THE ANTIMICROBIAL ACTIVITY OF HONEY FROM FOUR SPECIES OF MALAYSIAN STINGLESS BEES, TRIGONA APICALIS,TRIGONA CARNIFRONS, TRIGONA ITAMA AND TRIGONA THORACICA

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It is hereby certified that we have read this project paper entitled "The Antimicrobial Activity of Honey from Four Species of Malaysian Stingless Bees, *Trigona apicalis*, *Trigona carnifrons*, *Trigona itama* and*Trigona thoracica*" by Cheng Yee Hong and in our opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfilment of the requirement for the course VPD 4999- Project.

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ABSTRAK

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

AKTIVITI ANTIMIKROB EMPAT SPESIES LEBAH KELULUT MALAYSIA, *TRIGONA APICALIS,TRIGONA CARNIFRONS, TRIGONA ITAMA*DAN*TRIGONA THORACICA*

Oleh

Cheng Yee Hong

2018

Penyelia Utama: Dr. Siti Zubaidah Ramanoon

Penyelia Bersama: Prof. Madya Dr. Zunita Zakaria

Ketahanan antimikrob telah menjadi isu kesihatan awam yang amat penting di seluruh dunia kerana penggunaan sembarangan antimikrob yang akhirnya membawa kepada kegagalan rawatan seperti untuk luka terinfeksi. Madu daripada lebah Apis dan Meliponines (lebah madu tiada sengat) telah dilaporkan mempunyai ciri-ciri antimikrob. Perkembangan terkini ternakan lebah madu tiada sengat di Malaysia telah menjana minat bagi menjalankan kajian ini bertujuan untuk menentukan aktiviti antimikrob madu segar dari empat spesies lebah tiada sengat Malaysia iaitu *Trigona apicalis, Trigona carnifrons, Trigona itama* dan *Trigona thoracica*. Asai resapan *well* digunakan untuk menilai aktiviti antimikrob empat jenis madu terhadap tujuh bakteria yang biasa ditemui pada luka terinfeksi. Diameter zon perencatan (DZP) daripada empat jenis madu telah dipastikan terhadap *Bacilus subtilis* (0-29.3 mm), *Enterococci*

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fecalis (0-21.5mm), *Escherichia coli* (0-31.9mm), *Pasteurella multocida* (24.3-40mm), *Proteus mirabilis* (0-21.3mm), *Pseudomonas aeruginosa* (7-21mm) dan *Staphylococcus aureus* (10.6-30.7mm). Hasil kajian mendedahkan bahawa semua madu yang diuji menunjukkan aktiviti antimikrob. Walau bagaimanapun, analisis statistik menunjukkan tiada perbezaan yang signifikan. Salah satu sebab mungkin disebabkan oleh nektar dari jenis bunga yang sama telah digunakan oleh lebah. Walau bagaimanapun, DZP tertinggi berbanding semua bakteria ujian untuk madu *Trigona thoracica*yang ditemui dalam kajian ini memerlukan siasatan selanjutnya. Di samping itu, ciri-ciri lain seperti osmolariti, keasidan, hidrogen peroksida dan sebatian fenolik madu lebah tiada sengat Malaysia hendaklah juga disiasat.

Kata kunci: Antimikrob, Madu, Trigona, Lebah Tiada Sengat, Asai Resapan *Well*, Bakteria, Patogenik, Luka

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ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfilment of the course VPD 4999- Project.

THE ANTIMICROBIAL ACTIVITY OF HONEY FROM FOUR SPECIES OF MALAYSIAN STINGLESS BEES, *TRIGONA APICALIS,TRIGONA CARNIFRONS, TRIGONA ITAMA* AND *TRIGONA THORACICA*

By

Cheng Yee Hong

2018

Supervisor: Dr. Siti Zubaidah Ramanoon

Co-Supervisor: Assoc. Prof. Dr. Zunita Zakaria

Antimicrobial resistance (AMR) has become very important public health issue worldwide due to indiscriminate use of antimicrobials which eventually leads to failure of treatment such as for infected wounds. Honey from Apis bee and Meliponines (stingless honey bees) has been reported to have antimicrobial property. Recent development in stingless bee keeping in Malaysia has generated the interest to conduct this study aimed to determine the antimicrobial activity of fresh honey from four species of Malaysian stingless bees namely *Trigona apicalis, Trigona carnifrons, Trigona itama* and *Trigona thoracica.* A well diffusion assay was used to assess the antimicrobial activity of the four honeys against seven bacteria commonly found on infected wounds. The zone diameter of inhibition (ZDI) of the four honeys has been determined against *Bacilus subtilis* (0-29.3mm), *Enterococci*

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fecalis (0-21.5mm), *Escherichia coli* (0-31.9mm), *Pasteurella multocida* (24.3-40mm), *Proteus mirabilis* (0-21.3mm), *Pseudomonas aeruginosa* (7-21mm) and *Staphylococcus aureus* (10.6-30.7mm).The study findings revealed that all tested honeys showed antimicrobial activity. However, the statistical analysis indicated no significant difference. One of the reasons could be due to the similar type of flower nectar used by the bees. Nevertheless, the highest ZDI against all tested bacteria for *Trigona thoracica* honey found in this study warrants further investigation. Additionally, other characteristics such as the osmolarity, acidity, hydrogen peroxide and phenolic compound of the Malaysian stingless bee honey should also be investigated.

Keywords: Antimicrobial, Honey, Trigona, Stingless Bee, Well Diffusion Assay, Bacteria, Pathogenic, Wound

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1.0 INTRODUCTION

Honey is a thick liquid produced by bees, made from nectar of flowers. The nectar collected by the bees is stored and mixes with enzymes in the crop before being regurgitated out into the hive. The bees then continue with a process named fanning to dehydrate the mixture to make the final product – honey(Palermo, 2013).

Honey, the precious product has been used as both food and medicine since 8000 years ago which the fact is proved by Stone Age painting in the Rana Cave at Bicorp, near Valencia in Spain with the androgynous figure on vines trying to get honey out from a nest(Oskouei et. al., 2013). In The Edwin Smith Papyrus, an ancient Egyptian medical text, named after the dealer who bought it in 1862, and the oldest known surgical treatise on trauma describes 48 cases of injuries, fractures, wounds, dislocations and tumors, there is one case of describes a gaping eyebrow wound penetrating to the bone. The remedy was: "Now after you have stitched it, you should bind fresh meat upon it the first day. If you find the stitching of the wound is loose, draw it together and treat it with grease and honey every day until the patient recovers" (Manjo 1975). Another example of ancient use of honey can be traced back to Ming Dynasty (1368-1644) where there was a well-known pharmacist Li Shizhen who wrote the classical book of traditional Chinese medicine: Compendium of Materia Medica. Written in the book: "Honey can help dispel pathogenic heat, clear away toxins, relieve pain and combat dehydration." Li Shizhen showed that taking honey regularly resulted in clear sight and rosy cheeks. He also stated that consuming honey in the morning can help prevent constipation and it also relieves chronic coughing.

In contrast, for modern medicine, according to Pasupuleti (2017), honey has been shown to be the most effective natural products used for wound healing, as

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antimicrobial, antioxidant, anti-inflammatory, anticancer, antihyperlipidemic, and cardioprotective properties; the treatment of eye disorders, gastrointestinal tract diseases, neurological disorders, and fertility disorders and wound healing activity.Honey has been reported to be effective in a number of human pathologies which is suuported by clinical studies that have demonstrated healed of severely infected cutaneous wounds following topical application of honey as treatment. It was also shown that honey posses broad-spectrum antimicrobial (antibacterial, antifungal, antiviral, and antimycobacterial) properties, which may be attributed to the acidity (low pH), osmotic effect, high sugar concentration, presence of bacteriostatic and bactericidal factors (hydrogen peroxide, antioxidants, lysozyme, polyphenols, phenolic acids, flavonoids, methylglyoxal, and bee peptides) (Israili, 2014).

With the excessive and indiscriminate use of antibiotics over the past decades, multidrug resistant bacteria have developed which then become a global public health problem (Kacaniova et al. 2011; Mandal et al. 2009). Hence, alternative antimicrobial strategies such as the use of honey has been adopted to prevent antimicrobial resistance (AMR)(Oflosson et. al. 2014)

According to National Pest Management Association (2014), there are over 20,000 known species of bees but not all bees produce and store honey;only members of the genus *Apis* are considered as true honey bees in melittology. However, there are other types of bees that produce and store honey, one of them is stingless honey bees(meliponines) which can be found in most tropical or subtropical regions of the world, such as Australia, Africa, Southeast Asia, and tropical America (Michener, 2007).

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Similar to the *Apis* bees, stingless bee honey possess antimicrobial properties that could overcome the bacterial contamination, anti-inflammatory effect to protect the tissue from highly toxic inflammatory mediators and moisturizing properties to promote angiogenesis and oxygen circulation, thus improves healing rate(Jalil et al.,2017). Furthermore, according to Mussafeer et. al. (2015), among manuka, propolis, polyfloral and artificial honey, stingless bee honey has shown to be the most effective honey based on its lowest minimum inhibition concentration (MIC) value.

According to the news report by The Star by Wong(2017), local stingless bee,also known as *Lebah Kelulut*, has been reported to produce a lot more honey than even Australian bees and this local superfood is developed rapidly in Malaysia for their commercial, environmental, educational and eco-tourism values.

However, there are very limited studies conducted for the Malaysian stingless bee honey. To explore the medicinal property of the local stingless bee honey, this study was conducted to determine the antimicrobial activity of honey from four species of Malaysian stinglessbees namely*Trigona apicalis, Trigona carnifrons, Trigona itama,* and *Trigona thoracica.*

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