the non-peroxide activity however was determined via agar well diffusion. The MIC values for both honeys was between 10-25% on the 13 bacteria tested, putting Tualang's activity in the same range as that of Manuka's; Tualang also maintained its activity after catalase treatment, suggesting it has non-peroxide activity. Tze Tan et al. (2009) concluded that although Tualang activity was variable amongst the 13 bacteria, its potency against some of the micro-organisms gives it potential as an alternative to antibiotics.

Revamil (RS) honey is a medical grade honey created under control conditions in green houses. Kwakman et al. (2010) found that its broad spectrum activity is down to hydrogen peroxide, MGO and bee defensin-1. When comparing the time and dilutions needed to kill bacteria, it was found that RS honey killed most organisms in a more rapid manner than Manuka, but was comparatively less effective at lower concentrations (Kwakman et al., 2011).

Types of Bee
Boorn et al. (2009) observed that a majority of research was done on honey produced by the bee *Apis mellifera,* and therefore examined the differences between these honeys and honey produced by the stingless bee *Trigona carbonaria* from Australia. 11 stingless bee honey samples were used, with a combination of plants as the floral source; comparison honeys consisted of medical, table and artificial honey. Boorn et al. (2009) used several methods to determine the anti-microbial activity, as conflicting results have been observed in the past relating to this bee. Large differences were found between the 11 samples, but the honey did exhibit broad spectrum anti-bacterial activity comparable to medical grade honey, with the time-kill assay suggesting it had quicker activity; it was however ineffective at killing fungi. There are many differences between stingless bee honey and *Apis*; one of interest is the different storage methods within the hive. Stingless bee honey is kept in pots made of propolis and wax rather than combs made solely of wax, giving the honey a greater chance of being imbued with plant-derived anti-microbial agents. Irish et al. (2011) also suggested that different bee *colonies* could affect the activity

of the honey, as when testing floral sources for effects on activity, they found difference between honeys from the same floral source but different hives could vary widely in their activity against *S.aureus*. This could perhaps explain the differences between Manuka honey's as they can vary in potency from 10-25+ UMF.

Different floral sources and other factors that may influence activity
The differences between honeys are usually attributed to their different floral sources. Irish et al. (2011) sought to determine the activity of different honeys from various Australian floral sources by using an agar well diffusion method against *S.aureus*. 477 samples were collected and 57% showed activity greater than or equal to 10% phenol; 80 of these 477 samples showed non-peroxide activity and *Leptospermum* spp was the main or sole source of 77.5% of these honeys. However Australian *L. scoparium* (the Manuka bush) samples did not show non-peroxide activity, as it does in New Zealand; suggesting that other, as yet unidentified factors (such as environmental factors) contribute to the non-peroxide activity of New Zealand Manuka honey.

Lee et al. (2008) compared the antimicrobial activity of American honeys and Manuka; they found that American honeys exhibited lower activity. The majority of samples contained hydrogen peroxide and some were affected by proteolytic enzymes (Manuka was not), suggesting that active proteins and peptides play a part in their activity. Lee et al. (2008) also found bacterial isolates, in the American and Manuka honey, which exhibited antimicrobial properties that contribute to the activity observed.

The storage conditions and age of the honey samples were also considered in the Irish et al. (2011) study. While the age of the honeys (8 and 22 months) did not correlate with activity, storage at 4°C and 25°C resulted in loss of peroxide activity, with a greater loss seen at 25°C; an optimal storage temperature was not suggested. Temperature was also found to affect the activity of honey in Mulu et al.'s (2004) study, in which honey produced by *Apis mellifera* was used. They found that although honey was unaffected by storage at -10°C, the minimal bactericidal concentration (MBC) decreased by 1.2% following autoclaving (121°C) for 15 minutes. They concluded that autoclaving would have stopped the activity of the heat-liable phytochemicals, as well as enzymes such as glucose oxidase; this has implications in the results of other studies that have used autoclaving as a means of sterilisation, such as Mandal et al. (2010) who studied honey from west Bengal.

Clinical studies on Honey's activity in wound treatment
There have been many randomised and clinical trials on the use of honey in the treatment of wounds, with several producing favourable results; however many are criticised as being of low-quality due to lack of double-blinding (Moore et al., 2001; Molan, 2006). Molan (2006) argues however, that blinding is next to impossible to do, due to the very recognisable look and aroma of honey; however there is evidence to suggest the activity is not a result of the placebo effect. For example, multiple wound patients have been tested with honey and a comparative treatment simultaneously, and honey was shown to be significantly better; animal studies also show a positive result for honey without the influence of psychological belief (Molan, 2006).

The majority of evidence that supports the use of honey is where honey dressings were applied to partial thickness or mild to moderate superficial burns, while

evidence for benefits on ulcers has been contradictory (Majtan, 2011). Hyperosmolar sugar paste has proven to be effective in animal experiments, and to be superior to antiseptics; it explains some of the benefits seen when honey is used (Moore et al., 2001). When honey is compared to other treatments, Moore et al. (2001) found that of 7 randomised trials examined, 6 reported honey as being superior to other treatments (both conventional and alternative). But it was concluded that the evidence was of such low quality that caution should be taken when interpreting the results. Molan (2006) on the other hand, advises clinicians to consider the wide range of evidence supporting the use of honey; as many modern wound dressings have equally low quality evidence, and there may not be as many studies e.g. nanocrystalline silver dressing. When Molan (2006) investigated trials using honey, he found 17 randomised and 5 clinical trials (totalling more than 2060 participants) reported positive results when honey was used as a wound dressing.

There is also a vast amount of in vitro evidence supporting both honey's anti-bacterial activity (see *Table 1*) and its immunomodulatory roles in promoting healing.

Table 1: Summaries from a sample of studies that have found honey to have an antimicrobial activity in vitro. Minimum inhibitory concentration (MIC), Minimum bactericidal concentration (MBC).

Authors	Honey/s	Micro-organisms	Method	Results/conclusions
Cursons, 2010	Manuka (equivalent to 16.5% phenol)	Gram negative bacteria	Time-kill assay, with viable count measured hourly. Concentrations from 20% down.	Manuka was better than artificial honey and acted in a bactericidal manner. 20% killed most of the organisms between 2-6 hours after onset. Most organisms were inhibited at concentration <8% v/v. Concluded that different organisms have different susceptibility to the effects of Manuka.
Lusby et al., 2005	Local honeys (lavender, red stringy bark and Paterson's curse) vs. Commercial honeys (Manuka, Rewa rewa and Medihoney).	Used 13 bacterial organisms (gram positive and gram negative) and 1yeast.	Agar dilution assay; concentrations of 0.1%, 1%, 5%, 10% and 20% w/v.	The amount of inhibition observed increased with concentration, with most showing 75% inhibition at 20%. The local honeys were equivalent in activity to Manuka against some bacteria.

Badet and Quero, 2011	Manuka UMF 30+ and UMF 16+	Oral bacteria	Macro broth dilution for MICs and MBCs.	UMF 30+ always had better activity than UMF 16+. The MBC was always at least two times the MIC. Both UMF's inhibited adherence at levels below their MIC. Concluded that the anti-cariogenic effect of Manuka may counteract its high sugar content in oral health.
Sherlock et al., 2010	Ulmo 90 and Manuka UMF 25+	5 clinical isolates of *MRSA, E.coli* and *P.aeruginosa*.	Agar well diffusion assay using two-fold dilutions beginning at 50%. Used a spectrophotometer to assess MIC. Catalase used.	Ulmo and Manuka had similar effects on *E.coli* and *P.aeruginosa*. However some of the *MRSA* isolates were more sensitive to Ulmo. Catalase effected Ulmo activity and not Manuka.
Mandal et al., 2010	Honey from west Bengal.	*E.coli, P.aeruginosa* and *Samonella enterica* serotype *Typhi*.	Agar dilution assay; 0.25%-4% v/v concentrations. Honey was autoclaved.	MIC against: *P.aeruginosa* 3.5% v/v *E.coli* 3-3.5% v/v *S.enterica* 1.75-3% v/v
Boorn et al., 2009	Stingless bee honey (Australia), Medical, artificial and table honey.	Gram positive and gram negative bacteria, and fungi.	Agar dilution assay, broth microdilution assay, agar diffusion assay and time-kill assay.	Agar dilution MIC: 4->10% w/v for gram positive 6- >16% w/v for gram negative. 6- >10% w/v for fungi. Concluded that stingless bee honey has a similar activity to medical honey.

Tonks et al. (2001) studied the effects of Manuka and pasture honey (alongside an artificial control) on monocytes (Mono Mac 6 cells –MM6) and found that honey had both inhibitory and stimulatory activity on these cells. Reactive oxygen intermediates' (ROIs) production was inhibited by both honeys, but more so by pasture honey, and it is suggested this is due to its higher hydrogen peroxide content. TNF-α production was stimulated in resting monocytes, but not in the primed ones; TNF-α is known to be beneficial in healing processes, and ROIs are part of macrophage mediated

damage, thus these two activities may be behind the healing properties associated with honey.

Internal use
Honey is a traditional remedy for sore throats, but there is very little evidence investigating the effects of honey in vivo on upper respiratory tract (URT) pathogens, and no *in vitro* studies were found that selected micro-organisms based on their ability to infect the URT, although some studies contained these kinds of pathogens.

Two clinical studies which look at honey's abilities when taken internally are: firstly, Paul et al. (2007), who investigated the effect of honey on nocturnal coughing and sleep quality in children. They found that honey was significantly better than no treatment, but not significantly different from Dextromethorphan treatment, for the reduction in cough frequency and improved sleep. However, Oduwole at al. (2010) concluded, when they reviewed data on whether honey could be used to treat coughs in children, that there was inadequate evidence to support the use of honey; but neither was there evidence to suggest it shouldn't be used. Secondly, a study in Iran on the effect of honey on the common cold (Pourahmad and Sobhanian, 2008) found that out of the two groups, those receiving honey as well as conventional treatment had a smaller duration of signs and symptoms (1-2 days less) than those only receiving conventional treatment. They concluded that this is evidence that honey should be considered in more depth for the treatment of colds.

Conclusion
Honey is increasingly becoming a substance of interest, not only due to its sometimes potent activity against bacteria *in vitro;* but also due to promising results *in vivo*, where it also seems to promote healing, as well as sanitising the wound. Although there is still some question over its use, due to the low quality of the evidence produced by trials, because of the difficulty of blinding. However considering the increased rate of bacterial resistance being seen to antibiotics, and the expense of these drugs to non-industrial countries, it is worthy of consideration, as it addresses these problems. It is however clear that more clinical trials should be done using those honeys that having proven potent *in vitro* as a medical dressing, and on different wounds. As many honeys have shown activity against *MRSA* (e.g. Manuka and Ulmo), it would be interesting to see studies done using them as a dressing on *MRSA* abscesses in hospitals. Alongside this, other untested honeys should be assessed *in vitro* to determine if they have equivalent or better activity than those that have proven activity (i.e. Manuka).

Further studies should also be considered on the effects of ingested honey on infections of the URT and gastrointestinal tract.

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