

Antibacterial Activity of Indonesian Local Honey Against Strains of *P. Aeruginosa*, *S. Aureus* and MRSA

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Background: Honey has been used in wound care since ancient times. Many publications attest antibacterial activity of manuka honey against *Pseudomonas aeruginosa*, *S. aureus*, and Methicillin-resistant *S. aureus* (MRSA). However, antibacterial effect of local honey from Indonesia has never been studied and compared to medical grade honey before. This study aim to compare antibacterial activity of local honey and manuka honey against *P. aeruginosa*, *S. aureus* and MRSA

Methods: The honeys were tested for their antimicrobial activities with broth dilution method. Different concentrations of honey were prepared in reaction tubes. Strains of *P. aeruginosa*, *S. aureus*, and MRSA were grown in nutrient broth. These strains of bacteria then added to the different concentrations of honey. Minimum Inhibitory Concentrations (MIC) is the lowest concentration of the honey that yielded no growth of bacteria.

Results: MIC of local honey for *P. aeruginosa* is 50%, for *S. aureus* is 100% and for MRSA is 100%. MIC of manuka honey for *P. aeruginosa* is 12,5%, for *S. aureus* is 25%, and for MRSA is 12,5%.

Conclusion: Nusantara Manuka have the similar antibacterial activity spectrum against *P. aeruginosa*, MRSA and *S. aureus*, but it must be given in greater concentration to exert the same level of antibacterial activity with Manuka honey.

Keywords: *honey, wound care, antibacterial activity*

Latar Belakang : Madu telah digunakan dalam perawatan luka sejak dahulu kala. Banyak penelitian mengenai efek antibakteri madu terutama madu manuka. Saat ini, madu manuka hanya bisa didapatkan di kota besar dengan harga yang lebih mahal daripada madu lokal Indonesia. Sedangkan efek antibakteri madu lokal Indonesia belum pernah diteliti dan dibandingkan dengan madu manuka.

Metode: Efek antibakteri kedua madu ini diteliti dengan metode dilusi dengan kaldu. Konsentrasi berbeda dari kedua madu dibuat dalam beberapa tabung reaksi. Kuman *P. aeruginosa*, *S. aureus* dan MRSA yang telah dibiakkan pada media kaldu, ditambahkan ke dalam masing-masing konsentrasi. Tingkat kekeruhan pada tabung reaksi dibandingkan dengan tabung kontrol yang tidak ditambahkan kuman. Konsentrasi terendah dari madu yang menunjukkan tidak adanya kuman yang tumbuh disebut sebagai Minimum Inhibitory Concentration (MIC)

Hasil : MIC dari madu murni nusantara untuk *P. aeruginosa* adalah 50%, untuk *S. aureus* adalah 100% dan untuk MRSA adalah 100%. MIC dari madu manuka untuk *P. aeruginosa* adalah 12,5%, untuk *S. aureus* adalah 25% dan untuk MRSA adalah 12,5%

Kesimpulan : Madu Nusantara memiliki spektrum aktivitas antibakteri terhadap *P. aeruginosa*, *S. aureus*, dan MRSA yang sebanding dengan madu Manuka, namun perlu diberikan dalam konsentrasi lebih besar untuk mencapai efektivitas antibakterial yang sebanding dengan madu Manuka.

Kata kunci: *madu, perawatan luka, aktivitas antibakteri*

Honey, according to WHO definition, is a natural sweetener produced by *Apis mellifera* bee extracted from plants, collected, deposited, dehydrated inside the bee hive^{1, 2}. Honey has been used in wound care since ancient times^{3,4,5}. In the 1970's, its use in wound care has been reintroduced to the medi-

cal world once again, mainly for infected operative wound, burns, and pressure ulcer^{6,7,8}.

Honey has several important characteristics for wound care, such as anti-inflammatory activity, antibacterial activity, aid in debridement, odor reducing agent, maintaining moisture in wound, thus enhanced wound healing activity^{9,10,11,12,13,14}. Antibacterial activity in

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Table 1. Sensitivity test against *P. aeruginosa*, *S. aureus* and MRSA.

No	Antibiotics	<i>P. aeruginosa</i>	MRSA	<i>S. aureus</i>
1	Amoxicillin	-	R	R
2	Amox+Clav	R	I	R
3	Sulbenicillin	R	R	R
4	Carbenicilin	R	-	-
5	Cefotiam	R	-	-
6	Ceftazidime	R	-	-
7	Cefoperazone	I	-	-
8	Gentamycin	S	R	R
9	Tobramycin	S	-	-
10	Chloramphenicol	-	R	R
11	Tetracyclin	-	R	S
12	Cotrimoxazole	-	R	S
13	Clindamycyn	S	S	S
14	Ciprofloxacin	S	R	I
15	Amp Sulbactam	-	R	R
16	Aztreonam	S	-	-
17	Dibekacin	S	-	-
18	Ofloxacin	S	R	R
19	Clarithromycin	-	R	S
20	Vancomycin	-	S	S
21	Azithromycin	-	R	R
22	Oxacillin	-	R	S
23	Cefepime	S	-	-
24	Sulferazone	S	R	S
25	Imipenem	S	-	-
26	Piperacillin	S	-	-
27	Tigecycline	R	S	S
28	Levofloxacin	S	S	S
29	Meropenem	I	-	-
30	Fosfomycin	I	R	I
31	Linezolid	-	S	S
32	Moxifloxacin	-	S	S

honey is one of the main characteristics in honey for wound treatment. Its antibacterial activity is due to the low osmolarity, low acidity level (pH) and it produces hydrogen peroxide. Honey also has other unidentified components with antibacterial effect^{12,15}. Many publications attest to the antibacterial activity of honey against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Methicillin-resistant Staphylococcus Aureus (MRSA)*^{5,16}. Unfortunately, medical grade honey has not yet available nationwide and the cost of it are still very high for most of Indonesian people.

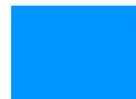
In Indonesia, local honey has been used by the non-medical society for various purposes. Despite many kinds of local honey produced in Indonesia, none of them had ever been proven for its efficacy in wound treatment. Antibacterial effect of local honey from Indonesia has

never been studied and compared to medical grade honey before.

In this study, we want to compare antibacterial activity of local honey and manuka honey against *P. aeruginosa*, *S. aureus* and MRSA.

METHODS

This study is an analytical descriptive study to compare antibacterial effect of Indonesian local honey and Manuka honey against *P. aeruginosa*, *S. aureus* and MRSA. The study was being conducted at the microbiology laboratory in the Medical Faculty of the Universitas Indonesia from March – April 2010. We used 3 bottles of “Manuka Honey MGO 400 (500 gram)” produced by Manuka Health New Zealand Ltd, compared to 3 bottles of Indonesian



local honey "Madu Murni Nusantara" produced by PT Madu Murni Nusantara. Both honeys have been certified by BPOM (Madu Nusantara - DEPKES RI 137611001072; Madu Manuka - BPOM RI 237606001074). Honey samples were taken with consecutive sampling from all the honey in the DKI Jakarta area on March 2010.

Antibacterial activity of these honeys was tested against *P. aeruginosa*, *Staphylococcus aureus* and MRSA, obtained from bacteria isolation of patients' wounds in Cipto Mangunkusumo hospital Jakarta.

Dilution method was used to obtain antibacterial effect of each honey. Honey was diluted with sterile Mueller Hinton broth to have various concentration of honey, from 1% to 100%. A broth jelly media without honey was used as a control for the bacterial growth. Phenol was also used against these strains of bacteria as a control. *P. aeruginosa*, *S. aureus* and MRSA were cultured each in broth jelly at 37°C for 4 hours, to then be added to the various honey concentration media. These media then incubated at 37°C for 24 hours and afterward examined for bacterial growth. Minimum Inhibitory Concentration (MIC) is the lowest concen-

tration of honey which can prevent the growth of bacteria in the media. MIC was obtained by comparing the clarity level of various media concentration to control.

Sensitivity test was also conducted against these strains of bacteria, to obtain level of resistancy against antibiotics of these bacteria.

RESULTS

P. aeruginosa used in this study have resistancy towards amoxycillin, amoxycillin clavulanat, sulbenicillin, carbenicillin, cefotiam, ceftazidim dan tigecyclin. MRSA only have sensitivity against clindamycin, vancomycin, tigecyclin, levofloxacin, linezolid dan moxifloxacin. *S. aureus* are resistant towards amoxycillin, amoxycillin clavulanat, sulbenicillin, gentamycin, chloramphenicol, ampicillin sulbactam, ofloxacin, azithromycin. (Table 1).

From the dilution method, MIC of *P. Aeruginosa* from manuka honey was 12,5%. The MIC for Indonesian local honey was 50%. (Table 2). The result for MRSA also available (Table 3).

Table 2. MIC for *P. aeruginosa*

<i>Pseudomonas aeruginosa</i>	100%	50%	25%	12,5%	6,25%	3,125%
Manuka honey	-	-	-	-	+	+
Local honey	-	-	+	+	+	+

Table 3. MIC for MRSA

MRSA	100%	50%	25%	12,5%	6,25%	3,125%
Manuka Honey	-	-	-	-	+	+
Local Honey	-	+	+	+	+	+

Table 4. MIC for *S. aureus*

<i>S. aureus</i>	100%	50%	25%	12,5%	6,25%	3,125%
Manuka Honey	-	-	-	+	+	+
Local Honey	-	+	+	+	+	+

Table 5. MIC of manuka honey and Indonesian local honey against *P. aeruginosa*, *S. aureus* and MRSA.

Bacteria strains	MIC for Manuka honey	MIC for local honey
<i>P. aeruginosa</i>	12,5 %	50%
<i>S. aureus</i>	25%	100%
MRSA	12,5%	100%



Figure 1. Reaction tube consist of MRSA in honey concentration media of 100% and 50%, In 100% tube, clarity level is the same as control, while in 50% tube, there were sedimentation.

From the dilution method, MIC of *S. Aureus* from manuka honey was 25% (Table 4). The MIC for Indonesian local honey was 100% (Table 5).

In this study, we found that the clarity level of *P. aeruginosa* for the local honey was uncertain. Thus, we cultured the bacteria from the local honey media to a blood jelly media. From this media, we can see that in 50% concentration of local honey, bacteria can not grow (Figure 1-2).

DISCUSSION

Antibacterial activity of honey is due to its low osmolarity, low pH, its hydrogen peroxide and other unidentified components^{12,15}. In this study, from the sensitivity test against 3 bacteria, we can see most of antibiotics commonly used have already been resistant. This is probably due to the unreasonable use of antibiotics in Cipto Mangunkusumo hospital causing resistancy of bacteria towards these antibiotics. The high resistancy of antibiotics will make wound healing in infected wound even harder to be achieved.



Figure 2. *P. aeruginosa* culture in blood jelly media. In 50% concentration of local honey, there were no bacteria growth.

From the dilution method, MIC of Indonesian local honey was highest achieved against *P. aeruginosa* which is 50%. This means even by diluting local honey to 1:2, it will still have antibacterial activity against *P. aeruginosa*. This bacteria can not live in acid condition. Honey has a pH of 3,5 – 4,5 causing an acid condition, and if this acidity can be maintain, then *P. aeruginosa* can not live in this condition^{6,8,9}.

Even if bacteria can live in acid condition, honey will cause plasmolysis due to its low osmolarity level. The high level of glucose in honey make a low osmolarity level, and may cause osmosis between intracellular liquid with extracellular liquid. If bacteria cell is suspended in high concentrate of glucose, water will come out of the intracellular, thus plasmolysis occurred. In this study, MIC for local honey against *P. aeruginosa* has more effectivity than against MRSA nor *S. aureus*. This is probably because honey antibacterial activity against *P. Aeruginosa* is achieved not only because of the low osmolarity level but also due to its acid condition.

From all MIC results, manuka honey has the MIC lower than local honey. In several literatures, it was found that there is methy-



lglioxil substance in manuka honey. This substance is said to be the main cause of manuka's great antibacterial effect. Our Indonesian local honey has never been tested for the availability of methylglyoxil.

MIC indicates that honey has antibacterial activity against these bacterias, and this effect can be used to treat infection, including wound infection. The high concentration of local honey MIC means that to obtain its antibacterial effect, honey cannot be diluted less than its MIC. This has correlation especially in wounds with high level of exudation. Exudation can dilute honey, causing honey to loose its antibacterial effect if diluted under the MIC concentration. Thus, for highly exudative wound, frequent change of dressing using honey is important to prevent dilution of honey, and to achieve its optimal antibacterial effect.

CONCLUSION

This study concluded that Manuka honey has MIC lower than Indonesian local honey (*madu lokal nusantara*). Indonesian local honey (*madu lokal nusantara*) has antibacterial activity against *P. aeruginosa*, MRSA and *S. aureus*. This effect has been tested against bacterias cultured from Cipto Mangunkusumo hospital.

Antibacterial effect of local honey can be used in treatment for infected wound, especially wounds infected by *P. aeruginosa*, MRSA and *S. aureus*. Furthermore, to obtain its antibacterial effect in infected wounds, honey needs to be applicated as wound dressing according to the wound condition itself, especially the amount of exudation which can dilute honey concentration.

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