# The Scientific Evidence Validating The Use Of Honey As A Medicinal Agent

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**Abstract:**

Honey has been used to treat wounds, infections, and other diseases since ancient times. Due to the widespread bacterial resistance to antibiotics, scientists have investigated the healing power of honey. Numerous studies have documented the broad-spectrum antibacterial effect of honey and its success in inhibiting MRSA. The use of honey as a wound dressing has proven to successfully heal chronic wounds in short amounts of time by inhibiting pathogens, reducing inflammation, rebuilding damaged tissue, and minimizing scarring. The mechanisms of action are directly related to the high hydrogen peroxide and rich polyphenol content present in honey. These two components account for much of the antibacterial, anti-inflammatory, and antioxidant activity of honey. Other significant components include acidic molecules and water and sugar content. Current research is exploring the antiproliferative effect of honey on cancer cells, and the results are positive. Many companies have patented medical-grade honey, and the scientific proof regarding the medicinal properties of honey demonstrates that honey should be considered as a treatment option for various diseases.

## Introduction

Humans have known the medicinal properties of honey since the origin of mankind. The use of honey to treat wounds, bacterial infections, and other ailments has been popular in the field of alternative medicine. Recently, honey has been experiencing a revival in modern medicine. The development of antimicrobial agents has always been a priority in reducing the casualties of infectious diseases. As a result of the overuse of antibiotics, pathogens are becoming resistant and no longer respond to traditional treatment. This emerging threat has led scientists to reevaluate the effectiveness of ancient remedies, including honey, and much research has been done proving the medicinal and antimicrobial properties of honey. (Mandal M, Mandal S, 2011)

Honey is composed of numerous unique qualities that allow it to ideally improve wound healing. Honey provides a moist healing environment due to its low water content. The high viscosity of honey serves as a protective barrier that prevents infection and cross contamination. Honey has a pH of 3.6-3.7, and most bacteria are unable to grow in that acidic environment. In addition, honey stimulates the production of lymphocytes, which aid in the body's immune response. One of the key antibacterial components present in honey is hydrogen peroxide; however, some types of honey are effectively inhibit bacteria despite their low levels of hydrogen peroxide. (Lotfi, 2008)

Although many of the mechanisms behind the success of honey are a mystery and are currently being delved into, scientists have proven that using honey as a wound dressing has multiple benefits.

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Rapid healing, especially in burns, was noted, and ulcers that were present for long periods of time were healed due to the ability of honey to stimulate the healing process. Unlike antibiotics, honey has no side effects, yet it is an effective antimicrobial agent for even resistant bacteria such as, methicillin-resistant *Staphylococcus aureus* (MRSA). Honey has powerful cleansing properties and is unique in the manner in which it rebuilds damaged tissue while minimizing scarring. In addition, honey has anti-inflammatory and antioxidant properties. (Molan, 1999)

Recently, the theory of using honey as a medicine has resurfaced and gained popularity. Multiple studies have proven that the once ancient remedy has considerable scientific evidence supporting the miraculous role of honey in the treatment of wounds and other diseases.

**The Origin and Composition of Honey**

Honeybees create honey to serve as a food source during the winter. A bee will fly 55,000 miles and collect nectar from 2 million flowers in order to produce one pound of honey. The worker bees will regurgitate the nectar so that it is partly digested before storing it in the honeycomb. There, the bees will fan the honeycomb with their wings in order to evaporate the nectar and to avoid the fermentation of the honey (Jaganathan, Mandal, 2009). Bees incorporate the antibacterial characteristics of honey during the gathering of pollen and the ripening process (Garcia, et al. 2001). Hundreds of variations of honey are documented, and the differences lie in their botanical origin.

In order to study the medicinal benefits of honey and its mechanisms of action, it is imperative to examine the composition of the substance. Although the nutrition found in honey is small compared to the recommended daily intake, its significance lies in its diverse physiological effects (Bogdanov, et al. 2008). Honey is primarily composed of carbohydrates that take up 95% of the dry weight. In addition, honey contains other compounds such as organic acids, proteins, amino acids, minerals, polyphenols, vitamins, and aroma compounds.

Figure 1 indicates the breakdown of the components present in honey. The primary sugars include the monosaccharides fructose and glucose. In addition, 25 oligosaccharides have been identified, significantly among them are the disaccharides sucrose, maltose, trehalose, turanose

and other nutritionally essential ones. During digestion, the carbohydrates fructose and glucose are absorbed into the blood and can be used as an energy source by the human body. Honey is comprised of 0.5% proteins that are primarily enzymes and free amino acids. Three important enzymes are diastase that breaks down starch or glycogen; invertase that decomposes sucrose into fructose and glucose; and glucose oxidase that produces hydrogen peroxide and gluconic acid from glucose. Another functional ingredient present in honey is polyphenols. The main polyphenols in honey are flavonoids and phenolic acids, which are responsible for the antioxidant properties of honey. (Bogdanov, et al. 2008)

**The Antimicrobial Activity of Honey**

Honey has been used to treat microbial infections and wounds since ancient times. In 1882, a Dutch scientist by the name of Van Ketel first documented the antibacterial properties of honey. Sacket followed in 1919 with the observation that the antibacterial potency of honey was increased by limited dilution. In 1937, Dold coined the term inhibine to describe the antibiotic feature present in honey (Molan, 1992). As explained by Bogdanov (1997), scientists debate the definition of inhibine. Some believe that the hydrogen peroxide produced by honey glucose oxidase is the main antibacterial agent. Others argue that the amount of peroxide present is not enough to inhibit bacteria, and that the non-peroxide activity plays a greater role. Honey is primarily composed of sugars, which kill bacteria by their osmotic effect. Research has also proven that the acids, pH levels, and flavonoids present in honey contribute to the antibiotic effect. Therefore, it can be concluded that a combination of these factors contributes to the antimicrobial effect of honey.

| | Blossom honey | | Honeydew honey | |
|---|---|---|---|---|
| | average | min.–max. | average | min.–max. |
| Water | 17.2 | 15–20 | 16.3 | 15–20 |
| Monosaccharides | | | | |
| fructose | 38.2 | 30–45 | 31.8 | 28–40 |
| glucose | 31.3 | 24–40 | 26.1 | 19–32 |
| Disaccharides | | | | |
| sucrose | 0.7 | 0.1–4.8 | 0.5 | 0.1–4.7 |
| others | 5.0 | 2–8 | 4.0 | 1–6 |
| Trisaccharides | | | | |
| melezitose | <0.1 | | 4.0 | 0.3–22.0 |
| erlose | 0.8 | 0.5–6 | 1.0 | 0.1–6 |
| others | 0.5 | 0.5–1 | 3.0 | 0.1–6 |
| undetermined oligosaccharides | 3.1 | | 10.1 | |
| Total sugars | 79.7 | | 80.5 | |
| Minerals | 0.2 | 0.1–0.5 | 0.9 | 0.6–2.0 |
| Amino acids, proteins | 0.3 | 0.2–0.4 | 0.6 | 0.4–0.7 |
| Acids | 0.5 | 0.2–0.8 | 1.1 | 0.8–1.5 |
| pH-value | 3.9 | 3.5–4.5 | 5.2 | 4.5–6.5 |

**Figure 1. Honey Composition (Data in g/100 g** (Bogdanov, et al. 2008)

There are two basic microbiological techniques, outlined by Molan (1992), that are used to measure the antibacterial activity of honey. This first is the agar diffusion assay technique. A small amount of honey or solution of honey is placed on a nutrient agar plate inoculated with a microbial culture. During incubation, the honey spreads out into the agar from its point of application. A clear zone is observed where the concentration of honey is high enough to inhibit growth. The size of the clear zone indicates the measure of potency of the honey. Because the honey gets diluted as it diffuses across the agar, the actual effective concentration is lower than the concentration of honey applied. The second type of assay involves incorporating the honey into the nutrient agar or nutrient broth in which the culture is grown. Using different concentrations will help identify the minimum inhibitory concentration (MIC) for each kind of honey. A honey that can retain its antibacterial activity while undergoing dilutions will be most effective.

The frequency of bacterial resistance to antibiotics and even last resort drugs is increasing at an alarming rate to the extent that drug companies have slowed research in the field of antibiotic drug discovery. Honey is emerging as a popular topical antimicrobial agent due to its effectiveness, and especially because bacteria resistance to honey has not yet been recognized (Kwakman, et al. 2008). Laboratory studies demonstrate that manuka honey is effective against several human pathogens, including *Escherichia coli*, *Enterobacter aerogenes*, *Salmonella typhimurium*, and *S. aureus*. Other experiments show that honey kills methicillin-resistant *S. aureus* (MRSA), *β-haemolytic streptococci* and vancomycin-resistant *Enterococci* (VRE). Figure 2 illustrates some bacteria that cause life-threatening diseases that are susceptible to honey(Mandal M, Mandal S, 2011). Willix, et al. (1992) studied seven major wound-infecting species of bacteria and compared their sensitivity to manuka honey and another type of honey. The two honeys differed in their known mechanism of action. In general, the high sugar content of honey controls infection by the osmotic effect; however, honey also inhibits bacteria by its hydrogen peroxide activity (standard honey) or by an unidentified floral source (manuka honey). At a concentration of 1.8% (v/v), the non-peroxide activity of manuka honey completely inhibited *Staphylococcus aureus* after an incubation of only 8 hours. All seven species of bacteria were completely inhibited by both manuka and standard honey at a concentration below 11% (v/v) (Willix, et al. 1992). In conclusion, the overall antibacterial activity of both peroxide and non-peroxide honeys successfully inhibit bacteria in only 8 hours. These studies bring undeniable proof that honey should be seriously considered as an antimicrobial agent.

In order to document the efficacy of therapeutic honeys, scientists from all around the world compare the effectiveness of commercial, medical-grade honey to honey that is locally produced. One study analyzed the pollen source and antibacterial activity of Spanish honeys. Twenty-five samples of honey from various botanical origins were tested using *Staphylococcus aureus* as the resistant microorganism. Honey originating from the labiate and rosemary families exhibited the greatest zone of inhibition against *S. aureus*, while heather honey proved to be ineffective (Garcia, 2001). Another study, conducted in Australia, compared the effectiveness of Medihoney® (medical grade manuka honey), manuka honey, and honey obtained from local beekeepers (Lusby, et al. 2005). Results indicated that

**Figure 2. Antibacterial activity of honey against bacteria causing life-threatening infection to humans.** (Mandal M, Mandal S, 2011)

| Bacterial strain | Clinical importance |
|---|---|
| *Proteus* spps. | Septicemia, urinary infections, woundinfections |
| *Serratia marcescens* | Septicemia, wound infections |
| *Vibrio cholerae* | Cholera |
| *S. aureus* | Community acquired and nosocomial infection |
| *E. coli* | Urinary tract infection, diarrhea, septicemia, wound infections |
| *P. aeruginosa* | Wound infection , diabetic foot ulcer, Urinary infections |
| *S. maltophilia* | Pneumonia, urinary tract infection, blood stream infection, nosocomial infection |
| *A. baumannii* | Opportunistic pathogen infects immunocompromised individuals through open wounds, catheters and breathing tubes |
| *A. schubertii*<br>*H. paraphrohaemlyticus*<br>*Micrococcus luteus*<br>*Cellulosimicrobium cellulans*<br>*Listonella anguillarum*<br>*A. baumannii* | Burn– wound infection |
| *H. pylori* | Chronic gastritis, peptic ulcer, gastric malignancies |
| *Salmonella enterica* serovar Typhi | Enteric fever |
| *Mycobacterium tuberculosis* | Tuberculosis |

twelve out of thirteen bacteria were inhibited by all honeys, with the exception of *Serratia marcescens* and the yeast *Candida albicans*. Evidently, local, unprocessed honey and medical-grade honey display antibacterial properties. However, three species of bacteria were more sensitive to Medihoney® proving that medical-grade honey possesses stronger antibacterial agents. In contrast to this, a study done in India comparing the antibacterial activity of manuka honey (Australia), heather honey (UK), and khadikraft honey (India) against 152 strains of resistant *Pseudomonas aeruginosa* isolated from chronic wound infections, proved that local khadikraft honey displayed the highest antibiotic power (Mullai, Menon, 2007). Despite the fact that some local honeys seemed more powerful than some medical grade honeys, it would be wise to use medical grade honey due to the consistency of its components.

The large variations in honey found in the natural environment can lead to inconsistent experimental results. An experiment conducted in Amsterdam used Revamil medical grade honey, which is produced by bees in closed greenhouses. The bactericidal spectrum of the honey was tested in vitro, and its usefulness in eliminating microbial skin colonization in healthy humans was measured by topical application. The activity of 11 batches of Revamil medical-grade honey was compared in a microdilution assay with *B. subtilis* as the target organism. At a concentration of 40% (v/v), the honey completely killed the inocula of resistant strains of *S. aureus*, *S. epidermis*, and *E. faecium* (gram-positive bacteria). At a lower concentration of 20% and 10% (v/v), most of the bacteria were killed as well. The honey was equally effective against gram-negative bacteria, including ESBL-producing strains that were killed after 24 hours of incubation in 20% (v/v) honey. The effectiveness of honey was tested in vivo by applying it to the forearm skin of healthy volunteers. After only 48 hours, the median level of skin colonization was reduced 100 fold (Kwakman, et al. 2008). It can be concluded that Revamil, which is produced under controlled conditions, is a reliable broad-spectrum antibacterial agent.

**The Antibacterial Activity of Honey against MRSA**

One of the most acclaimed benefits of using honey as an antimicrobial agent is its success in inhibiting methicillin-resistant *Staphylococcus aureus* (MRSA). Cooper, et al. (2002) tested 18 strains of MRSA isolated from infected wounds against manuka honey, pasture honey and artificial honey. The minimum inhibitory concentration (MIC) values for all the strains were between 2.7-4% (v/v) for the manuka and pasture honey, yet none of the bacteria were inhibited by the artificial honey even at concentrations of >30% (v/v) (Cooper, et al. 2002).

A study investigated the effect of manuka honey on the cell cycle of MRSA (Jenkins, et al. 2011). In staphylococci, cell division is preceded by the formation of a septum at the cell equator, followed by cleavage, which separates the two daughter cells. Murein hydrolases, also called autolysins, are the enzymes responsible for hydrolyzing structural components in the cell wall, such as peptidoglycan. A decrease in murein hydrolases will prevent cell cleavage from occurring. These enzymes are encoded by the *atl* gene, and sensitivity to manuka honey in *S. aureus atl* mutants cause an accumulation of cells containing septa. After treating MRSA cultures with various concentrations of manuka honey, results showed significantly higher proportions of cells with partial and complete septa (64-67%). Furthermore, murein hydrolase activity was not detected in MRSA treated with manuka honey (Jenkins, et al. 2011). Figure 3 indicates that enlarged cells containing septa were observed in MRSA that was treated with honey. In addition, treating MRSA with manuka honey reversed oxacillin resistance and down-regulated the mecR1 pathway responsible for oxacillin resistance (Jenkins, Cooper, 2012). Evidently, treating MRSA with honey and antibiotic combinations may restore the bacteria's susceptibility to a particular drug.

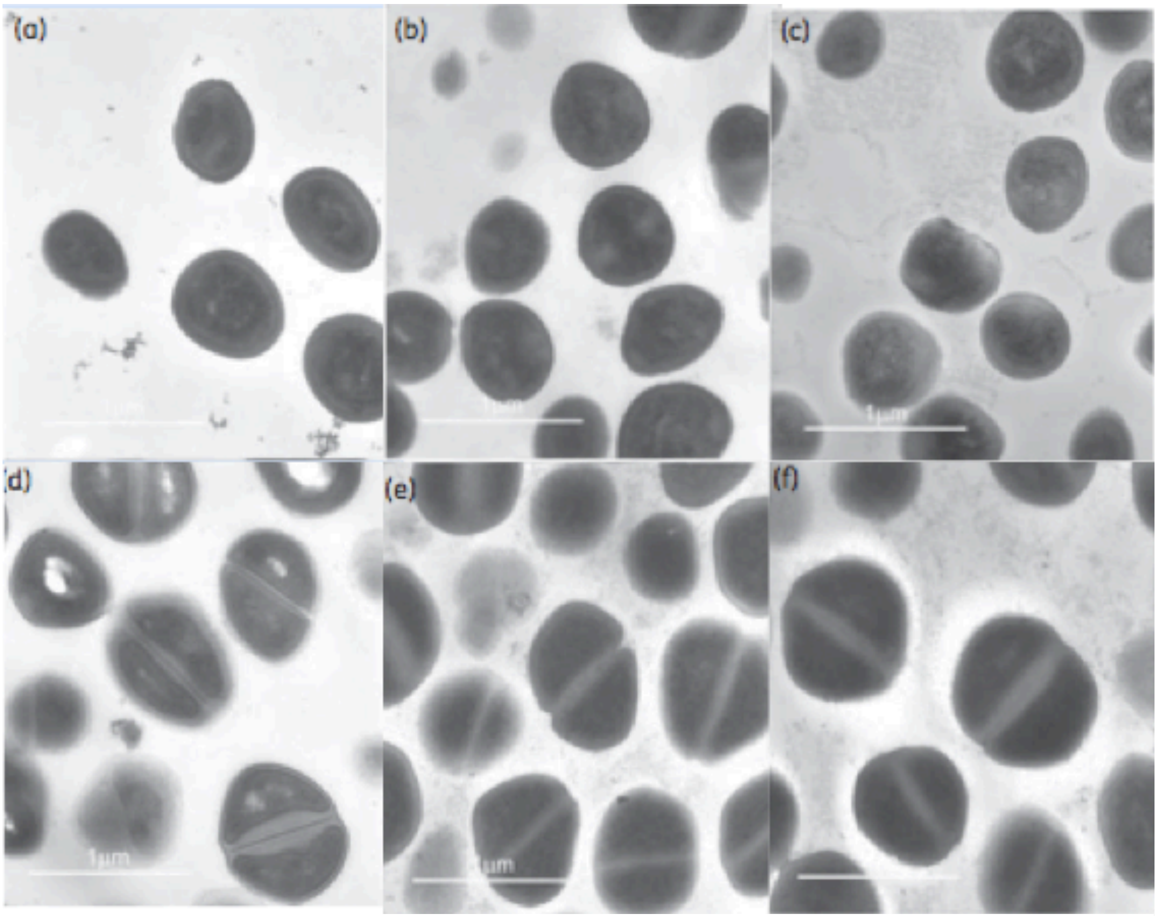


**Figure 3. Effect of honey on the structure of MRSA cells. Transmission electron micrographs show MRSA at x32000 magnification following incubation for 120 min in (a) nutrient broth (NB), (b) NB+10% (w/v) artificial honey, (c) NB+2.5% (w/v) manuka honey (MH), (d) NB+5% (w/v) MH, (e) NB+10% (w/v) MH, and (f) NB+20% (w/v). The percentage of cells with septa was significantly increased following treatment with concentrations of MH >2.5% (w/v), and cells were also significantly larger than control cells.** (Jenkins, et al. 2011)

## Honey in Wound Care

In order to convince skeptical clinicians that honey is more than a therapeutic healing agent, Molan (2006a) collected all the experimental evidence proving the effectiveness of using honey as a wound dressing. In total, positive findings on honey in wound care have been documented from 17 randomized controlled trials involving 1965 participants, and 5 clinical trials involving 97 participants treated with honey. Similar results were also seen in over 533 wounds on experimental animals. Wounds included burns of all degrees, chronic ulcers, surgical wounds, incisions plus others (Molan, 2006a). In a study surveying these patients, most reported complete healing of 99% within 2-9 weeks. However, in controlled clinical trials, the healing effect of honey was only 56% in 4-12 weeks (Medhi, et al. 2008).

The role of honey in pediatric wound management was evaluated by Bittmann, et al. (2010). Despite the fact that there is little research in this area, 15 pediatric oncology patients with infected wounds were successfully treated with Medihoney (Bittmann, et al. 2010). Therefore, it is clear that using honey as a wound dressing shows positive results; however, further research is needed to establish its true efficacy.

Part of the success in using honey as a wound dressing lies in its antibacterial properties. A wound will not heal if it is heavily saturated with bacteria. Bacteria produce toxins that inhibit growth of repair tissue; proteases produced by bacteria will digest connective tissue; and bacterial endotoxins are known to stimulate inflammatory responses that restrict blood flow to the wound site (Molan, 2006b). The low water content component draws water away from the wound by osmosis, thereby inhibiting bacterial growth. Yet the watery layer present in honey will not adhere to the newly formed skin and will provide for painless dressing changes (Molan, 2006b). The glucose oxidase present in honey produces gluconic acid and hydrogen peroxide, which kills bacteria without harmful side effects (Bittmann, et al. 2010). These qualities depict honey as an ideal wound dressing. The antibacterial activity of honey has been widely established, and further research has been done concerning wounds that are specifically caused by resistant bacteria.

The effectiveness of honey against antibiotic-resistant strains of coagulase-negative staphylococci was determined in a study by done Cooper, et al. (1999). Fifty-eight strains of coagulase-negative staphylococci, isolated from wounds, were inhibited by manuka and pasture honeys at concentrations of 2-4% (v/v), with manuka honey being more powerful. A similar study was done by French, et al. (2005), testing honey against coagulase-negative staphylococci present in biofilms on the surface of medical equipment. These pathogens are included in the top five causative agents of hospital-acquired infection caused by the insertion of temporary and permanent invasive medical devices. Despite the fact that these devices are initially sterilized, skin organisms contribute to contamination during implantation and following use. Both manuka and pasteurized honeys inhibited 18 isolates of coagulase-negative staphylococcus at a concentration of 2.7-5% (v/v) (French, et al. 2005), proving that honey has great potential as an antimicrobial agent to prevent infection. Additionally, there are great advantages in applying honey to the damaged tissue around medical devices. Honey's anti-inflammatory properties prevent serous exudates, which often supplies a medium for bacteria to grow; honey provides a moist environment to stimulate the growth of tissues; and honey has no harmful side effects on the tissue (Molan, 1999).

Numerous studies conducted on animals prove the healing power of honey (Lotfi, 2008). Of ten dogs suffering burns to part of their skin, those that received honey dressings showed 98% recovery in 21 days. Their wounds displayed less bacterial growth in comparison with the group that received saline solution as a wound dressing (Jalali, et al. 2007). In an experiment done on 24 mice with skin excisions, the ones that received honey as a wound dressing showed more extensive epithelization and a greater thickness of granulation tissue in the center of the wounds compared to the control group (Bergman, et al. 1983). Gupta, et al. (1992) studied the effect of topical honey on the healing of 90 infected skin wounds in buffalo calves. Surprisingly, the authors claim that honey was more effective than ampicillin in speeding up the healing process. This statement is hard to believe, but it does illustrate that honey is a powerful healing agent. The honey treated wounds displayed less neutrophilic infiltration and greater formation of angioblasts and fibroblasts (Gupta, et al. 1992). Studies that compared the use of honey and silver sulfadiazine as a burn dressing for pigs and dogs reported more rapid results when using honey (Lotfi, 2008). These studies provide evidence for the success of using honey as a wound dressing over other conventional medications.

The efficacy of using honey in wound dressings has also been documented in humans. One hospital in the UK has adopted the use of honey-impregnated dressings for over a year (Visavadia, et al. 2008). An 80-year-old man with a split skin graft from his upper arm suffered from a MRSA infected wounds for 6 months. Figure 4 illustrates that the wound healed after only 2 weeks of applying a manuka honey dressing. A woman with a 3-year-old recalcitrant wound had tried conventional medicine and four surgeries to treat her wound with no success. After using a manuka honey dressing for one week, the wound became smaller and less

inflamed, the scarred area became more pliable, and bacteria ceased to grow. The infection stopped, and the wound completely healed within 4 months (Cooper, et al. 2001).

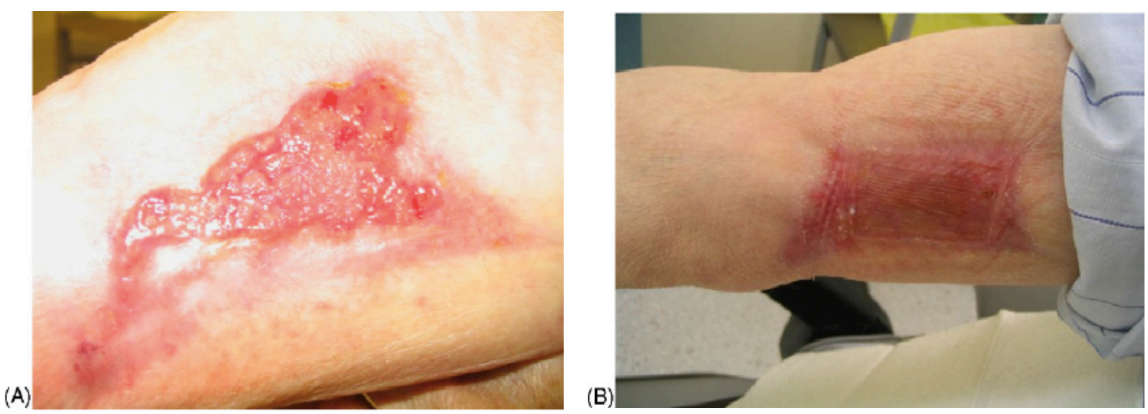


**Figure 4. (A) Infected split skin donor site in the upper arm. (B) Upper arm healed after 2 weeks. (Visavadia, et al. 2008)**

Another study was done on 8 patients with leg ulcerations. Over a 4-week period, the wounds decreased by an average of 50% with the use of manuka honey dressings. Malodor, which is common in chronic wounds, was absent after one week. However, the 2 patients with arterial wounds did not report as successful results, leading to the conclusion that manuka honey may not be the best course of treatment for arterial wounds (Gethin, Cowman, 2005). Another study involves a 47 year old woman with a MRSA colonized, hydroxyurea-induced leg ulcer (Natarajan, et al. 2001). After 3 months of using topical medications with no results, gamma-irradiated manuka honey was applied to the wound. The wound completely healed within 3 weeks, and the MRSA was not present, despite the patient continuing hydroxyurea therapy, the direct cause of the ulcer. Figure 5 illustrates the progress over a 3-week period. In addition to wound healing, using honey as a wound dressing promotes patient comfort, safety and quality of life as reported by 3 individuals undergoing treatment using Medihoney along with other antibiotics (Sare, 2008). Although a physician should always treat serious wounds, these studies all indicate that the use of honey as a wound dressing should be seriously considered among the treatment options for chronic wound infections.

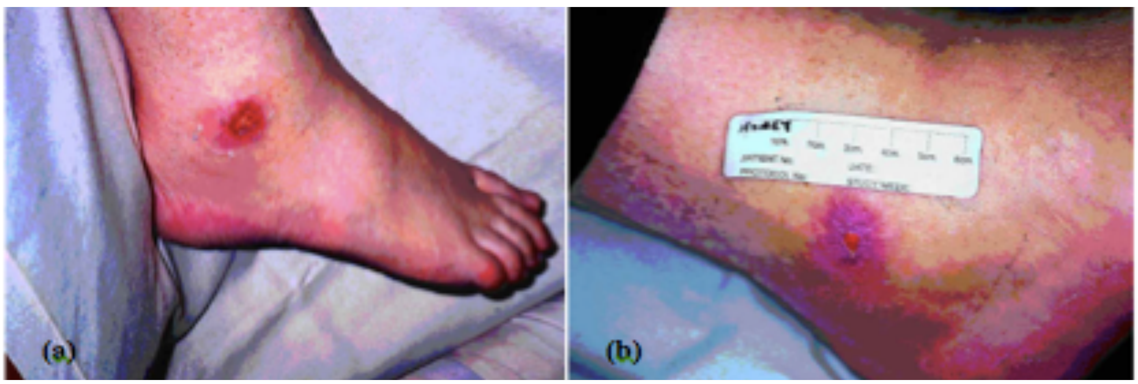



**Figure 5. (a) Hydroxyurea-induced ulcer over the right lateral malleolus, (b) Ulcer 1 week following commencement of honey treatment, (c) Healed ulcer 3 weeks following commencement of honey treatment.** (Natarajan, et. al. 2001)

**Honey in Oral Health**

effects of honey is thought to counteract its cariogenic effects. In an experiment

Several studies have explored the efficacy of using honey to limit oral pathogens. *Streptococcus mutans*, the main bacteria contributing to dental caries, along with other bacteria, forms a microbial community on the tooth surface called dental biofilm (plaque). The bacteria present produce lactic acid, which demineralizes the tooth (Nassar, et al. 2011). Despite the fact that honey contains 70% sugar and that high sugar consumption leads to tooth decay, the antibacterial testing two kinds of manuka honey against oral bacteria in their planktonic and biofilm states, the honey inhibited the bacteria at a high concentration (Badet, Quero, 2011). Another study measured the antimicrobial activity of natural honey (NH) versus artificial honey (AH) against the growth and biofilm formation of *S. mutans*. The NH wells indicated less growth and a greater zone of inhibition than the AH wells (Nassar, et al. 2011). Scientists have also evaluated the effectiveness of honey as a root canal medication against endodontic bacteria in comparison to standard drugs (Mittal, et al. 2012). At a concentration of 100%, honey displayed impressive results against *S. aureus*, *P. aeriginosa*, and other bacteria present in an infected root canal. Despite the fact that the antibiotics Ampicillin and Gentamycin revealed the maximum mean bacterial inhibition, honey did inhibit common endodontic microflora at a level deserving consideration for use as an intracanal medication. Further studies need to determine whether oral bacteria can be inhibited at levels lower than 100%, since such a high concentration of honey may potentially erode the tooth.

**Mechanisms of Action**

The efficacy of honey has much to do with its medicinal properties such as its antibacterial activity, anti-inflammatory action, and antioxidant activity. In addition, honey boosts the immune system and stimulates cell growth (Molan, 2001). Some of the mechanisms contributing to the healing power of honey have been briefly mentioned, and in this section they will be explored in greater depth.

Scientists have argued over the component present in honey that contributes to its antibacterial effect. The main theories are the osmotic effect, low acidity, and hydrogen peroxide activity. Honey is a saturated or supersaturated solution mainly comprised of sugar and partially made up of water. The sugar and water molecules interact strongly leaving very few water molecules available for other microorganisms. The free water is measured as the *water activity* ($a_w$), and its values are as low as 0.562-0.62 in honey. This environment inhibits bacteria; however, results of studies comparing the effectiveness of natural honey with artificial honey containing identical sugar and water concentrations, prove that honey contains additional factors responsible for inhibiting bacteria (Molan, 1992). The low acidity of honey (pH3.2-4.5) is caused by the presence of gluconolactone/gluconic acid, which is produced as a result of the enzymatic action in the ripening nectar. The low pH of honey would inhibit most organisms that grow under an optimum pH of 7.2-7.4; however, under experimental conditions, the growth medium can create a neutralizing effect on the honey, preventing inhibition. This is less likely to occur in a wound dressing where acidity plays a more significant role in inhibiting bacteria (Bogdanov, 1997, Molan, 1992).

White, et al. (1963) first identified hydrogen peroxide as the primary antibacterial agent present in honey. Hydrogen peroxide is produced by the enzyme glucose oxidase (found in the hypopharyngeal glands in honey bees), and upon dilution, its activity increases by a factor of 2,500-50,000 (Bang, et al.

2003, Molan, 1992). Catalase, an enzyme present in honey that originates from pollen, effectively destroys hydrogen peroxide. Therefore, the levels of hydrogen peroxide present in honey will be determined by the amount of glucose oxidase and catalase (Weston, 2000). At a very high concentration, hydrogen peroxide can cause cellular and protein damage in tissues by producing oxygen radicals (Bang, et al. 2003). Experiments done by Taormina, et al. (2001) and Bang, et al. (2003) have validated that upon dilution, hydrogen peroxide levels in honey are high enough to inhibit foodborne and wound pathogens without causing damage. However, certain dark honeys successfully inhibited bacteria despite the addition of catalase proving that other non-peroxide factors were in effect (Taormina, et al. 2001).

Manuka honey exhibits non-peroxide antibacterial activity, and the primary factor that destroys the bacteria is yet to be determined. However, this component is believed to derive from the unique floral sources that the honey originated from. In an experiment done by Westen, et al. (1999), active phenolic compounds such as methyl syringate, phenyllactic acid, and flavonoid components were extracted. These products were determined to have antibiotic properties, but are only partly responsible for the non-peroxide antibacterial effects of manuka honey. Honeys are labeled ‘non-peroxide’ when they exhibit antibacterial activity despite being exposed to catalase, which destroys hydrogen peroxide. Weston (2000) hypothesized that the catalase added was insufficient to effectively destroy the hydrogen peroxide present in manuka honey, and that the honey contained abnormally high amounts of hydrogen peroxide. An experiment done by Snow and Manley-Harris (2004) compared the effect of a 10-fold excess of catalase and the normal amount of catalase used to destroy the hydrogen peroxide. No statistical difference was observed between the two, indicating that the non-peroxide antibacterial activity was not due to residual hydrogen peroxide present in manuka honey. Therefore, the healing mechanisms of manuka honey are still unknown, but are most probably derived from the honey’s large range of phytochemicals.

It has been recently discovered that the activity of hydrogen peroxide alone does not cause DNA strand breaks, but rather, it is the coupling chemistry between the hydrogen peroxide and phenolic components present in honey. Hydroxyl radicals (OH□) are created as a result of the coupling chemistry between hydrogen peroxide and metal ions (Fe(II) or Cu(II)) via the Fenton reaction. When adding metal ions to honey, the hydroxyl radical content increases by 30-fold, and the resistant bacteria is inhibited by only 0.78% v/v, a much lower concentration than normally required (Brudzynski, Lanigan, 2012a). Further experimentation determined that the removal of hydrogen peroxide by catalase prevented DNA degradation in bacteria, but the polyphenols extracted from honey degraded plasmid DNA in the presence of hydrogen peroxide and Cu(II) via the Fenton reaction. At low content, honey polyphenols exhibited pro-oxidant activity damaging to DNA (Brudzynski, et al. 2012b). Therefore, phenolic/hydrogen peroxide-induced-oxidative stress explains the mechanism of honey antibacterial and DNA damaging activities. This recent study proves that many unknown mechanisms regarding the activity of honey will be unraveled with systematic and advanced levels of experimentation.

Honey inflammation is the immunological and pathological response of tissues, and it is triggered by infectious organisms, cancer, autoimmune diseases, toxic chemical substances, or physical injury (Kassim, et al. 2010). Typical wound healing is a complex process in which damaged tissue is removed and slowly replaced by restorative tissue. Inflammation is an important step in wound healing, and honey has been shown to stimulate monocytes in cells to release cytokines TNF-a, 1, and IL-6, which are the cell messengers that mediate the immune response (Tonks, et al. 2003). In addition, honey stimulates the production of B-lymphocytes, T-lymphocytes, and neutrophils, which aid in immunity (Abuharfeil, 1999). In the case of a chronic wound, prolonged inflammation can prevent healing and cause damage. During excessive inflammation, leukocytes release prostaglandins which cause pain; other chemical messengers stimulate swelling, which restricts the flow of blood through the capillaries and starves the wounded tissues of much needed oxygen and nutrients; reactive oxygen species are produced, which can potentially erode body tissues; and excessive fibroblast activity leads to fibrosis and scarring (Molan, 2001). Honey demonstrates strong anti-inflammatory action, and when honey is applied to wounds, leukocyte action is reduced, minimizing pain, wound exudate, and scarring. The anti-inflammatory activity of honey is attributed to its phenolic content, and the results of an experiment done on the inflammation of rats' paws showed that the methanol and ethyl acetate extracts of honey reduced inflammatory signs and markers, observed through the inhibition of swelling, decrease in pain, and reduction of the mediators of inflammation tested (NO and $PGE_2$) (Kassim, 2012). Extensive research is yet to be done regarding the anti-inflammatory effects of honey on a cellular level, however, the present evidence provides proof that the honey does work as an anti-inflammatory agent.

The presence of flavonoids and polyphenols in honey contribute to its antioxidant activity, which is defined as the ability of honey to scavenge free radicals. Free radicals are dangerous to the human body, because they attack DNA and proteins, leading to cell injury (Fujita, 2002). The antioxidant effect of honey is not only due to its ability to scavenge free radicals, but is also due to its initial inhibition of the formation of free radicals. Inflammation, the body's natural response to infection or injury, creates superoxide that is then converted to hydrogen peroxide, which generates the extremely reactive peroxide radical. The peroxide radical is generated by the Fenton reaction and is catalyzed by metal ions such as iron and copper. The flavonoids and polyphenols present in honey sequester these metal ions in complexes with organic molecules, and through this mechanism, honey is a powerful antioxidant. (Molan, 2001)

Apitherapy is the use of honey to treat wounds, burns, skin ulcers, dyspepsia, and peptic ulcers, specifically utilizing honey's antioxidant activity, caused by its phenolic compounds. Polyphenols prevent serious chronic disease such as cancer, cardiovascular diseases, and diabetes, which are caused by oxidative stress. Oxidative stress is defined as the imbalance between free radical production and the antioxidant defense system. Specifically, the polyphenols in honey have proven to suppress oxidative degradative reactions. In a study done by Inoue, et al. (2005), the antioxidant activities of various honeys were evaluated with 1,1-diphenyl-2-picrylhydroazyl (DPPH) radical and free radical (methyl ($CH_3$□), hydroxyl (OH□), and superoxide anions ($O_2$□$^-$)) scavenging systems. Buckwheat honey displayed the highest scavenging activity for DPPH and hydroxyl radicals, and manuka honey specifically scavenged superoxide anion radicals due to its high content of methyl syringate (Inoue, et al. 2005). Evidently, the polyphenols present in honey have much to do with the antibacterial, anti-inflammatory, and antioxidant activity of honey.

**Conclusion**

Historically, honey has been utilized as a vital medicinal agent since ancient times. With the discovery of antibiotics, drug companies have shifted their focus to the development of expensive and potentially harmful antibiotics. Recent antibacterial resistance has led scientists to seriously consider the validity of ancient remedies. Upon experimentation, honey has proven itself to be a powerful broad-spectrum antimicrobial agent, even against resistant bacteria that is no longer responding to conventional medicine (Mandal M, Mandal S, 2011). Honey has revolutionized wound care, and upon using honey dressings, chronic wounds have been cured in relatively short periods of time without adverse side effects (Molan, 2006a). Some hospitals have incorporated honey wound dressings in their treatment plans (Visavadia, et al. 2008), and many companies have patented medical grade honey (Kwakman, et al. 2008, Lusby, et al. 2005). Scientists are currently delving into the mechanisms of action of honey, and many have successfully verified the biochemical explanation of these effects. The hydrogen peroxide content coupled with the phenolic content of honey successfully degrades bacterial DNA (Brudzynski, et al. 2012b). Other components present in honey contribute to its antibacterial, anti-inflammatory, and antioxidant activity. Scientists have begun to prove that the rich phenolic content present in honey inhibits cancer cell proliferation and provides anti-tumor activity (Jaganathan, et al. 2010). The effectiveness of honey as a medicinal agent has been unequivocally demonstrated, and the once ancient remedy has gained widespread acceptance as a proven cure.

**References**

Abuharfeil N, Al-Oran R, Aboshehada M. 1999. The effect of bee honey on the proliferative activity of human B- and T-lymphocytes and the activity of phagocytes. Food Agric Immunol 11:169-177

Badet C, Quero F. 2011. The in vitro effect of manuka honeys on growth and adherence of oral bacteria. Anaerobe 17(1):19-22

Bang LM, Buntting C, Molan P. 2003. The effect of dilution on the rate of hydrogen peroxide production in honey and its implications for wound healing. J Altern Complement Med 9(2):267-273

Bergman A, Yanai J, Weiss J, Bell D, David MP. 1983. Acceleration of wound healing by topical application of honey: An animal model. The American Journal of Surgery 145(3):374-376

Bittmann S, Luchter E, Thiel M, Kameda G, Hanano R, Langler A. 2010. Does honey have a role in paediatric wound management? Br J Nurs 19(15)S19-S20, S22, S24

Bogdanov S. 1997. Nature and origin of the antibacterial substances in honey. LWT-Food Sci Technol 30(7):748-753

Bogdanov S, Jurendic T, Sieber R, Gallmann P. 2008. Honey for nutrition and health: A review. J Am Coll Nutr 27(6):677-689

Brudzynski K, Lannigan R. 2012a. Mechanism of honey bacteriostatic action against MRSA and VRE involves hydroxyl radicals generated from honey's hydrogen peroxide. Front Microbiol 3(36):1-8

Brudzynski K, Abubaker K, Miotto D. 2012b. Unraveling a mechanism of honey antibacterial action: Polyphenol/$H_2O_2$-induced oxidative effect on bacterial cell growth and on DNA degradation. Food Chem 133:329-336

Cooper RA, Molan PC, Harding KG. 1999. Antibacterial activity of honey against strains of *Staphylococcus aureus* from infected wounds. J R Soc Med 92:283-285

Cooper RA, Molan PC, Krishnamoorthy L, Harding KG. 2001. Manuka honey used to heal a recalcitrant surgical wound. Eur J Clin Microbiol Infect Dis 20(10):758-759

Cooper RA, Molan PC, Harding KG. 2002. The sensitivity to honey of Gram-positive cocci of clinical significance isolated from wounds. J Appl Microbiol 93:857-863

French VM, Cooper RA, Molan PC. 2005. The antibacterial activity of honey against coagulase-negative staphylococci. J Antimicrob Chemother 56(1):228-231

Fujita N. 2002. Formation and removal of reactive oxygen species, lipid peroxides and free radicals, and their biological effects. J Pharm Soc Japan 122:203-218

Garcia M, Perez-Arquillue C, Juan T, Juan MI, Herrera A. 2001. Pollen analysis and antibacterial activity of Spanish honeys. Food Sci Technol Int 7(2):155-158

Gethin G, Cowman S. 2005. Case series of use of Manuka honey in leg ulceration. Int Wound J 2(1):10-15

Gupta SK, Singh H, Varshney AC, Prakash P. 1992. Therapeutic efficacy of honey in infected wounds in buffaloes. Indian J Anim Sci 62(6):521-523

Inoue K, Murayama S, Seshimo F, Takeba K, Yoshimura Y, Nakzazwa H. 2005. Identification of phenolic compound in manuka honey as specific superoxide anion radical scavenger using electron spin resonance (ESR) and liquid chromatography with coulometric array detection. J Sci Food Agric 85:872-878

Jaganathan SK, Mandal M. 2009. Antiproliferative effects of honey and of its polyphenols: A review. J Biomed Biotechnol Jan:1-13

Jaganathan SK, Mondhe D, Wani ZA, Pal HC, Mandal M. 2010. Effect of honey on eugenol and Ehrlich ascites and solid carcinoma. J Biomed Biotechnol Mar:1-5

Jalali JSS, Trajik H, Saifzadeh S, Fartash B. 2007. Topical application of natural Urmia honey on experimental burn wounds in the dog: Clinical and microbiological studies. Asian J Anim Vet Adv 2(3)133-139

Jenkins R, Burton N, Cooper R. 2011. Manuka honey inhibits cell division in methicillin-resistant *Staphylococcus aureus*. J Antimicrob Chemother 66:2536-2542

Jenkins RE, Cooper R. 2012. Synergy between oxacillin and manuka honey sensitizes methicillin-resistant *Staphylococcus aureus* to oxacillin. J Antimicrob Chemother 67:1405-1407

Kassim M, Achoui M, Mansor M, Yusoff KM. 2010. The inhibitory effects of Gelam honey and its extracts on nitric oxide and prostaglandin $E_2$ in inflammatory tissues. Fitoterapia 81:1196-1201

Kwakman PHS, Van den Akker JPC, Güçlü A, Aslami H, Binnekade JM, De Boer L, Boszhard L, Paulus F, Middelhoek P, Te Velde A, Vandenbroucke-Grauls CMJE, Schultz MJ, Zaat SAJ. 2008. Medical-grade honey kills antibiotic-resistant bacteria in vitro and eradicates skin colonization. Clin Infect Dis 46(11): 1677-1682

Lotfi A. 2008. Use of honey as a medicinal product in wound dressing (human and animal studies): A review. Res J Biol Sci 3(1):136-140

Lusby PE, Coombes AL, Wilkinson JM. 2005. Bactericidal activity of different honeys against pathogenic bacteria. Arch Med Res 36(5):464-467

Mandal MD, Mandal S. 2011. Honey: Its medicinal property and antibacterial activity. Asian Pac J Trop Biomed 1(2):154-160

Medhi B, Puri A, Upadhyay S, Kaman L. 2008. Topical Application of honey in the treatment of wound healing: A metaanalysis. JK Sci 10(4)166-169

Mittal L, Kakkar P, Verma A, Dixit KK, Mehrotra MM. 2012. Anti microbial activity of honey against various endodontic micro organisms: An in vitro study. J Int Dent Med Res 5(1):9-13

Molan PC. 1992. The antibacterial activity of honey: 1. The nature of the antibacterial activity. Bee World 73(1)5-28

Molan PC. 1999. Why honey is effective as a medicine. 1. Its use in modern medicine. Bee World 80(2):80-92

Molan PC. 2001. Why honey is effective as a medicine. 2. The scientific explanation of its effects. Bee World 82(1):22-40

Molan PC. 2006a. The evidence supporting the use of honey as a wound dressing. Low Extrem Wounds 5(1):40-54

Molan PC. 2006b. Using honey in wound care. Int J Clin Aromather 3(2):21-24

Mullai V, Menon T. 2007. Bactericidal activity of different types of honey against clinical and environmental isolates of *Pseudomonas aeruginosa*. J Altern Complement Med 13(4):439-441

Natarajan S, Williamson D, Grey J, Harding KG, Cooper RA. 2001. Healing of an MRSA-colonized, hydroxyurea-induced leg ulcer with honey. J Dermatolog Treat 12:33-36

Nassar HM, Li M, Gregory RL. 2011. Effect of honey on *Streptococcus mutans* growth and biofilm formation. Appl Environ Microbio 78(2):536-540

Sare JL. 2008. Leg ulcer management with topical medical honey. Br J Community Nurs 13(9):S22-S32

Snow MJ, Manley-Harris M. 2004. On the nature of non-peroxide antibacterial activity in New Zealand manuka honey. Food Chem 84:145-147

Taormina PJ, Neimira BA, Beuchat LR. 2001. Inhibitory activity of honey against foodborne pathogens as influenced by the presence of hydrogen peroxide and level of antioxidant power. Int J Food Microbiol 69:217-225

Tonks AJ, Cooper RA, Jones KP, Blair S, Parton J, Tonks A. 2003. Honey stimulates inflammatory cytokine production from monocytes. Cytokine 21:242-247

Visavadia BG, Honeysett J, Danford MH. 2008. Manuka honey dressing: An effective treatment for chronic wound infections. Br J Oral Maxillofac Surg 46:55-56

Weston RJ, Mitchell KR, Allen KL. 1999. Antibacterial phenolic components of New Zealand manuka honey. Food Chem 64:295-301

Weston RJ. 2000. The contribution of catalase and other natural products to the antibacterial activity of honey: a review. Food Chem 71:235-239

White JW, Subers MH, Schepartz AI. 1963. The identification of inhibine, the antibacterial factor in honey, as hydrogen peroxide and its origin in a honey glucose-oxidase system. Biochim Biophys Acta 73:57-70

Willix DJ, Molan PC, Harfoot CG. 1992. A comparison of the sensitivity of wound-infecting species of bacteria to the antibacterial activity of Manuka honey and other honey. J Appl Microbiol 73:388-394