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Honey: its medicinal property and antibacterial activity

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ABSTRACT

Indeed, medicinal importance of honey has been documented in the world´s oldest medical literatures, and since the ancient times, it has been known to possess antimicrobial property as well as wound-healing activity. The healing property of honey is due to the fact that it offers antibacterial activity, maintains a moist wound condition, and its high viscosity helps to provide a protective barrier to prevent infection. Its immunomodulatory property is relevant to wound repair too. The antimicrobial activity in most honeys is due to the enzymatic production of hydrogen peroxide. However, another kind of honey, called non-peroxide honey (*viz.*, manuka honey), displays significant antibacterial effects even when the hydrogen peroxide activity is blocked. Its mechanism may be related to the low pH level of honey and its high sugar content (high osmolarity) that is enough to hinder the growth of microbes. The medical grade honeys have potent *in vitro* bactericidal activity against antibiotic-resistant bacteria causing several life-threatening infections to humans. But, there is a large variation in the antimicrobial activity of some natural honeys, which is due to spatial and temporal variation in sources of nectar. Thus, identification and characterization of the active principle(s) may provide valuable information on the quality and possible therapeutic potential of honeys (against several health disorders of humans), and hence we discussed the medicinal property of honeys with emphasis on their antibacterial activities.

1. Introduction

Antimicrobial agents are essentially important in reducing the global burden of infectious diseases. However, as resistant pathogens develop and spread, the effectiveness of the antibiotics is diminished. This type of bacterial resistance to the antimicrobial agents poses a very serious threat to public health, and for all kinds of antibiotics, including the major last-resort drugs, the frequencies of resistance are increasing worldwide[1,2]. Therefore, alternative antimicrobial strategies are urgently needed, and thus this situation has led to a re-evaluation of the therapeutic use of ancient remedies, such as plants and plant-based products, including honey[3-5].

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The use of traditional medicine to treat infection has been practiced since the origin of mankind, and honey produced by *Apis mellifera* (*A. mellifera*) is one of the oldest traditional medicines considered to be important in the treatment of several human ailments. Currently, many researchers have reported the antibacterial activity of honey and found that natural unheated honey has some broad-spectrum antibacterial activity when tested against pathogenic bacteria, oral bacteria as well as food spoilage bacteria[6,7]. In most ancient cultures honey has been used for both nutritional and medical purposes. The belief that honey is a nutrient, a drug and an ointment has been carried into our days, and thus, an alternative medicine branch, called apitherapy, has been developed in recent years, offering treatments based on honey and other bee products against many diseases including bacterial infections. At present a number of honeys are sold with standardized levels of antibacterial activity. The *Leptospermum scoparium* (*L. scoparium*) honey,the best known of the honeys, has been reported to have an inhibitory effect on around 60 species of bacteria, including aerobes and anaerobes, grampositives and gram-negatives[8]. Tan *et al*[9] reported that Tualang honey has variable but broad-spectrum activities against many different kinds of wound and enteric bacteria. Unlike glucose oxidase, the antibacterial properties from *Leptospermum* spp. honeys are light- and heat-stable. Natural honey of other sources can vary as much as 100-fold in the potency of their antibacterial activities, which is due to hydrogen peroxide[6,10]. In addition, honey is hygroscopic, which means that it can draw moisture out of the environment and dehydrate bacteria, and its high sugar content and low level pH can also prevent the microbes from growth.

Based upon the extensive searches in several biomedical science journals and web-based reports, we discussed the updated facts and phenomena related to the medicinal property of honeys with emphasis on their antibacterial activities in this review.

2. Medicinal property

Honey is an ancient remedy for the treatment of infected wounds, which has recently been 'rediscovered' by the medical profession, particularly where conventional modern therapeutic agents fail. The first written reference to honey, a Sumerian tablet writing, dating back to 2100-2000 BC, mentions honey's use as a drug and an ointment. Aristotle (384-322 BC), when discussing different honeys, referred to pale honey as being "good as a salve for sore eyes and wounds". Manuka honey has been reported to exhibit antimicrobial activity against pathogenic bacteria such as *Staphylococcus aureus* (*S. aureus*) and *Helicobacter pylori* (*H. pylori*) making this honey a promising functional food for the treatment of wounds or stomach ulcers[10].

The honey has been used from ancient times as a method of accelerating wound healing[11], and the potential of honey to assist with wound healing has been demonstrated repeatedly[12,13]. Honey is gaining acceptance as an agent for the treatment of ulcers , bed sores and other skin infections resulting from burns and wounds[14,15]. The healing properties of honey can be ascribed to the fact that it offers antibacterial activity, maintains a moist wound environment that promotes healing, and has a high viscosity which helps to provide a protective barrier to prevent infection[6]. There are many reports of honey being very effective as dressing of wounds, burns, skin ulcers and inflammations; the antibacterial properties of honey speed up the growth of new tissue to heal the wound[16]. The medihoney and manuka honey have been shown to have *in vivo* activity and are suitable for the treatment of ulcers, infected wounds and burns[6,17].

The honey, when applied topically, rapidly clears wound infection to facilitate healing of deep surgical wounds with infection[18]. The application of honey can promote the healing in infected wounds that do not respond to the conventional therapy, *i.e.*, antibiotics and antiseptics[18], including wounds infected with methicillin-resistant *S. aureus*[19,20]. Moreover, it can be used on skin grafts and infected skin graft donor sites successfully[21].

The manuka, jelly bush and pasture honeys are capable of stimulating the monocytes, the precursors of macrophages, to secrete TNF-α [22,23]. On the other hand, glycosylated proteins can induce TNF-α secretion by macrophages, and this cytokine is known to induce the mechanism of wound repairing.Furthermore, the ability of honey to reduce 'reactive intermediates release'[23] may well limit tissue damage by activated macrophages during wound healing. Thus, the immunomodulatory property of honey is relevant to wound repair.

The support for using honey as a treatment regimen for peptic ulcers and gastritis comes from traditional folklore as well as from reports in modern times[24]. Honey may promote the repair of damaged intestinal mucosa, stimulate the growth of new tissues and work as an anti-inflammatory agent[24,25]. Raw honey contains copious amounts of compounds such as flavonoids and other polyphenols which may function as antioxidants[26]. Clinical observations have been reported of reduced symptoms of inflammation when honey is applied to wounds. The removal of exudate in wounds dressed with honey is of help in managing inflamed wounds[18].

3. Antibacterial activity

3.1. Potential antibacterial agent

The use of honey as a traditional remedy for microbial infections dates back to ancient times[8]. Research has been conducted on manuka (*L. scoparium*) honey[27], which has been demonstrated to be effective against several human pathogens, including *Escherichia coli* (*E. coli*), *Enterobacter aerogenes*, *Salmonella typhimurium*, *S. aureus*[6,27]. Laboratory studies have revealed that the honey is effective against methicillin-resistant *S. aureus* (MRSA), β-*haemolytic streptococci* and vancomycin-resistant *Enterococci* (VRE)[28,29]. However, the newly identified honeys may have advantages over or similarities with manuka honey due to enhanced antimicrobial activity, local production (thus availability), and greater selectivity against medically important organisms[6]. The coagulase-negative *staphylococci* are very similar to *S. aureus*[14,30] in their susceptibility to honey of similar antibacterial potency and more susceptible than *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Enterococcus* species[14].

The disc diffusion method is mainly a qualitative test for detecting the susceptibility of bacteria to antimicrobial substances; however, the minimum inhibitory concentration (MIC) reflects the quantity needed for bacterial inhibition. Following the *in vitro* methods, several bacteria (mostly

Table 1
Antibacterial activity of honey against bacteria causing life-threatening infection to humans.

Bacterial strain	Clinical importance	Authors
Proteus spps.	Septicemia, urinary infections, woundinfections	Molan[8] Agbagwa and Frank-Peterside[33]
Serratia marcescens	Septicemia, wound infections	Molan[8]
Vibrio cholerae	Cholera	Molan[8]
S. aureus	Community acquired and nosocomial infection	Taormina *et al*[50] Chauhan *et al*[34] Sherlock *et al*[35]
E. coli	Urinary tract infection, diarrhea, septicemia, wound infections	Chauhan *et al*[34] Sherlock *et al*[35]
P. aeruginosa	Wound infection , diabetic foot ulcer, Urinary infections	Chauhan *et al*[34] Sherlock *et al*[35] Mullai and Menon[36]
S. maltophilia	Pneumonia, urinary tract infection, blood stream infection, nosocomial infection	Tan *et al*[9]
A. baumannii	Opportunistic pathogen infects immunocompromised individuals through open wounds, catheters and breathing tubes	Tan *et al*[9]
A. schubertii *H. paraphrohaemlyticus* *Micrococcus luteus* *Cellulosimicrobium cellulans* *Listonella anguillarum* *A. baumannii*	Burn- wound infection	Hassanein *et al*[38]
H. pylori	Chronic gastritis, peptic ulcer, gastric malignancies	Ndip *et al*[57]
Salmonella enterica serovar Typhi	Enteric fever	Mulu *et al*[58] Chauhan *et al*[34] Molan[8]
Mycobacterium tuberculosis	Tuberculosis	Asadi-Pooya *et al*[59]

multidrug resistant; MDR) causing human infections that were found susceptible to honeys are presented in Table 1.

3.2. Zone diameter of inhibition

The zone diameter of inhibition (ZDI) of different honey samples (5%-20%) has been determined against *E. coli* O157: H7 (12 mm -24 mm) and *S. typhimurium* (0 mm -20 mm)[31]. The ZDIs of Nilgiris honeys were found to be (20-21) mm, (15-16) mm and (13-14) mm for *S. aureus*, *P. aeruginosa* and *E. coli*, respectively[32]. Agbagwa and Frank-Peterside[33] examined different honey samples: Western Nigerian honey, Southern Nigerian honey, Eastern Nigerian honey and Northern Nigerian honey, and compared their abilities to inhibit the growth of *S. aureus*, *P. aeruginosa*, *E. coli* and *Proteus mirabilis* (*P. mirabilis*) with an average of ZDIs (5.3-11.6) mm, (1.4-15.4) mm , (4.4-13.5) mm and (9.1-17) mm, respectively, and with honey concentrations of 80%-100%. The extracts of raw and processed honey showed ZDI (6.94-37.94) mm, against gram-positive bacteria *viz*., *S. aureus*, *Bacillus subtilis*, *Bacillus cereus*, as well as gram-negative bacteria like *E. coli*, *P. aeruginosa* and *S. enterica* serovar Typhi[34]. Figure 1 represents the ZDIs for gram-negative and gram-positive bacterial strains due to ulmo and manuka honeys.

3.3. Minimum inhibitory concentration

The MIC assay showed that a lower MIC was observed with ulmo (*Eucryphia cordifolia*) honey (3.1% - 6.3% v/v) than with manuka honey (12.5% v/v) for MRSA isolates; for the *E. coli* and *Pseudomonas* strains equivalent MICs were observed (12.5% v/v)[35]. The MICs for Tualang honey ranged 8.75% - 25%, while those for manuka honey ranged 8.75% - 20% against many pathogenic gram-positive and gram-negative bacteria[9]. The MICs of manuka, heather, khadikraft and local honeys against clinical and environmental isolates of *P. aeruginosa* were recorded as

10% – 20%, 10% – 20%, 11% and 10% – 20%, respectively[36]. The MICs of *A. mellifera* honey ranged (126.23 – 185.70) mg/mL and of *Tetragonisca angustula* honey (142.87 – 214.33) mg/mL against *S. aureus*[37]. The *Egyptian clover* honey MIC was 100 mg/mL for *S. typhimurium* and *E. coli* O157:H7[31]. The Nilgiri honey MICs were 25%, 35% and 40% for *S. aureus*, *P. aeruginosa* and *E. coli*, respectively[32]. MIC values of honey extracts were found in the range of (0.625–5.000) mg/mL, for *S. aureus*, *B. subtilis*, *B. cereus*, and gram-negative bacteria (*E. coli*, *P. aeruginosa* and *S. typhi*[34].

By visual inspection, the MICs of Tualang honey ranged 8.75% – 25% compared with those of manuka honey (8.75% – 20%) against wound and enteric microorganisms: *Streptococcus pyogenes* (*S. pyogenes*), coagulase-negative *Staphylococci*, MRSA, *Streptococcus agalactiae*, *S. aureus*, *Stenotrophomonas maltophilia* (*S. maltophilia*), *Acinetobacter baumannii* (*A. baumannii*), *S. Typhi*, *P. aeruginosa*, *Proteus mirabilis*, *Shigella flexneri*, *E. coli* , *Enterobacter cloacae* (*E. cloacae*)[9]. Six bacterial strains from burn- wound patients, namely, *Aeromonas schubertii* (*A. schubertii*), *Haemophilius paraphrohaemlyticus* (*H. paraphrohaemlyticus*), *Micrococcus luteus* (*M. luteus*), *Cellulosimicrobium cellulans* (*C. cellulans*), *Listonella anguillarum* (*L. anguillarum*) and *A. baumannii* had MICs of Cirtrus, Clover, Nigella and Eljabaly honeys 35%–40%, 35%–40%, 35%–40%, 25%–30%, respectively, as has been reported by Hassanein *et al* . The honeys were inhibitory at dilutions down to 3.6% – 0.7 % (v/v), for the pasture honey, 3.4% – 0.5% (v/v), and for the manuka honey, against coagulase-negative *Staphylococci*[10]. The MICs of various types of honeys for various pathogenic bacterial strains have been determined by many authors[39]; in this article for oral bacterial strains and bacterial strains causing wound infections, honey MICs are depicted in Figure 2 and 3.

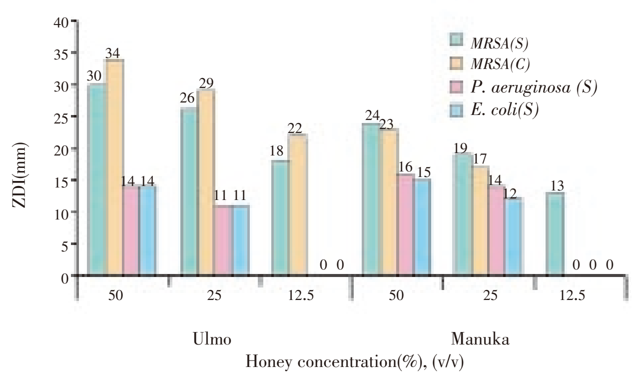

Figure 1. Antibacterial activity of ulmo and manuka honeys based on the ZDI produced for clinical (C) MRSA and standard (S) MRSA, *E. coli* and *P. aeruginosa* isolates.

3.4. Time-kill study

The kill kinetics provides more accurate description of antimicrobial activity of antimicrobial agents than does the MIC[2]. In our earlier study, we explored the time-kill activity of autoclaved honey against *E. coli*, *P. aeruginosa* and *S. Typhi* in order to establish the potential efficacy of such local honey (not studied before) collected from a village of the West Bengal state, India[5]. Antibiotic susceptible and resistant isolates of *S. aureus*, *S. epidermidis*, *Enterococcus faecium*, *E. coli*, *P. aeruginosa*, *E. cloacae*, and *Klebsiella oxytoca* were killed within 24 h by 10%–40% (v/v) honey[40]. Thus, more studies are required to establish various local honeys based upon kill kinetics and their effective *in vivo* application against MDR infections.

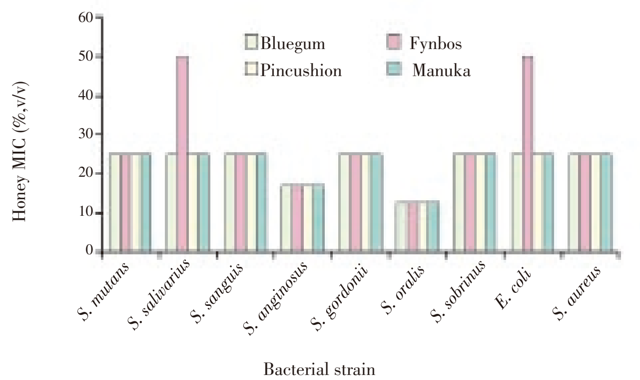

Figure 2. MIC of four different honeys (as shown in the figure) to oral bacterial strains (*Streptococcus* spp., *E. coli* and *S. aureus*).

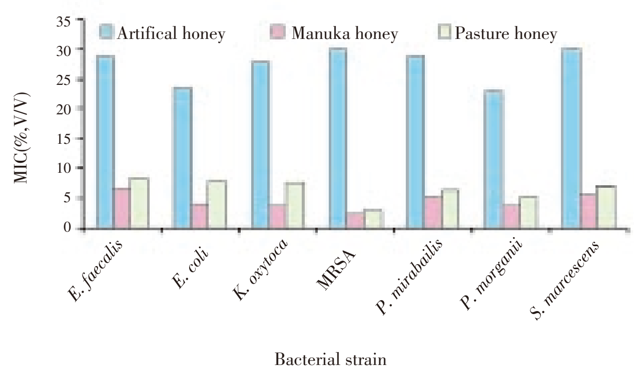

Figure 3. MIC of different honey types for bacterial strains causing wound infections.

4. Mechanism and factors affecting antibacterial activity

4.1. Mechanism of antibacterial activity

The beneficial role of honey is attributed to its antibacterial property with regards to its high osmolarity, acidity (low pH) and content of hydrogen peroxide (H_2O_2) and non-peroxide components, *i.e.*, the presence of phytochemical components like methylglyoxal (MGO)[41,42]. The antimicrobial agents in honey are predominantly hydrogen peroxide, of which the concentration is determined by relative levels of glucose oxidase, synthesized by the bee and catalase originating from flower pollen[41]. Most types of honey generate H_2O_2 when diluted, because of the activation of the enzyme glucose oxidase that oxidizes glucose to gluconic acid and H_2O_2, which thus attributes the antimicrobial activity[43]. But, in some cases, the peroxide activity in honey can be destroyed easily by heat or the presence of catalase.

Besides H_2O_2, which is produced in most conventional honeys by the endogenous enzyme glucose oxidase, several other non-peroxide factors have been found to be responsible for the unique antibacterial activity of honey[13]. Honey may retain its antimicrobial activity even in the presence of catalase (absence of glucose oxidase), and thus this type of honey is regarded as "non-peroxide honey"[8,13]. Several components are known to contribute the non-peroxide activity, such as the presence of methyl syringate and methylglyoxal, which have been extensively studied in manuka honey that is derived from the manuka tree (*L. scoparium*)[42,44]. Unlike manuka honey, the activity of ulmo honey is largely due to H_2O_2 production: 25 % (v/v) solution of ulmo honey had no detectable antibacterial activity when tested in presence of catalase, while, at the same concentration the manuka honey retained its antibacterial activity in the presence of catalase (absence of H_2O_2)[35]. Neither type of activity is influenced by the sterilizing procedure of gamma-irradiation[13].

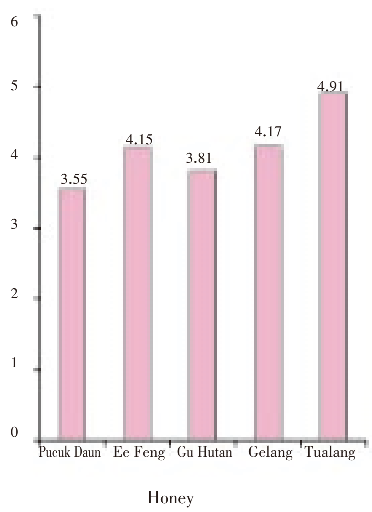

Figure 4. The pH values of different honeys having antibacterial activity

Honey is characteristically acidic with pH between 3.2 and 4.5, which is low enough to be inhibitory to several bacterial pathogens[45]; Figure 4 depicts the pH values of different honeys. The minimum pH values for growth of some common pathogenic bacteria are: *E. coli* (4.3), *Salmonella* spp. (4.0), *P. aeruginosa* (4.4), *S. pyogenes* (4.5)[46], and thus in undiluted honey the acidity is a significant antibacterial factor. The antibacterial property of honey is also derived from the osmotic effect of its high sugar content and low moisture content, along with its acidic properties of gluconic acid and the antiseptic properties of its H_2O_2[47]. A recent study examining the antimicrobial properties of honey *in vitro* found that H_2O_2, MGO and an antimicrobial peptide, bee defensin-1, are distinct mechanisms involved in the bactericidal activity of honey[48].

4. 2. Factors affecting antibacterial nature of honey

Molan and Cooper[49] reported that the difference in antimicrobial potency among the different honeys can be more than 100-fold, depending on its geographical, seasonal and botanical source as well as harvesting, processing and storage conditions. The antibacterial nature of honey is dependent on various factors working either singularly or synergistically, the most salient of which are H_2O_2, phenolic compounds, wound pH, pH of honey and osmotic pressure exerted by the honey. Hydrogen peroxide is the major contributor to the antimicrobial activity of honey, and the different concentrations of this compound in different honeys result in their varying antimicrobial effects[8]. It has further been reported that physical property along with geographical distribution and different floral sources may play important role in the antimicrobial activity of honey[50]. Several authors reported that different honeys vary substantially in the potency of their antibacterial activity, which varies with the plant source[6,7,51]. Thus, it has been shown that the antimicrobial activity of honey may range from concentrations $< 3\ \%$ to $50\ \%$ and higher[6,10,51]. The bactericidal effect of honey is reported to be dependent on concentration of honey used and the nature of the bacteria[4,52]. The concentration of honey has an impact on antibacterial activity; the higher the concentration of honey the greater its usefulness as an antibacterial agent[31]. Taormina *et al*[50] reported that the concentration of honey needed for complete inhibition of *S. typhimurium* growth is $<25\%$.

5. Conclusion

Microbial resistance to honey has never been reported[53], which makes it a very promising topical antimicrobial agent against the infection of antibiotic-resistant bacteria (*e.g.*, MDR *S. maltophilia*) and in the treatment of chronic wound infections that do not respond to antibiotic therapy. Hence honey has been used as a last-resort medication. Manuka honey has been widely researched and its antibacterial potential is renowned worldwide. The potency of honeys, such as Tualang honey, against microorganisms suggests its potential to be used as an alternative therapeutic agent in certain medical conditions, particularly wound infection.

Lusby *et al*[6] reported that honeys other than the commercially available antibacterial honeys (*e.g.*, manuka honey) can have equivalent antibacterial activity against bacterial pathogens. The growth of bacterial species that cause gastric infections, such as *S. typhi*, *S. flexneri* and *E. coli*, are inhibited by Tualang honey at the low concentrations. The Tualang honey has been reported to be effective against *E. coli*, *S. typhi* and *S. pyogenes*[54], and thus, when taken orally in its pure undiluted form, this honey may help speed up recovery from such infections. Honey is effective when used as a substitute for glucose in oral rehydration and its antibacterial activity shortened the duration of bacterial diarrhoea.

Currently, the emerging antimicrobial resistance trends in burn wound bacterial pathogens are a serious challenge[55]. Thus, honey with effective antimicrobial properties against antibiotic-resistant organisms such as MRSA and MDR *P. aeruginosa*, *Acinetobacter* spp. and members of the family *Enterobacteriaceae*, which have been associated with infections of burn wounds and in nosocomial infections, is much anticipated[55,56].

Overall, the unpredictable antibacterial activity of non-standardized honey may hamper its introduction as an antimicrobial agent due to variation in the *in vitro* antibacterial activity of various honeys. At present a number of honeys are sold with standardized levels of antibacterial activity, of which the best known is manuka (*Leptospermum*) honey as well as Tualang (*Koompassia excelsa*) honey. The medical-grade honey (Revamil, medihoney), which has the potential to be a topical antibacterial prophylaxis because of its broad-spectrum bactericidal activity, or to be a treatment for topical infections caused by antibiotic-resistant as well as antibiotic-sensitive bacteria, should be considered for therapeutic use. Moreover, mountain, manuka, capillano and eco-honeys have exhibited inhibitory activity against *H. pylori* isolates at concentration 10% (v/v)[57], demonstrating that locally produced honeys possess excellent antibacterial activity comparable to the commercial honeys. Therefore it is necessary to study other locally produced but yet untested honeys for their antimicrobial activities.

Conflict of interest statement

We declare that we have no conflict of interest.

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