Faculty of Resource Science and Technology

Chemical Profiling and Biological Activities of Stingless Bee Honey and Propolis from the Selected Areas in Sarawak

Easter Sirah Anak Kelabo

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Chemical Profiling and Biological Activities of Stingless Bee Honey and Propolis from the Selected Areas in Sarawak

Easter Sirah Anak Kelabo

A thesis submitted

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DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Malaysia Sarawak. Except where due acknowledgements have been made, the work is that of the author alone. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

……

Signature

Name: Easter Sirah Anak Kelabo

Matric No.: 18020121

Faculty of Resource Science and Technology

Universiti Malaysia Sarawak

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ABSTRACT

Stingless bees products such as honey and propolis have been widely studied due to their profound therapeutic properties. The increase in commercial values of stingless bee products has made it susceptible to adulteration practices, such as the addition of either natural or scientific sweeteners into stingless bee honey. This study was conducted to analyse stingless bee honey and propolis from Serapi Garden, Siniawan Farm, and PoliKu Farm and their chemical constituents. The chemical composition of stingless bee honey and propolis were then compared with the extracts of predominant flowers from the respective locations. The glucose content, heavy metals-environmental contaminants, and biological activities of stingless bee honey and propolis were also analysed for the authenticity and quality determination of the stingless bee products. Analysis of chemical constituents was conducted using gas chromatography-mass spectrometer (GC-MS). The analysis depicted various chemical components in honey and propolis, which interestingly correlated with the chemical composition of the predominant flowering at the respective locations. Glucose content in honey analysed by using high-performance liquid chromatography (HPLC) showed low glucose content (61.19 – 174.53 g/L), while heavy metals content for environmental contaminants analysis conducted using inductively coupled plasma-optical emission spectroscopy (ICP-OES) showed the predominant elements Zn (0.069 – 1.653 ppm) that is below the permissible limit. The ^{1}H Nuclear Magnetic Resonance (^{1}H-NMR) spectra obtained from the honey samples depicted strong fatty alcohols signals present at 3.5 – 4.5 ppm, which can be used as potential chemical biomarkers. The presence of phenolic, aliphatic acids and fatty alcohols in the honey and propolis has contributed to the excellent antibacterial activities against *Escherichia coli* (MIC = 144; inhibition zone = 19mm) and *Staphylococcus aureus* (MIC = 170; inhibition zone = 9mm). The antioxidant activities of

honey and propolis showed excellent antioxidant properties with IC_{50} values of 5.068 and 4.083 ppm, respectively. These results indicated that stingless bee honey and propolis in Sarawak possessed unique chemical composition that can be used as potential biomarkers, antimicrobial and antioxidant agents.

Keywords: *Heterotrigona itama*, stingless bee honey, propolis, honey adulteration, honey quality

Profil Kimia dan Aktiviti Biologi bagi Madu dan Propolis Lebah Kelulut dari Kawasan Terpilih di Sarawak

ABSTRAK

Produk lebah kelulut seperti madu dan propolis telah banyak dikaji kerana sifat terapeutiknya yang amat baik. Peningkatan pada nilai komersial produk lebah kelulut telah menjadikannya lebih terdedah kepada amalan pemalsuan seperti penambahan pemanis semula jadi atau saintifik dalam madu lebah kelulut. Sehubungan dengan itu, kajian ini dilakukan untuk menganalisis madu kelulut dan propolis untuk kandungan kimia. Komposisi kimia madu kelulut dan propolis kemudianya dibandingkan dengan estrak bunga utama dari lokasi masing-masing. Kandungan glukosa, logam berat bahan cemar alam sekitar, dan aktiviti biologi madu kelulut dan propolis juga dianalisis untuk kesahihan dan penentuan kualiti produk lebah kelulut. Analisis kandungan kimia dilakukan menggunakan kromatografi gas-spektrometri jisim (GC-MS) dan analisis menunjukkan pelbagai kandungan kimia dalam madu dan propolis yang berkait rapat dengan kandungan kimia pembungaan utama di setiap lokasi. Kandungan glukosa dalam madu yang dianalisis dengan menggunakan kromatografi cecair berprestasi tinggi (HPLC) menunjukkan kandungan glukosa yang rendah (61.19 - 174.53 g/L), sementara analisis logam berat untuk bahan cemar alam sekitar yang dilakukan menggunakan kaedah spektrofotometer pancaran optik plasma gandingan aruhan (ICP-OES) menunjukkan Zn sebagai unsur dominan (0.069 - 1.653 ppm) tetapi di bawah had yang dibenarkan. Spektrum ^{1}H Resonans Magnetik Nuklear (^{1}H-NMR) yang diperolehi daripada sampel madu menunjukkan isyarat alkohol lemak yang kuat pada 3.5 – 4.5 ppm yang berpotensi sebagai biomarker kimia. Kehadiran fenolik, asid alifatik dan alkohol berlemak dalam madu dan propolis telah menyumbang kepada aktiviti antibakteria yang sangat baik terhadap Escherichia coli (MIC = 144; zon

perencatan = 19 mm) dan Staphylococcus aureus (MIC = 170; zon perencatan = 9 mm). Aktiviti antioksidan madu dan propolis menunjukkan sifat antioksidan yang sangat baik dengan nilai IC_{50} masing-masing pada 5.068 ppm dan 4.083 ppm. Hasil kajian ini menunjukkan madu dan propolis lebah kelulut di Sarawak memiliki komposisi kimia yang unik dan berpotensi untuk digunakan sebagai ejen biomarker kimia, antimikrob dan antioksidan.

Kata kunci: *Heterotrigona itama, madu kelulut, propolis, pemalsuan madu, kualiti madu*

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LIST OF ABBREVIATIONS

$CDCl_3$	Deuterated chloroform
DMSO	Dimethyl sulfoxide
DPPH	2,2-diphenyl-1-picrylhydrazyl
EEP	Ethanolic extracts of propolis
FTIR	Fourier Transform Infrared Spectrometer
GC-MS	Gas Chromatography Mass Spectrometer
^{1}H-NMR	Proton Nuclear Magnetic Resonance
HPLC	High Performance Liquid Chromatography
IC_{50}	Concentration needed for 50% reduction of response
ICP-OES	Inductively Coupled Plasma-Optical Emission Spectroscopy
MIC	Minimum inhibitory concentration
NIST	National Institute Standard and Technology
ppm	parts per million
rpm	retention per minute
UV	Ultra-violet

CHAPTER 1

INTRODUCTION

1.1 Study Background

Stingless bees are known as *kelulut* in Malaysia. Stingless bees are good pollinators and are commonly found in tropical dry and humid forests (Harun et al., 2015). Stingless bees have been largely farmed for their honey and have become one of the important income-generating activities in Malaysia. In meliponiculture, the stingless bees' colonies are kept in a log hive as shown in Figure 1.1 (Jailani et al., 2019).

Figure 1.1: Stingless bee nest in Serapi Garden, Sarawak

The distinctive characteristic of stingless bees which is distinguished from other bees is their small sizes (2-5 mm) with a dysfunctional stinger (Streinzer et al., 2016). Due to their smaller sizes, stingless bees can retrieve pollen and nectars in smaller herbal plants more efficiently than other honey bees (Abd Jalil et al., 2017). This is one of the reasons that make stingless bee honey more nutrients than regular honey. Stingless bees also produce a high quantity of propolis as honey storage (Ibrahim et al., 2016). The products of stingless

bees such as honey and propolis have a modest commercial scale around the world due to their high medicinal benefits.

Honey is a natural sweetener derived from honey bees and is known for its remedial values (Chan-Rodríguez et al., 2012). Honey has been traditionally used around the globe to heal wounds, sore throat, cough and earaches (Rao et al., 2016). The honey from the stingless bee is unique as it originates from rich vegetation in native environments, which is typically harvested directly from a forest or in a well-established meliponary (Rao et al., 2016; Abd Jalil et al., 2017). Modern research has discovered that the traditional application of stingless bees honey has great potential to be used as an added value in modern medicine and is considered to have higher medicinal value than other bees species (Yaacob et al., 2017).

Stingless bees, however, produce a small amount of honey production compared to other honey bees (Ibrahim et al., 2016). Nevertheless, stingless bees honey has a unique sweetness taste, which is a mixture of sour and acidic tastes (Abd Jalil et al., 2017). Stingless bees honey has been used as a natural remedy by various tribes and ethnic communities around the world and is claimed to be medicinally potent (Biswa et al., 2017). Despite having sugars (fructose and glucose) as the major component in stingless bees honey, there are also other chemical compounds such as phenolic acids, flavonoids, proteins, amino acids, enzymes, vitamins and minerals present in the stingless bees honey (Habib et al., 2014). Various studies had suggested that the bioactive compounds in stingless bees honey possess a wide spectrum of biological activities such as anti-inflammatory, antimicrobial, antioxidant as well as possess anticancer properties (Boorn et al., 2010; Borsato et al., 2014; Yazan et al., 2016; Yaacob et al., 2017). There is great variability in the chemical

composition of honey from stingless bees considering the type of bees, the predominant flowering and geographical origin (Biluca et al., 2016).

Stingless bees stored the honey in clusters of small resin pots called propolis, which is different from *Apis* sp. that stored the honey in hexagonal-shaped combs (Yazan et al., 2016). Propolis is a sticky dark-coloured material that stingless bees collect from living plants, mix with wax which plays a crucial role in protecting the hive against pathogenic agents (Sarıçoban & Yerlikaya, 2016). Propolis is an apicultural product that has been used in traditional and complementary medicine since ancient times for its biological properties (Sanpa et al., 2015; Baltas et al., 2016).

The chemical compositions of propolis depend on the geographical location, climate, type of bees, and availability of vegetation in the locality (Shashikala, 2016). The bioactive compounds present in propolis are aromatic acid, phenolic compounds, and phenolic acids. These bioactive compounds are correlated with compounds present in plants that exhibit many biological active features such as antioxidant, antibacterial, antifungal, anti-inflammatory and anticancer properties (Dziedzic et al., 2013; Kothai & Jayanthi, 2014).

1.2 Problem Statement

Stingless bee products such as honey and propolis have been widely commercialised in Malaysia due to their beneficial properties and ability to generate additional income for the stingless beekeepers (Mustafa et al., 2018). Investigation on the chemical composition and biological activities of stingless bee honey and propolis has been widely reported (Ibrahim et al., 2016; Ab Rahim et al., 2018). The geographical and floral origins of honey and propolis are some of the important parameters that have contributed to the quality of honey and propolis (Wong et al., 2019). The humid tropical climate with dipterocarp forests

and a thick canopy of indigenous plants in Sarawak could provide preferable conditions for stingless bees (Abu Hassan et al., 2017). Conditions such as high humidity and minimum sunlight exposure will provide healthy hives for the stingless bee to produce nutritional and low contamination residue of honey and propolis with high antioxidant activity (Harun et al., 2015).

However, there is still lack of scientific support on the chemical profile and quality standards of stingless bee products from Borneo, particularly in Sarawak (Ibrahim et al., 2016; Wong et al., 2019). The studies on stingless bee products in Sarawak were mostly focused on the honey (Tuksitha et al., 2018; Maringgal et al., 2019; Wong et al., 2019; Badrulhisham et al., 2020; Ismail et al., 2020). However, fewer studies reported on the correlation of stingless bee honey and propolis (Zhao et al., 2017) with the surrounding flora and fauna. A comprehensive study on both stingless bee honey and propolis would provide a better insight into the significant potential of these two stingless bee products (Mohd & Md Zin, 2020). An assessment of sugar and heavy metals content using spectroscopic methods is also essential to evaluate the nutritive quality of honey and propolis in Sarawak. (Korn et al., 2013; Chan et al., 2017). Besides, the determination of the chemical constituents based on locality and microbiological characteristics of the stingless bee honey and propolis are also required to ensure the quality of the stingless bee for application in value-added products (Khalil et al., 2012).

Evaluation on the chemical compositions, purity (without adulteration), and biological properties of the stingless bee honey and propolis are necessary to ensure both stingless bee products are safe for daily consumption and medicinal purposes. The current standards for bee products set by Codex Alimentarius Commission (2001) are specifically

applied for *Apis mellifera*, hence the standards could not be applied on stingless bee products. The main reference point for checking on physicochemical and nutritional properties of stingless bee products from Sarawak is currently following the standards set by Malaysian Standard (MS2683: 2017) (Wong et al., 2019). Setting a quality standard specifically for stingless bee honey and propolis over honeybees is important for sustainable income generation and potential commodities in Sarawak (Mustafa et al., 2018; Al-Hatamleh et al., 2020). It is hoped that the data to be generated from this study can be used as scientific support and become a standard reference for the optimum quality and purity of Sarawak stingless bee honey and propolis which later can be used as a foundation for future work on the potential of developing stingless bee products from Sarawak for health-related products in Malaysia.

1.3 Objectives

This study embarks on the following objectives:

i. To analyse the chemical composition in stingless bee honey, propolis, and compare with plant extracts from different geographical locations in Sarawak namely Serapi Garden, Siniawan Farm, and PoliKu Farm;

ii. To determine the glucose content, heavy metals-environmental contaminants in stingless bee honey and propolis;

iii. To evaluate antibacterial activities of stingless bee honey and propolis against selected bacteria, i.e., *Escherichia coli* and *Staphylococcus aureus*; and

iv. To study the antioxidant activities of stingless bee honey and propolis.

1.4 Scope of Study

The purpose of this study was to perform chemical profiling of *Heterotrigonna itama* stingless bee honey and propolis from selected areas in Sarawak (Serapi Garden, Siniawan Farm, and PoliKu Farm), and to evaluate the biological activities of honey and propolis of the stingless bee.

The first part of the study was focused on determining the chemical composition of the honey and propolis. The chemical constituents of the honey, propolis, and plant extracts in this study were identified using gas chromatography-mass spectrometry (GC-MS) analysis to gain insight into the correlation between the chemical constituent of the honey, propolis and predominant flowerings based on different localities. The study on the chemical profiling of honey was further investigated using ^{1}H Nuclear Magnetic Resonance (^{1}H-NMR) profiling to identify the chemical markers of honey from three different sample locations, honey from different bee species, and adulterated honey. The honey and propolis were also analysed by using high performance liquid chromatography (HPLC) to determine the glucose content. Analysis of the heavy metals content in the honey and propolis were conducted by using inductively coupled plasma-optical emission spectroscopy (ICP-OES) to compare the concentrations of environmental contaminants in the honey and propolis from different environments.

The second part of the study was conducted to evaluate the biological activities of honey and propolis. Antibacterial and antioxidant properties have been studied as two of the most bio-attributions possessed by stingless bee honey and propolis. The antibacterial properties of the stingless bee propolis were investigated by using turbidimetry kinetic method, while disc diffusion assay was performed to evaluate the antibacterial activities of honey. The free radical scavenging activities of stingless bee honey and propolis were

determined by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay to evaluate the antioxidant capacity of the honey and propolis. This study will provide insight into how the honey and propolis of *H. itama* from Sarawak can perform as valuable sources for pharmaceutical and nutraceutical products.

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