



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

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**FPV 2015 61**

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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

Jiavendrasingh Mussafeer

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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  $20.96 \pm 3.56$  mm in diameter), compared to *P. mirabilis* ( $5.71 \pm 3.86$  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  $(18.13 \pm 5.82)$  %, followed by Propolis ( $31.25 \pm 8.44$ ) %, Manuka ( $34.38 \pm 9.38$ ) % and lastly artificial honey ( $34.38 \pm 9.38$ ) %. 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 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  $20.96 \pm 3.56$  mm diameter) berbanding dengan yang paling rintang *P. mirabilis* ( $5.71 \pm 3.86$  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 ( $18.13 \pm 5.82$ ) %, diikuti oleh Propolis ( $31.25 \pm 8.44$ ) %, Manuka ( $34.38 \pm 9.38$ ) % dan yang terakhir adalah madu tiruan ( $34.38 \pm 9.38$ ) %. 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.

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