

PHYSICOCHEMICAL PROPERTIES AND ANTIMICROBIAL  
ACTIVITY OF HONEY FROM THE MALAYSIAN  
STINGLESS BEE AGAINST SELECTED  
PATHOGENIC BACTERIA

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**PHYSICOCHEMICAL PROPERTIES AND ANTIMICROBIAL ACTIVITY OF  
HONEY FROM THE MALAYSIAN STINGLESS BEE AGAINST SELECTED  
PATHOGENIC BACTERIA**

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**Nilai**

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## AUTHOR DECLARATION

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

I hereby declare that the work in this dissertation is my own except for quotations and summaries which have been duly acknowledged.

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## ABSTRAK

Kajian ini mengkaji ciri-ciri fiziko-kimia dan aktiviti antimikrob madu kelulut (SBH) yang dihasilkan oleh lima spesis lebah kelulut (*Trigona* spp., sampel A; *Hetrotringona laeviceap*, sampel B; *Heterotringona thoracica*, sampel C, *Heterotringona itama*, sampel D; dan *Hetrotringona terminata*, sampel E) keatas bakteria Gram positif dan negatif, iaitu *Escherichia coli* ATCC 25922, *Bacillus subtilis* ATCC 21332, *Serratia marcescens* ATCC 13880, *Staphylococcus aureus* ATCC 25923, *Salmonella* Typhimurium ATCC 13311 dan *Proteus vulgaris* ATCC 8427. Kandungan kelembapan SBH adalah pelbagai iaitu antara 29.04 dan 37.07%, sementara lingkungan  $a_w$  adalah antara 0.208 untuk sampel A dan 0.821 untuk sampel C. Kebanyakan madu kelulut adalah dalam lingkungan warna kekuningan dan *amber* dengan jumlah pepejal terlarut ( $^{\circ}$ Brix)  $75.6 \pm 7$  untuk sampel E dan meningkat kepada  $88.6 \pm 6$  untuk sampel A. Aktiviti antimikrob telah dikaji menggunakan kaedah difusi telaga dan piring mikrotiter. Aktiviti antibakteria kelulut didapati berbeza dengan spesis-spesis lebah dan bakteria-bakteria yang telah dipilih. Pada kepekatan madu 50% (w/v) untuk sampel C dan D, semua bakteria itu dihalang pembiakannya; sampel A tidak menghalang pembiakan *E.coli*, sampel C, D dan E gagal untuk menghalang pembiakan *Salmonella* Typhimurium. Nilai pH pada madu kelulut adalah diantara pH  $2.95 \pm 0.2$  hingga  $3.57 \pm 0$  dengan sedikit penurunan pH setelah dicairkan kepada 10 to 50%. Kajian ini telah menunjukkan bahawa madu kelulut Malaysia mempunyai ciri-ciri antimikrob yang menghalang tumbesaran bakteria yang dipilih dan pH madu kelulut yang rendah bertanggungjawab dalam aktiviti anitmikrob tersebut.

Kata kunci: Lebah kelulut, madu kelulut, aktiviti antimikrob, sifat kimia-fisiko

## ملخص البحث

في ماليزيا، يمكن جمع العسل عادة من نوع من النحل يسمى (sting bees)، يطلق علي هذا العسل اسم ( madu kelulut). تقم هذه الدراسة الخصائص الفيزيائية و الكيميائية لعسل (madu kelulut)، إضافة الي الخصائص المضادة لنمو الميكروبات. تشمل هذه الدراسة علي خمس انواع من العسل تم جمعها من انواع مختلفة من النحل و هي (العينة A: *Trigona spp.*; العينة B: *Hetrotringona-laeviceap*; العينة C: *eterotringona-thoracica*; العينة D: *Hetero-tringona-Itama*; العينة E: *Hetrotringona-terminata*). هذه العينات تم اختبار فعاليتها ضد بكتيريا سالبة الجرام و اخري موجبة الجرام. تشمل: (*Escherichia coli* ATCC 25922, *Bacillus subtilis* ATCC 21332, *Serratia marcescens* ATCC 13880, *Staphylococcus aureus* ATCC 25923, *Salmonella Typhimurium* ATCC 13311 and *Proteus vulgaris* ATCC 8427). تتراوح نسبة الرطوبة لعينات العسل من 29.04 الي 37.07; في حين يتراوح قيمة aw من 0.280 للعينة A الي 0.821 للعينة E. معظم عينات العسل ذات لون اصفر غامق مائلا الي اللون البرتقالي. و تتراوح نسبة المواد الدائبة حوالي  $75.6 \pm 7$  للعينة E. لتصل الي  $88.6 \pm 6$  للعينة A. . تتفاوت الخصائص المضادة لنمو البكتيريا من نوع الي اخر و من بكتيريا الي اخري، و قد تم قياسها باستخدام طريقة *well diffusion and microtitre plate assay*. اعتمادا علي استخدام محاليل ذات تراكيز 50%. العينة C و العينة D منعت نمو جميع انواع البكتيريا. في حين A منعت نمو *E. coli*, العينة C و D فشلت في تثبيط نمو *Salmonella Typhimurium*. التركيز الأدنى للبكتيريا *S. aureus* هو 10% للعينة A و B, في حين يتراوح الحد الأدنى للعينات الاخرى ما بين 30% و 50%. جميع عينات العسل ذات درجة حموضة منخفضة ما بين 2.95 و 3.97 مع انخفاض ضئيل بعد تخفيف المحاليل من 50% الي 10%. اثبتت هذه الدراسة علي أن العسل المستخلص من stingless bee يحتوي علي مواد فعالة ضد نمو البكتيريا المستخدمة في هذه الدراسة، و قد تكون نسبة الحموضة المنخفضة هي العامل الرئيسي في كبح نمو البكتيريا.

الكلمات المفتاحية للبحث: stingless bee honey, madu kelulut, الخصائص المضادة لنمو الميكروبات, الحد الأدنى لنمو البكتيريا, الحد الأدنى للتركيز الفعال ضد نمو الميكروبات.

## ABSTRACT

This study evaluate the physiochemical and antimicrobial activity of stingless bee honey (SBH) or “madu kelulut” produced by five Malaysian stingless bee species (*Trigona* spp., sample A; *Hetrotringona laeviceap*, sample B; *Heterotringona thoracica*, sample C; *Hetero-tringona itama*, sample D; and *Hetrotringona terminata*, sample E) against Gram negative and positive bacteria namely, *Escherichia coli* ATCC 25922, *Bacillus subtilis* ATCC 21332, *Serratia marcescens* ATCC 13880, *Staphylococcus aureus* ATCC 25923, *Salmonella* Typhimurium ATCC 13311 and *Proteus vulgaris* ATCC 8427. The moisture content of SBH varied between 29.04 and 37.07%, whereas  $a_w$  ranged between 0.280 for sample A and 0.821 for sample C. Most of the SBH are within the range of yellow shades and amber with total soluble solids (°Brix) of  $75.6 \pm 7$  for sample E and  $88.6 \pm 6$  for sample A. The antimicrobial activity was evaluated using well diffusion and microtiter plate assay. The antibacterial activity of SBH varied with bee species and the target bacteria. At 50 % (w/v) of honey sample C and D inhibited all the bacteria; sample A did not inhibit *E. coli*; Sample C, D and sample E failed to inhibit the growth of *S. Typhimurium*. The pH of raw SBH samples ranged from pH  $2.95 \pm 0.2$  to  $3.57 \pm 0$  with slight reduction in pH after dilution from 10 to 50%. This study has demonstrated that Malaysian stingless bee honey has antimicrobial properties against the selected micro-organism, and the low pH of SBH may be responsible for the antimicrobial activity.

**Key words:** Stingless bee, madu kelulut, antimicrobial activity

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## ABBREVIATIONS

SBH	stingless bee honey
MIC	minimum inhibition concentration
OD	optical density
NA	nutrient agar
NB	nutrient broth
TSS	total soluble solids
ZDI	zone diameter of inhibition
cm	centimeter
$\mu$ l	microliter
$\mu$ m	micrometer
w/v	weight per volume
%	Percentage

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