



UNIVERSITI PUTRA MALAYSIA

***ANTIMICROBIAL PROPERTIES OF LOCAL HONEY AGAINST
PATHOGENIC WOUND CAUSING BACTERIA IN ANIMALS***

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PATHOGENIC WOUND CAUSING BACTERIA IN ANIMALS**



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It is hereby certified that I have read that this project paper entitled “Antimicrobial properties of local honey against pathogenic wound causing bacteria in animals” by Jiavendrasingh Mussafeer and in my opinion it is satisfactory in terms of scope, quality and presentation as a partial fulfillment of the requirement of the course VPD4999-Final year project.



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Dedications

I dedicate this thesis to all the animals who have been suffering from chronic non-healing wounds and constantly in pain, until they were treated with honey.

This also goes to Mom and Dad, who went through all the ups and downs in order to make their son achieve his dream of being a veterinarian.

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E.coli: *Escherichia coli*

MIC: Minimum Inhibitory concentration

°C: Degree Celsius

Abstract

An abstract of the paper presented to Faculty of Veterinary Medicine in partial fulfilment of the course VPD4999-Final year project.

Antimicrobial properties of local honey against pathogenic wound causing bacteria in animals

By

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Supervisor: Assoc. Prof. Dr. Zunita Zakaria

Honey has been used for medicinal purposes since the times of ancient civilisations because of its known antimicrobial, anti-inflammatory and healing properties. This study aimed to investigate the antimicrobial properties of local Malaysian honey against common pathogenic wound associated bacteria in animals. Five types of honey which comprised of local honey; Kelulut, multifloral, Propolis, artificial and imported honey; Manuka, were tested for their antibacterial activity against eight bacteria among which four were Gram positive (Methicillin Resistant *Staphylococcus aureus* (MRSA), Vancomycin Resistant Enterococci (VRE), *Bacillus*

subtilis and *Staphylococcus intermedius* and four were Gram negative bacteria (*Proteus mirabilis*, *E.coli*, *Pseudomonas aeruginosa* and *Pasturella multocida*). The well diffusion method was performed to screen pure undiluted honeys against bacteria, and the zones of inhibition were measured. The Minimum Inhibitory Concentration (MIC) for each honey was then determined using the microbroth dilution method. The most susceptible bacteria was *P. multocida* (mean inhibition zone of 20.96 ± 3.56 mm in diameter), compared to *P. mirabilis* (5.71 ± 3.86 mm), being the least susceptible. There was a significant difference among the types of honey and the inhibition zone ($p < 0.05$), with the most effective honey being Manuka, followed by Propolis, kelulut, Multifloral and lastly artificial honey. Minimum inhibition concentration results revealed that Kelulut was the most effective honey with mean MIC of (18.13 ± 5.82) %, followed by Propolis (31.25 ± 8.44 %), Manuka (34.38 ± 9.38 %) and lastly artificial honey (34.38 ± 9.38 %). Polyfloral honey barely inhibited bacteria when diluted. It was noted that few of the honeys which are not efficient in pure form were effective when diluted. This may be due to the release and action of hydrogen peroxide when the honeys are diluted. In conclusion, Malaysian honey (Kelulut, Multifloral, Propolis) do possess antimicrobial properties against important pathogens and has the potential to be used for wound treatment.

Key words: Wound pathogens, Animals, Honey, Antimicrobial properties

Abstrak

Abstrak ini daripada kertas kerja projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999-Projek.

Ciri-ciri antimikrobial madu tempatan terhadap bakteria patogenik yang menyebabkan jangkitan luka dalam haiwan

Jiavendrasingh Mussafeer

2015

Penyelia: Prof Madya Dr. Zunita Zakaria

Madu telah digunakan untuk perubatan sejak tamadun purba kerana ianya diketahui untuk ciri-ciri antimikrob, anti-inflamasi dan penyembuhan. Tujuan pengajian ini adalah untuk menyiasat ciri-ciri antimikrob madu tempatan Malaysia terhadap bakteria patogenik umum yang terlibat dengan luka dalam haiwan. Lima jenis madu yang terdiri daripada madu tempatan; Kelulut, multiflora, propolis, madu tiruan dan madu yang diimport; Manuka, telah diuji untuk aktiviti antibakteria terhadap lapan bakteria yang terdiri daripada empat bakteria Gram positif iaitu Methicillin Resistant *Staphylococcus aureus* (MRSA), Vancomycin Resistant Enterococci (VRE), *Bacillus subtilis* dan *Staphylococcus intermedius* dan empat bakteria Gram

negatif (*Proteus mirabilis*, *E.coli*, *Pseudomonas aeruginosa* dan *Pasturella multocida*). Teknik 'agar well diffusion' telah digunakan bagi menyaring madu tulen terhadap bakteria dan zon rintangan telah diukur. 'Minimum Inhibitory Concentration' (MIC) untuk setiap madu juga telah ditentukan dengan menggunakan teknik pencairan 'microbroth'. Bakteria yang paling sensitif adalah *P. multocida* (purata zon rintangan 20.96 ± 3.56 mm diameter) berbanding dengan yang paling rintang *P. mirabilis* (5.71 ± 3.86 mm). Perbezaan ketara dapat dilihat antara jenis madu yang digunakan dan zon rintangan ($p < 0.05$), dengan Manuka madu yang paling efektif, diikuti oleh Propolis, Kelulut, Multifloral dan madu tiruan. Antara 5 madu yang telah diuji, Kelulut adalah yang paling efisien terhadap semua bakteria dengan nilai MIC antara (18.13 ± 5.82) %, diikuti oleh Propolis (31.25 ± 8.44) %, Manuka (34.38 ± 9.38) % dan yang terakhir adalah madu tiruan (34.38 ± 9.38) %. Madu multiflora tidak dapat merencat bakteria apabila ianya dicairkan. Ini mungkin kerana kesan pembebasan dan aktiviti hidrogen peroksida apabila madu dicairkan. Beberapa madu yang tidak efisien dalam bentuk tulen adalah efektif apabila ianya dicairkan. Kesimpulannya, madu tempatan Malaysia (Kelulut, multiflora, Propolis) mempunyai ciri-ciri antimikrob terhadap patogen yang penting dan mempunyai potensi untuk digunakan dalam pengubatan luka.

Kata Kunci: Patogen luka, Haiwan, Madu, Ciri-ciri antimikrob

1.0 INTRODUCTION

"Grind to a powder river dust and... (here the words are missing)... then knead it in water and honey and let plain oil and hot cedar oil be spread over it". These words dating from 2000BC, are the oldest of any written prescription record, probably a cure for a skin disease, which was written on a clay tablet from Nippur which the Sumerians consider as a religious centre in the Euphrates Valley, Circa (Kramer and Levey, 1954). In fact, besides being a food supplement, a treat and a product for religious purpose, honey has long been considered as a treatment drug. Amazingly, other scriptures mentioning the use of honey as means of treatment were found in sacred books of people from the Indus valley and Ganges valleys dating from over 1000BC, as well as in Egypt whereby many hieroglyphics and Papyrus give a remarkable picture of the wide use of honey (Jones, 2009). In addition, holy books like the bible and the Koran both make reference to honey as being a cure for man (Jones, 2009). These historical findings have one thing in common; honey was used specifically for treatments, that is, for dressing wounds and burns, for conjunctivitis, for gastric ulcers and diarrhoea. A question mark hereby hangs behind what makes this natural substance so renowned to the point that until today countless researchers are trying to elucidate its properties. In light of modern science, it has now been so far demonstrated that honey is in fact an antimicrobial agent, acts as an anti-inflammatory, helps in wound debridement, and fastens wound healing as demonstrated by Sekham and Cooper, 2013.

In the recent years, there has been a steep rise of multidrug resistance among the pathogenic bacteria and is regarded as this century's most pressing problem. In the effort to look for affordable and easily accessible alternative medicine to overcome multidrug resistant bacteria, this study was carried out to determine the antibacterial properties of selected locally produced honey and Manuka honey (from Australia) against pathogenic bacteria. The effectiveness of the tested honey was also determined by measuring the minimum inhibitory concentration (MIC).

2.0 LITERATURE REVIEW

As new pharmaceutical drugs emerged to be part of modern medicine, honey gradually went out of fashion. However, as a result of overuse or misuse of antimicrobial, one of the biggest threats in veterinary medicine is the emergence of antibiotic resistance which is currently gaining grounds at an alarming rate. The management of a chronic non-healing wound contaminated with a resistant bacteria can be sometimes be very frustrating, coupled with the fact that it causes prolonged discomfort to the animal. It is thus high time to seek for alternative medicine, being the reason of why honey is now being rediscovered.

2.1 Properties of honey

Honey has been known to have anti-inflammatory properties, wound debriding properties, tissue growth stimulation and most importantly antibacterial property.

Inflammation means pain, exudate and oedema and honey helps to prevent these; it actually contains a protein the bees add to the nectar they collect when making honey. This protein is called Albumin-1 and it suppresses the action of white blood cells engulfing bacteria which will trigger an immune response. It also has a debriding action; in a chronic non-healing wound there is usually a high level of proteases which actually degrades the wound further, and this works best in an alkaline pH. However honey is acidic and also honey has an osmotic effect and this will encourage lymphatic flow towards the wound and devitalised tissues. This promotes autolytic debridement by bringing plasminogen into the environment. Lastly, honey contains a wide range of amino acids, trace minerals and vitamins, It promotes formation of clean healthy granulation tissue and hastens epithelisation, and thus there is no need for skin graft (Molan, 2002). Besides all these factors stated, honey has most importantly an antimicrobial property which will be the main highlight of this study.

Indeed, honey has an established function as an antibacterial agent that has a broad spectrum of activity against gram-positive and gram-negative bacteria (Brudzynski et al., 2012). There are several factors that contribute to this. The high sugar content and low water content makes honey a substance with high osmolarity and coupled with this, honey also has an acidic pH and therefore these make it a very unsuitable environment for bacteria to grow. The high osmolarity feature also plays a role in drawing lymph from the wound by the osmosis process (along the concentration gradient) and also the high sugar content is used by the infecting bacteria in

preference to amino acids and this results in production of amino acids which makes the environment even more acidic (Molan, 2002). However the most important factor is the presence of inhibine which has been proven to exist in several honeys from different floral origins (Mathews and Binnington, 2012). Inhibine which comes from the bee's pharyngeal glands breaks down to hydrogen peroxide, which is the main factor for antimicrobial property. Also honey contains glucose oxidase enzyme which continuously produces further hydrogen peroxide once diluted, as well as gluconic acid (main acid in honey) (Mathews and Binnington, 2002). In addition to that, honey which is a thick viscous substance provides a physical barrier against bacteria from penetrating the wound from the outside environment.

2.2 Types of honey

2.2.1 Manuka

Manuka honey is a monofloral honey derived from the manuka tree (*Leptospermum scoparium*) in New Zealand and has greatly attracted the attention of researchers for its superior antimicrobial properties. Manuka is described as a non-peroxide honey, unlike the common honeys described previously and hence has a differently unique mechanism for its antimicrobial component. This honey has a range of photochemicals, one of which is methylglyoxal, which gives it a more sustained antimicrobial action (Hollis, 2012). Honey producers gave manuka a rating called as UMF (Unique Manuka Factor). A medical grade honey must have at least a UMF of 10+, which is equal to 10% of phenol (Hollis, 2012). According to Molan, 2012, Manuka was effective against 7 major wound isolated bacteria and a MIC ranging

from 1.8-10.8 %, although, Hollis, 2012 claims that same honey to work against over 70 wound pathogens. In the same line of thought, Kelulut honey was used in this study.

2.2.2 Kelulut

Kelulut is produced by a tiny stingless bee (*Trigona* spp.) which is commonly found in Malaysia. An experiment carried out by Zainol et al., 2013, showed that this honey had an MIC of 20% against *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* and *Pseudomonas aeruginosa*.

2.2.3 Polyfloral

A polyfloral honey is made from a variety of non-specific flowers, however very few studies have been carried out regarding polyfloral honeys. This is probably because these kind of honeys are based on the flowers normally found in a particular country, which varies from one place to another.

2.2.4 Propolis

Propolis is a mixture of resinous material with hypo-pharyngeal secretions, pollen and beeswax. Propolis in Greek literally means “protect the city” and indeed, the bees use it in their hive for the comb’s construction and polishing and since it is rich in flavonoids (nature’s antibiotic), it provides an aseptic environment inside the nest.

The chemical composition will however vary from regions to regions depending on the bees involved and flowers used (Muli et al., 2008).

2.2.5 Artificial honey

Artificial honey are usually cheaply priced honey that are sold in supermarkets. Some of the producers claim that the honey are pure, however its purity is doubtful.

3.0 MATERIALS AND METHOD

3.1 Honey samples

Five (5) honeys, Kelulut, Polyfloral, Propolis, artificial honey and Manuka honey were selected for this experiment (Table 1). Selection was made among the locally produced honey (Malaysia) on a random basis. All honeys were obtained from a local hypermarket, with the exception of Kelulut honey which was obtained from a beekeeper at the Malaysia Agriculture, Horticulture and Agrotourism International Show (MAHA) 2014. The honeys were stored in a dark place at room temperature, and once opened, the honey bottles/containers were wrapped with aluminium foil and kept at 4°C. When the honeys had to be used in the experiment, an aliquot was transferred into a laboratory glass bottle as the working solution.

Table 1: The Different Types of Honeys Tested

Brand name	Honey name	Origin
Syairtimur	Kelulut	Local
Madu Unggul	Polyfloral	Local
Madu Unggul	Propolis	Local
Rosebee	Artificial honey (Fraser's Hill)	Local
Waitemata	Manuka UMF 20+	New Zealand

3.2 Physiochemical study

The appearances of the honeys were observed and their pH was determined.

3.3 Bacterial isolates

Eight (8) bacteria known to be pathogenic and commonly isolated from animals' wounds were chosen to be tested in this study. The bacteria comprised Methicillin-resistant *Staphylococcus aureus* (MRSA), Vancomycin resistant enterococci (VRE), *Staphylococcus intermedius*, *Escherichia coli*, *Pasteurella multocida*, *Proteus mirabilis*, *Bacillus subtilis*, and *Pseudomonas aeruginosa*. The pure cultures of these bacteria were obtained from the culture collection at the Bacteriology laboratory of Faculty of Veterinary Medicine, Universiti Putra Malaysia. These pure cultures were subcultured onto blood agar and incubated at 37°C for 24 hours. The cultures were stored at 4°C. Laboratory biochemical tests were carried out to reconfirm the

identity of these bacteria. Gram staining for each bacteria was first carried out to classify them as gram positive or gram negative. Once the bacteria were classified by gram staining, the appropriate corresponding biochemical tests were carried out. The gram positive bacteria were subjected to catalase, coagulase, urease, glucose, nitrate reduction, sucrose, hemolysis, trehalose, NaCl, lactose, sorbitol and SIM tests. Gram negative bacteria were subjected to citrate, urease, triple sugar iron (TSI) and (Sulphide indole motility) SIM tests.

3.4 Antibiotic sensitivity test

The standard Kirby bauer method was employed, whereby the above mentioned bacteria were tested for their sensitivity against common antibiotics. Commercially available antibiotic discs (Oxoid Ltd., UK) were used and these included Streptomycin (25 µg), Gentamicin (30 µg), Enrofloxacin (5 µg), Tetracycline (30 µg), Ampicillin (10 µg) and Chloramphenicol (30 µg). Bacterial suspension for each bacteria was prepared by mixing the bacteria with sterile saline in a test tube and the turbidity of the suspension was adjusted to make it equivalent to 0.5 Mc Farland making the suspension contained approximately 10^8 cells/ml. A sterile swab was dipped inside the test tube, soaked in the suspension and any excess moisture was expressed by pressing the swab against the side of the tube. The Mueller-Hinton (Oxoid Ltd., UK) plate was entirely swabbed onto the plate 3 times, rotating the plate 90° each time to obtain a uniform inoculation. The plates were allowed to dry for 5 min, after which the discs were dispensed on the plates using a discs dispenser. A sterile forceps was used to press the discs to make sure they are in contact with the

agar surface. The plates were incubated overnight at 37⁰C and the diameter of the zone of inhibition was measured using a Vernier calliper. The determination of the susceptibility pattern of the tested organism towards the antibiotics were determined by comparing the zone of inhibition to the Clinical Laboratory Standards Institute (CLSI) (2013).

3.5 Antimicrobial property of honey

3.5.1 Well diffusion assay

The well diffusion assay was employed to determine the antibacterial properties of the tested honeys. This method was adapted from Khalil et al., 2014. Briefly, two Mueller-Hinton (MH) plates were used for one bacteria; three wells were made for 3 honeys on the first plate and two wells were made for the other two honeys on the other plate. Both plates contained a positive control with enrofloxacin antibiotic disc and a negative control with a blank disc inoculated with sterile saline. The wells were punched using a 6mm diameter sterile cork borer. Following to that, each plate was seeded with the respective inoculum of 1×10^8 CFU/ml as described previously. A 60 μ l honey samples, freshly aliquoted, were pipetted into the allocated wells. The control discs were placed gently using sterile forceps. All the plates assay were done in duplicates. The plates were incubated overnight at 37⁰C and the diameter of the zone of inhibition was measured using a Vernier calliper.

3.5.2 Minimum inhibitory concentration

The microbroth dilution method was adapted from Cooper et al., 2010, using a 96-well microtitre plate. Each honey were diluted, using sterile distilled water, with concentrations ranging from 90% to 5%, with a variation of 5% between each concentration. The test organisms were prepared in nutrient broth (approximately 1×10^8 cfu/ml). Each well was filled with the corresponding dilutions of honey and test organisms. Figure 1 shows the plate layout of the experiment. The plate was gently stirred to allow the contents to mix before incubating at 37°C for 24 h. The next day, a loopful from each wells was taken to make a pin-point inoculation on labelled Mueller-Hinton plates. The MH plates were incubated at 37°C for 24 h. The MIC was determined by determining the concentrations at which the bacteria started to grow on the MH plates as shown in Figure 1.

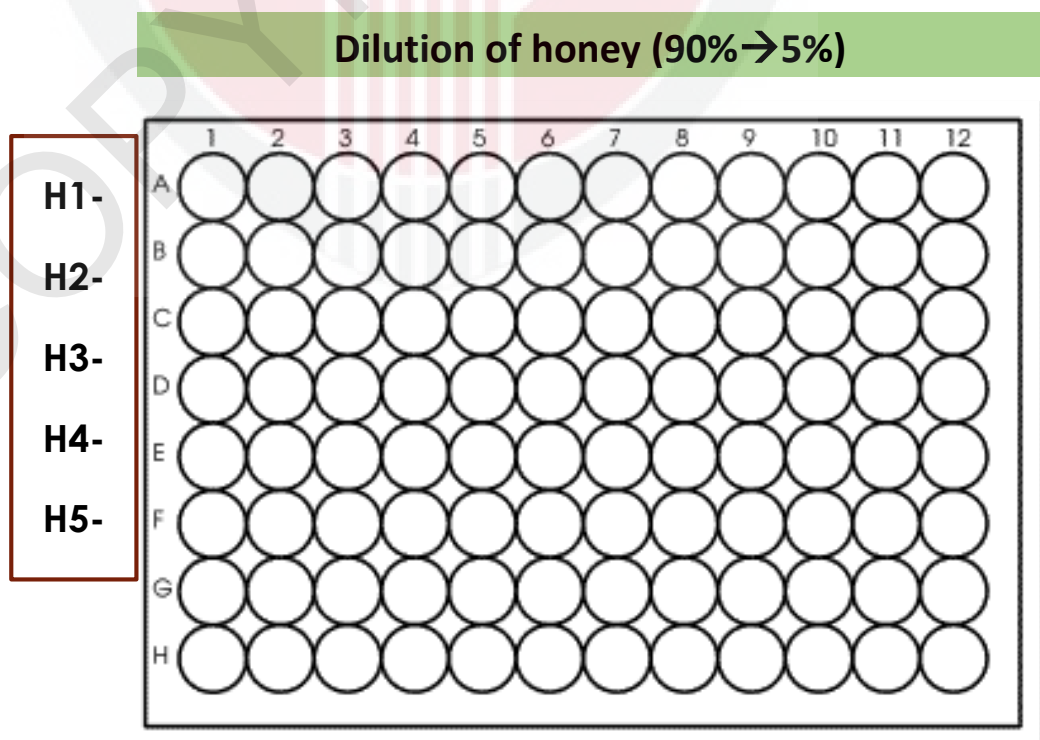


Figure 1. Microtiter plate (One plate is used for one bacteria)

H1: Kelulut; H2: Polyfloral; H3: Propolis; H4: Artificial; H5: Manuka

4.0 RESULTS

4.1 Physiochemical results

The colour of the honey depends entirely on the flowers from which the honey was collected. In this study, it was seen that kelulut had a golden yellow colour with thin viscosity and artificial honey had an almost similar bright yellow colour with the same viscosity. The multifloral and propolis honey had both a dark amber colour and a very thick viscosity as well. Manuka on the other hand had a darker brown colour, slightly granulated and its viscosity was the thickest among all. The pH of each of the honeys is shown in Table 2. The values ranged from 2.17 to 5.03.

Table 2: Appearance and pH of Honey

Honey	pH	Appearance
Kelulut	2.17	Golden yellow Thin viscosity
Multifloral	3.65	Dark amber Very thick viscosity
Propolis	5.03	Dark amber Very thick viscosity
Artificial	3.26	Dark amber Very thick viscosity
Manuka	4.02	Darker brown & slightly granulated Thickest viscosity

4.2 Bacteria identification

The identity of all tested bacteria were confirmed as Methicillin-resistant *Staphylococcus aureus* (MRSA), Vancomycin resistant enterococci (VRE), *Staphylococcus intermedius*, *Escherichia coli*, *Pasteurella multocida*, *Proteus mirabilis*, *Bacillus subtilis*, and *Pseudomonas aeruginosa* based gram staining and biochemical characteristics (Appendix 1 and 2).

4.3 Antibiotic sensitivity

The most effective antibiotic was Streptomycin followed by Enrofloxacin; they both inhibited most of the bacteria. The most resistant bacteria was *Pasteurella multocida* and *Pseudomonas aeruginosa* as they both were resistant to 2 or more antibiotics.

Table 3: Antibiotic sensitivity test results

	Strepto.	Genta.	Enroflo.	Tetracy.	Ampi.	Chloramph.
MRSA	28 (S)	28 (S)	26 (I)	10 (R)	12 (R)	16 (R)
VRE	28 (-)	22 (-)	28 (-)	8 (R)	18 (R)	10 (R)
<i>E.coli</i>	22 (S)	28 (S)	18 (R)	26 (S)	8 (R)	24 (R)
<i>Proteus mirabilis</i>	14 (S)	28 (S)	32 (S)	0 (R)	26 (S)	12 (R)
<i>Pasturella multocida</i>	14 (-)	0 (R)	37 (-)	0 (R)	24 (S)	0 (R)
<i>Bacillus subtilis</i>	16 (S)	36 (S)	36 (S)	32 (S)	14 (R)	36 (S)
<i>Pseudomonas aeruginosa</i>	18 (-)	28 (S)	18 (S)	8 (-)	0 (R)	0 (R)
<i>Staphylococcus intermedius</i>	22 (S)	22 (S)	22 (R)	8 (R)	16 (R)	18 (I)

(S: susceptible; I: intermediate; R: resistant)

4.4 Well diffusion assay

This part of the experiment was to determine the antibacterial activity of each of the tested honey, being in pure and undiluted. *Pasteurella multocida* was the most susceptible bacteria when tested against honey. It was sensitive against 4 honeys (except against artificial honey) with the highest zone against Propolis, followed by polyfloral, Manuka and Kelulut. The mean inhibition zone was in fact (20.96 ± 3.56) mm in diameter. On the other hand, the least susceptible bacteria was *P. mirabilis* which was resistant to all the 5 honeys, and hereby had a mean inhibition zone of (5.71 ± 3.86) mm diameter.



Figure 2: Measuring the zone of inhibition using Vernier callipers

Table 4: Well diffusion assay of honeys against bacteria

	Kelulut	Polyfloral	Propolis	Artificial	Manuka	Enrofloxacin
	Mean (mm)					
MRSA	0	0	12.46	0	14.40	31.50
VRE	7.00	0	0	0	8.50	27.72
<i>E.coli</i>	13.00	0	4.59	0	13.96	33.68
<i>Proteus mirabilis</i>	0	0	0	0	0	34.31
<i>Pasturella multocida</i>	16.00	23.96	24.38	0	21.995	39.39
<i>Bacillus subtilis</i>	14.50	0	11.49	0	16.07	40.63
<i>Pseudomonas aeruginosa</i>	12.00	24.53	15.34	30.35	17.14	14.35
<i>Staphylococcus intermedius</i>	6.00	0	8.77	0	16.56	32.66

Based on the Kruskal-Wallis test, there was a significant difference among the types of honey and the inhibition zone ($p < 0.05$), with the most effective honey being Manuka, followed by Propolis, kelulut, polyfloral and lastly artificial honey. Manuka inhibited all the bacteria except *P. mirabilis* while artificial honey inhibited *P.aeruginosa* only (Appendix 3).

4.5 Minimum Inhibitory Concentration

MIC is the lowest concentration of an antimicrobial (honey) that will inhibit the visible growth of a microorganism after overnight incubation. Therefore, honeys with lower MIC is more effective in inhibiting bacteria compared to honeys with higher MIC. Among the 5 honeys, Kelulut was most effective with mean MIC of (18.13 ± 5.82) %, followed by Propolis (31.25 ± 8.44) %, Manuka (34.38 ± 9.38) % and lastly artificial honey (34.38 ± 9.38) %. Polyfloral honey barely inhibited bacteria when diluted. It was noted that few of the honeys which are not efficient in pure form were actually effective when diluted. Moreover, when compared statistically using one-way anova, there was no significant difference between Kelulut and Manuka ($p=0.05$) in terms of MIC (Appendix 5).

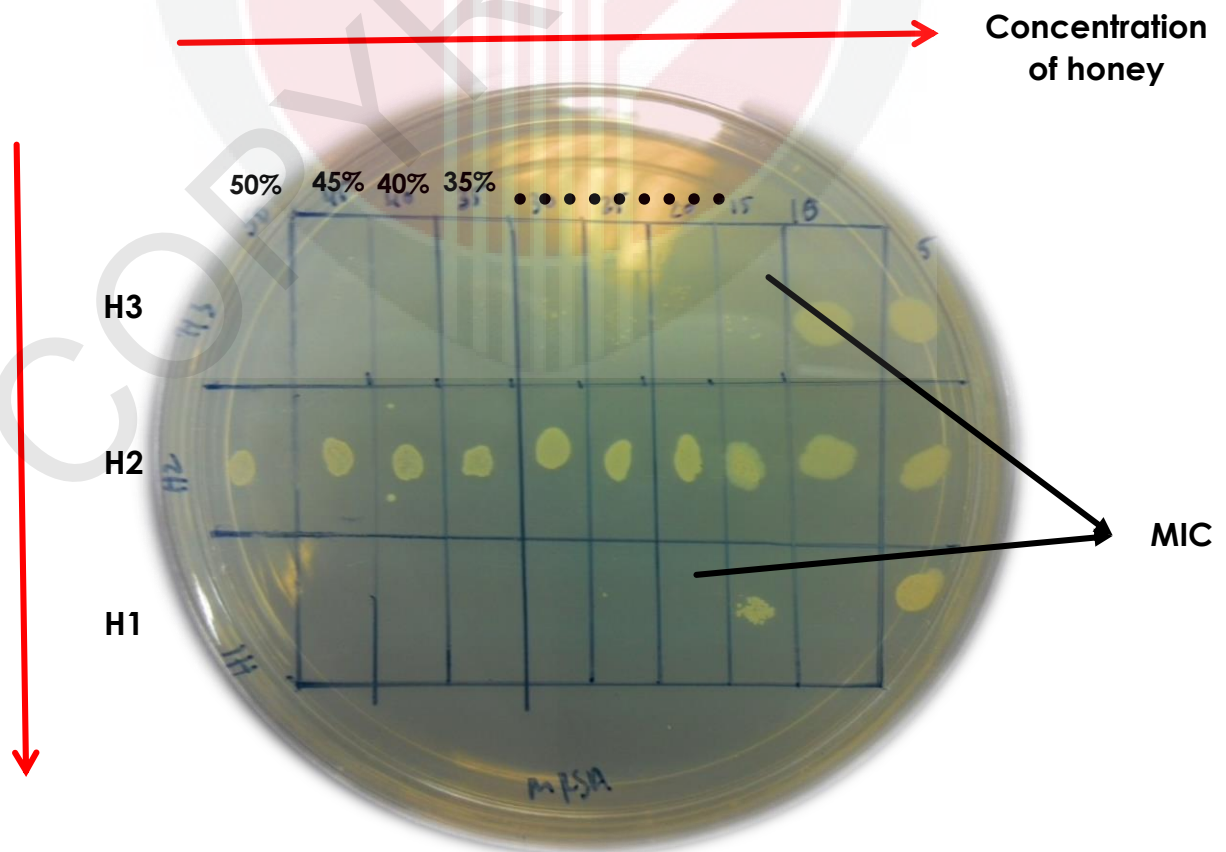


Figure 3. Determination of minimum inhibitory concentration

5.0 DISCUSSION

It has been well established that honey confers antibacterial activity, using various different methods across the globe. Lusby et al., 2005, reported that honeys other than the commercially available medical grade honeys (e.g., manuka honey) can have equivalent antibacterial activity against some bacteria, whereas Basson and Grobler, 2008, found no exceptionally high antimicrobial activity of honeys from indigenous wild flowers from South Africa. In this study, the well diffusion method and the microbroth dilution method to determine the MIC were employed and the results obtained were satisfactory to demonstrate that locally produced honey does possess antibacterial activity.

It was interesting to note that four of the honeys (Kelulut, Polyfloral, Propolis and Artificial) which gave no inhibition zone when tested against the various bacteria. However, using diluted honey in the MIC method, these honeys produced low MIC values, thus showing that those honeys can work effectively when diluted to a certain dilution. This phenomenon occurs when water is added to honey which triggers the hydrogen peroxide production cascade (Sekham & Cooper, 2013). A substantial correlation has been found between the level of endogenous hydrogen peroxide and the extent of inhibition of bacterial growth by honey (Brudzynski, 2006). When honey is pre-treated with catalase, it restored to a certain extent, the bacterial growth, hereby suggesting that endogenous H₂O₂ was associated in the growth inhibition (Brudzynski, 2006). Catalase efficiently hydrolyses hydrogen peroxide to oxygen and water. Bang et al., 2003, conducted a study about comparison of the levels of

hydrogen peroxide accumulated by a number of honey samples and found out that the maximum accumulation of hydrogen hydroxide for most of the honey samples tested occurred at 30-40% dilution of the honeys. Additionally, the use of hydrogen peroxide as a means of clearing infections in wounds often discouraged due to its cytotoxicity at millimolar concentrations but honey has shown to be an effective antimicrobial agent by supplying low levels of hydrogen peroxide to wounds continuously over time rather than as a large amount at the time of dressing (Bang et al., 2003).

In this study, Kelulut was concluded to be the most effective honey, based on its low MIC values for almost all the tested bacteria. This finding is consistent with another study done in Malaysia by Zainol et al., 2013 whereby Kelulut honey appeared most efficient in inhibiting bacterial growth in general regardless of their species. It had an MIC of 20% against *S. aureus*, *E. coli*, *P. aeruginosa* and *B. cereus*. Results from the current study showed almost similar MIC readings for similar bacteria; 15% for MRSA, 25% for *E. coli*, 15% for *P. aeruginosa*. On the other hand, Carnwath et al., 2014 conducted MIC of manuka 20+ and found MIC readings of 4% for MRSA, 5% for *E. coli* and 8% for *P. aeruginosa*. This is very much lower than the findings from this study whereby MRSA had MIC of 20%, *E. coli* 25% and *P. aeruginosa* 15%. Discrepancies in the results may be due to several reasons which include type of honey used which could relate the differences (Nzeako and Hamdi, 2000). Other reasons could also be attributed to the different floral origins of the honey or the different species of bees. In addition, natural factors like temperature and humidity

of the location from which the honey was produced play an important role (Tumin et al., 2005).

A study done by Isla et al., 2011 in Argentina, the second largest producer after China and accounting for 20% of the world production, concluded that the highest content of flavonoid and phenolic compounds are found in multifloral honeys. Flavonoids are like nature's 'antibiotic' and anti-oxidant. In our case however, multifloral (or polyfloral) honey gave the highest MIC values and barely inhibited bacteria. The differences can be attributed to the factors mentioned previously.

In short, honey consists of several factors which occur naturally and bring about this antimicrobial property and hydrogen peroxide was seen to be the major contributor. It is therefore perhaps safe to say that honey can be used as a potential alternative medicine, especially for wounds, when dealing with multidrug resistant bacteria.

6.0 RECOMMENDATION

Honey is sweet, it's a gift from nature. Malaysia is blessed with a vast variety of honeys, therefore they should be explored further in terms of their benefits and properties. Their efficacy should be tested against a wider range of pathogenic bacteria and It is recommended that the purity of the honey be confirmed prior to

experiments on its potency to avoid using adulterated honeys. Lastly, a concurrent in-vivo test should also be carried out to determine the effectiveness of honey in the host itself.

7.0 CONCLUSION

Locally produced honey (Kululut, Propolis and Artificial) do possess antimicrobial properties against bacteria, including multi-drug resistant bacteria MRSA and VRE. Based on MIC of the honeys in inhibiting the growth of pathogenic bacteria, Kelulut was the most effective among the tested honeys. This followed by Propolis, Manuka, artificial honey and lastly polyfloral honey.

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9.0 APPENDIX

Appendix 1: Biochemical test of Gram positive bacteria

Gram positive bacteria																		
	Hemolysis	6.5% NaCl	Bile esc.	Lactose	Sorbitol	Trehalose	Hemolysis	VP	Maltose	Mannitol	ADH	Plasma Tube	Slide coagulase	Urease	Glucose	Nitrate	Sucrose	Trehalose
<i>Bacillus subtilis</i>	+ve						+ve							+ve/-ve	+ve	+ve	+ve	+ve
Enterococcus group	-ve	+ve	+ve	-ve	+ve	+ve												
<i>Staphylococcus intermedius</i>							+ve	-ve	Weak	Weak -	-ve	+ve	-ve					
<i>Staphylococcus aureus</i>							+ve	+ve	+ve	+ve	-ve	+ve	-ve					

Appendix 2: Biochemical tests of Gram negative bacteria

Gram negative bacteria					
	Triple Sugar Iron (butt/slant/gas/H ₂ S)	Oxidase	Sulfide Indole- Motility	Urease	Citrate
<i>Proteus mirabilis</i>	Acid/alkaline/-ve/+ve	/	+ve/-ve/+ve	+ve	/
<i>E.coli</i>	Alkaline/acid/+ve/-ve	-ve	-ve/ +ve/ +ve	-ve	-ve
<i>Pasteurella multocida</i>	Acid/acid/-ve/-ve	+ve	-ve/ +ve/ -ve	-ve	/
<i>Pseudomonas aeruginosa</i>	Alkaline/alkaline// -ve/-ve	+ve	-ve/+ve/+ve	+ve	+ve

Appendix 3: Inhibition zone compared to bacteria

Kruskal-Wallis Test

Ranks			
	Bacteria	N	Mean Rank
I_Zone	MRSA	12	42.25
	VRE	12	37.00
	E.coli	12	45.29
	Proteus mirabilis	12	31.75
	Pasturella multocida	12	68.33
	bacillus subtilis	12	51.21
	Pseudomonas aeruginosa	12	66.96
	Staphylococcus intermedius	12	45.21
	Total		96

Test Statistics^{a,b}

	I_Zone
Chi-Square	20.130
df	7
Asymp. Sig.	.005

a. Kruskal Wallis Test

b. Grouping Variable: Bacteria

There is a significant difference due to bacteria on the inhibition zone. (P=0.05)

Appendix 4: inhibition zone compared to types of honeys

Kruskal-Wallis Test

Ranks		
Honey	N	Mean Rank
I_Zone Kelulut honey	16	43.06
Multifloral honey	16	34.31
Propolis honey	16	43.94
Artificial honey	16	27.94
Manuka honey	16	56.34
Enrofloxacin	16	85.41
Total	96	

Test Statistics^{a,b}

	I_Zone
Chi-Square	46.370
df	5
Asymp. Sig.	.000

a. Kruskal Wallis Test

b. Grouping Variable: Honey

There is a significant difference due to the honey type on the inhibition zone. (P=0.05)

Appendix 5: Relationship between Kelulut and Manuka honey using one-way Anova

Post Hoc Tests

Multiple Comparisons						
Dependent Variable: MIC						
Tukey HSD						
(I) Honey	(J) Honey	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Kelulut	Polyfloral	-56.250*	12.684	.001	-92.72	-19.78
	Propolis	-13.125	12.684	.838	-49.59	23.34
	Artificial	-35.000	12.684	.065	-71.47	1.47
	Manuka	-16.250	12.684	.704	-52.72	20.22
Polyfloral	Kelulut	56.250*	12.684	.001	19.78	92.72
	Propolis	43.125*	12.684	.014	6.66	79.59
	Artificial	21.250	12.684	.462	-15.22	57.72
	Manuka	40.000*	12.684	.026	3.53	76.47
Propolis	Kelulut	13.125	12.684	.838	-23.34	49.59
	Polyfloral	-43.125*	12.684	.014	-79.59	-6.66
	Artificial	-21.875	12.684	.433	-58.34	14.59
	Manuka	-3.125	12.684	.999	-39.59	33.34
Artificial	Kelulut	35.000	12.684	.065	-1.47	71.47
	Polyfloral	-21.250	12.684	.462	-57.72	15.22
	Propolis	21.875	12.684	.433	-14.59	58.34
	Manuka	18.750	12.684	.583	-17.72	55.22
Manuka	Kelulut	16.250	12.684	.704	-20.22	52.72
	Polyfloral	-40.000*	12.684	.026	-76.47	-3.53
	Propolis	3.125	12.684	.999	-33.34	39.59
	Artificial	-18.750	12.684	.583	-55.22	17.72

*. The mean difference is significant at the 0.05 level.

There is no significant difference between Kelulut and Manuka honey.