Comparison of Physical–Chemical Characteristic and Antibacterial Effect Between Manuka Honey and Local Honey

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Background: Honey has positive effects on wound healing due to its properties to decrease inflammatory reaction, assist wound debridement, lessen wound odor, help moisturize the wound, promote wound healing and has antibacterial effect. In this research we try to confirm the benefit of using Indonesian local honey by comparing its efficacy with Manuka honey, which is already known yet still costly and not readily available in Indonesia.
Methods: Two kinds of honey above were compared for its physical and chemical properties of antibacterial effects and safety, its acidity (pH) using pH meter, osmotic pressure with indirect measurement using hygroscope and hydrogen peroxide concentration using hydrogen peroxide strip test, also for its bacterial contents using standardized microbiological culture.
Results: The measurements of the water activity, pH level,production of hydrogen peroxide, also the bacterial content, showed that there is no significant difference between the manuka honey and the local ones.
Discussion: Both of the honey pH levels are still within the normal range of honey pH level (3.5-4.5) which still have the antibacterial properties to inhibit bacterial growth. Both of the honey water activity level are still within the normal range of honey water activity level (0.500–0.650) which still have the antibacterial properties to inhibit bacterial growth. Manuka honey has been known as non peroxide honey that didn't produce any hydrogen peroxide.
Conclusion: Indonesian local honey which has been used in this research has a similar physical–chemical characteristic as Manuka honey; making it a very good alternative source of medicinal honey because of its affordability and availability.
Keywords: *honey, antibacterial activity, pH, osmotic pressure, water activity, hydrogen peroxide, bacterial contents*

Latar Belakang: Madu memiliki efek positif terhadap penyembuhan berdasarkan komponennya yg memiliki efek menurunkan inflamasi, membersihkan luka, menghilangkan bau, dan memacu penyembuhan luka serta efek anti bakteri. Pada penelitian ini kami ingin membandingkan keberdayagunaan madu lokal Indonesia yang lebih terjangkau harganya dan mudah didapat; dengan madu Manuka yang lebih dulu dikenal.
Metode: kedua madu dibandingkan berdasarkan sifat kimia dan fisik dari efek antibakteri dan keamanannya, tingkat keasamannya menggunakan pH meter, tekanan osmotik dengan pengukuran tidak langsung menggunakan higroskop, konsentrasi hidrogen peroksida menggunakan hidrogen peroksida strip tes, serta jumlah bakteri menggunakan kultur mikrobiologi yang terstandarisasi.
Hasil: Berdasarkan tigkat aktivitas pencairan, derajat keasaman, produksi hidrogen peroksida, serta kontaminasi bakteri, tidak terdapat perbedaan yang signifikan antara madu Manuka dan lokal.
Diskusi: Tingkat keasaman dari kedua madu masih dalam batas normal keasaman madu (3.5-4.5), yang berarti masih memiliki efek antibakteri dengan menghambat pertumbuhan bakteri. Aktivitas pencairan madu masih dalam batas normal, yang berati masih memiliki efek antibakteri dengan menghambat pertumbuhan bakteri.
Kesimpulan: Madu lokal yang digunakan pada penelitian ini, memiliki karakteristik kimiawi dan fisik yang serupa dengan madu Manuka. Sehingga disimpulkan madu lokal Indonesia sama efektifnya dan menjadi alternatif yang baik karena mudah didapat dan terjangkau harganya.

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Honey is the natural sweet substance produced by honey bees from nectar or blossoms or from the secretion of living parts of plants or excretions of plants, which honey bees collect, transform, and combine with specific substances of their own to ripen and mature, according to WHO definition.[1] Quran and Bible have told us about the use of honey as a medicine.[2] Since 1970, honey has been rediscovered as an agent for wound treatment, and there are many sources that confirmed the use of honey as medicine. It has been used to treat skin diseases such as rotten and hollow ulcers[3], respiratory illness, peptic ulcer, eye infection and as oral rehydration for children.[4] The advantages and disadvantages of honey application in wound treatment are shown in Table 1.

Table 1. The advantages and disadvantages of using honey.

Advantages
- It provides a protective barrier to prevent cross-infection - It creates an antibacterial moist healing environment - It rapidly dears infecting bacteria, including antibiotic-resistant strains - It has a debridement effect and its osmotic action causes an outflow of lymph, lifting debris from the wound - It rapidly removes malodor - It hastens healing - It prevents hypertrophic scar - It minimizes the need for skin grafting - It is non-adherent and therefore minimizes trauma and pain during dressing changes
Disadvantages
-Becomes more fluid at higher temperatures -Risk of liquefaction restricts body site -In some patients, stinging sensation in the wound

Because of its natural composition, honey almost doesn't have side effect. The adverse reactions that can happen on honey application are honey allergies and stinging sensation on topical application.[12] This stinging sensation may be due to the acidity of honey as it has not been reported when the acidity is neutralized.

Manuka honey had been widely accepted as a standardized medical honey. It has been known as non peroxide honey with at least 10 unique Manuka factor (UMF) for antibacterial properties.[14,15] The Unique Manuka Factor is the non-peroxide antibacterial activity in Manuka honey, and the numbers used are the concentration of phenol with the same antibacterial activity as the honey. There are a lot of researches in New Zealand and Australia that compare Manuka honey with local honey for its wound healing or antibacterial properties. The results mostly confirmed that there are no significant difference between Manuka honey and local honey.[16-22] This research has never been confirmed in Indonesia, although honey has been accepted as a medicinal herb in here. In other hand, Medical grade honey has not been used widely in Indonesia due to its high cost and scarcity. Even though Indonesia has produced various kinds of commercial honey; none of those are standardized to be used as medicine. In this research we would like to compare the efficacy of Manuka honey with the local ones, which is more affordable and reachable to use.

METHODS

This research is an analytical descriptive study to compare the physical-chemical properties and microbial contents between Manuka honey and local honey. Research is held in Cipto Mangunkusumo Hospital, Physical–Chemical laboratory of Medical Faculty of University Indonesia during March–April 2010.

This research used "Manuka Honey MGO 400 (500 grams)" from Manuka Health New Zealand Ltd. Local honey that have been used for comparison are "Madu Murni Nusantara" from PT Madu Murni Nusantara. Both of honeys had been approved by BPOM (Nusantara Honey–DEPKES RI 137611001072 and Manuka Honey–BPOM RI ML 237606001074). Consecutive sampling of honey was done in Jakarta area during March 2010.

Sample size was determined based on honey avaibility in Jakarta area during March 2010. According to the distributor of Manuka Honey, there are only 3 bottles of Manuka Honey MGO 400 @ 500 grams available during March 2010 in Jakarta area. So this research compared 3 bottles of Manuka Honey (MGO 400, 500 gr) and 3 bottles of local honey (Madu Murni Nusantara, 250 cc).

Honey with high osmotic pressure has been known for its antibacterial effect because the sugar molecules 'tie up' water molecules so that bacteria have insufficient water to support their growth. Researchers had concluded that 0.90 water activity level was need for bacterial growth inhibition.[2,23-25] The water activity of honey is within a range of 0.5–0.65.[24,13] This low water activity is due to the high content of monosacharides (fructose and glucose) and relatively low moisture content.[13] For comparing the osmotic pressure between two honeys, ten grams of honey was taken using analytical balance (AND analytical scale, GR 120, scale of + 0.001 gram). Honey is then observed in a closed compartment with a hygrometer (ABBEON relative humidity indicator, model AB62B). Temperature was kept at 28 degrees Celcius (+1 degree). We observed every 30 minutes until equilibrium point was reached. When equilibrium is reached the reading was constant after 3 x 30 minutes. The equilibrium point was reached after 2 – 3 hours of observations. It will determine the relative humidity that has direct relation to water activity using equation :

$$\pi = (RT/V) \times \log(1/a_w)$$

$$Aw = \frac{RH}{100}$$

Where π is osmotic pressure, R is a constant value, T is temperature, V is partial molal of water, RH is relative humidity.[27,28]

The pH environment of chronic wounds has been recorded within the range of 7.15–8.9, while the normal skin adult pH is in the range of 5.4 to 5.9.[19,20] Usually bacteria growth needs pH level between 7.2 -7.4.[29-31] Low pH level on wound will inhibit bacterial growth. Especially if the pH level was 3.5–4.5.[12] Ten grams of honey was taken using analytical balance (AND analytical scale, GR 120, scale of + 0.001 gram). Honey was than diluted with aquadest until 100 cc. pH level of 20 cc of honey was measured using pH meter (METTLER TOLEDO, MP 220 pH Meter, scale 0.01) that had been calibrated with standardized buffer liquid (pH 4.0 and pH 7.0). The Liquid was measured 3 times, the means value was noted. Hydrogen peroxide that had been known as a antimicrobial agent, is produced in honey because of the glucose oxidase enzyme secreted from the hypopharyngeal gland of the bee into the nectar to assist in the formation of honey from the nectar, and it is explained by this reaction [12,32]:

glucose oxidase

Glucose + H2O + O2 --> Gluconic acid + H2O2

In some honeys that had been treated with catalase to remove the hydrogen peroxide activity, additional non-peroxide antibacterial factors have been identified.[10,33] However, the hydrogen peroxide concentration produced in honey and activated by dilution is typically around 1 mmol/l, about 1000 times less than in the 3% solution commonly used as an antiseptic but still effective as an antimicrobial agent. For measuring hydrogen peroxyde, 10 grams of honey was taken using analytical balance (and analytical scale, GR 120, scale of + 0.001 gram), and then diluted with aquadest until 50%, 20%, 10% and 5% of concentration was achieved. Hydrogen peroxide contents measured qualitatively using Peroxide Strip Test (Quantofix, Macherey–Nagel) after 1 hour. With scale of 0.5 – 2 – 5 – 10 – 25 mg/L. the honey was measured 3 times. Honey has distinctive properties that inhibit or kill most microorganisms. Hence, microbes of interest to the honey processing industry are those that withstand the concentrated sugar, acidity and antimicrobial character of honey. Usually bacteria could not live in honey after 8–24 days.[8] Except for spore producing bacteria such as *Bacillus sp* and *Clostridium sp*. Spores of *Clostridium sp*. could live in honey for 1 year with 4 degrees Celcius.[8] Honey sample was

cultured in Brucella, Kanamycin and Thioglucoid media with metronidazole disc. The media is then placed on anaerobic environment for 2 x 24 hours. Growth zone then was observed for *Clostridium sp.* using HE coloring. If there were not a growth for two weeks, the honey then considered sterile of *Clostridium sp.* Honey was processed with standardized TPC methods to confirm whether there are *Bacillus sp* contamination.

RESULTS

On Table 2, there are the results of relative humidity and water activity measurement. With Manuka honey and local honey water activity means are 0.620 and 0.632.

Table 2. Water activity measurement

Honey	Relative Humidity / RH (%)	*Water activity* / A_w
Manuka A	62.5	0.625
Manuka B	62.0	0.620
Manuka C	61.5	0.615
Local A	62.5	0.625
Local B	63.5	0.635
Local C	63.5	0.635

For measuring pH, 10 grams of honey was measured three times using analytical balances, then being diluted with aquadest until 100 cc. The pH level of 20 cc of the honey was measured using pH meter that had been calibrated to standardized buffer (pH 4.0 and pH 7.0). The measurement was taken 3 times, and the means value was noted. From Table 3, the pH level means of Manuka honey was 4.115 and local honey was 4.225. From Table 3, the pH level means of Manuka honey was 4.115 and local honey was 4.225

Measurement of hydrogen peroxide was done with 10 grams of honey that measured three times using analytical balance, then being diluted with aquadest until 50%, 20%, 10% and 5% of concentrations. The hydrogen peroxide production then measured using the peroxide strip test after 1 hour of observation.

Table 3. Measurement of pH level

Honey	Mean value of pH level
Manuka A	4.12
Manuka B	4.10
Manuka C	4.12
Local A	4.31
Local B	4.22
Local C	4.24

The measurement was done 3 times, and the result was noted. From Table 4, we can see that from all of the honey samples and concentrations, there were no hydrogen peroxide production was found.

Table 4. Measurement of hydrogen peroxide production

Honey	Hydrogen Peroxide			
	50 %	20%	10%	5%
Manuka A	-	-	-	-
Manuka B	-	-	-	-
Manuka C	-	-	-	-
Local A	-	-	-	-
Local B	-	-	-	-
Local C	-	-	-	-

For testing the contain of *Clostridium sp* bacteria, a sample of honey was taken when the bottle was opened the first time to prevent secondary contamination. The sterility examination was done using TPC methods. From Table 5, we could see that one of Manuka honey (Manuka A) contain *Bacillus sp* (1,25 x 102 CFU). And all of local honeys contain *Bacillus sp* (3x 101 - 1.85 x 102 CFU). From all of

Table 5. TPC examination for *Bacillus sp* and *Clostridium*

Honey	TPC *Bacillus sp* (colony / ml)	TPC *Clostridium sp* (colony / ml)
Manuka A	$1{,}25 \times 10^2$	Steril
Manuka B	Steril	Steril
Manuka C	Steril	Steril
Local A	3×10^1	Steril
Local B	1.85×10^2	Steril
Local C	1.6×10^2	Steril

the honey samples in this research, none of them contains *Clostridium sp* bacteria.

DISCUSSION

From this research, we measured the mean value of water activity level from Manuka honey and local honey is 0.620 and 0.632. Although the local honey water activity level it is not too far different from the Manuka honey water activity level. Both of the honey water activity level are still within the normal range of honey water activity level (0.500 – 0.650) which still have the antibacterial properties to inhibit bacterial growth.[2,3,15] Bacterial growth needs an environment with at least 0.900 of water activity level.[4,16-18]

Measurement of honey pH level using pH meter had been done by many other researchers.[40-42] From this research, mean pH level value of Manuka honey and local honey are 4.115 and 4.225.

Although the local honey pH level it's not too far different from the Manuka honey's pH level. Both of the honey pH levels are still within the normal range of honey pH level (3.5 - 4.5) which still have the antibacterial properties to inhibit bacterial growth.[6,8,40] Bacteria usually live in alkali environment (7.2-7.4).[21-23] Method of measurement of hydrogen peroxide contents had been done by other researchers.[33] From this research, there were no hydrogen peroxide productions. It is however possible that the hydrogen peroxide production was not at its peak at the time of testing. It has been shown that hydrogen peroxide production can peak at different times for different honeys. Some may take as long as 24 hours.[33] Exposure of honey to heat and light also deactivates the enzyme that produce hydrogen peroxide.[24] And Manuka honey has been known not to produce any hydrogen peroxide.[29]

From the sterility examination with TPC methods, this research found that there are *Bacillus sp* content on Manuka honey and local honey (3 x 101 – 1.86 x 102 CFU). But this micro-biological examination can only describe the genus of the microbes, because we could not further describe the species name. This research also found no *Clostridium sp* bacteria on both honeys. We did not find any other microbes due to honey antibacterial properties.[21] Although we found a number of *Bacillus sp* contamination on both honeys, the quantity of bacteria is still not enough to cause infection process on wound. Bowler et al confirmed that microbacteria needed minimally 1 x 104 CFU of bacteria to cause infection process.[44]

There is no *Clostridium sp* content in this research, because it rarely exists on honey.[29,43,46] *Clostridium sp* can survive as spores in honey but cannot multiply or produce toxins due to the inhibitory properties of honey.[45] *Bacillus sp* that was found in both of honeys, was commonly found on commercial honeys that was sold in the free market. This bacterial content is usually caused by primary sources include pollen, the digestive tracts of honey bees, dust, air, soil and nectar.[21,46] Even though we still can not eliminate the possibilities of secondary contamination. Sterilized honey that had been radiated with gamma radiation do not have bacterial contamination that can be used to treat wounds.[13] One example of sterilized honey to treat wounds is Medihoney.[47,48]

CONCLUSION

The local honey that had been used in this research as a new armament in treating wounds, is safe, cheaper and available nationwide. The local honey that been used in this research has a similar physical–chemical characteristic a Manuka honey. So the local honey that been used in this research could be use for wound treatment with the honey attributes of anti-inflammatory activity, antimicrobial activity, promotes debridement, reduces malodors, maintains moist wound environment and stimulates healing.

Medical health provider could use local honey that been used in this research for wound treatment. Further research is needed to determine the advantages of local honey in this research especially in treating chronic wounds.

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