Journal of Asia-Pacific Entomology 21 (2018) 563–570

Contents lists available at ScienceDirect

Journal of Asia-Pacific Entomology

journal homepage: www.elsevier.com/locate/jape

Full Length Article

Antioxidant and antibacterial capacity of stingless bee honey from Borneo (Sarawak)

Lalita Tuksitha[a], Yi-Lin Sophia Chen[b], Yi-Ling Chen[a], Kie-Yiong Wong[c], Chi-Chung Peng[a,*]

[a] Department of Biotechnology, National Formosa University, Yunlin, Taiwan
[b] Department of Biotechnology and Animal Science, National Ilan University, Ilan, Taiwan
[c] BEE EXC SCI TECK SDN. BHD. Sibu, Sarawak, Malaysia

ARTICLE INFO

Keywords:
Stingless bee honey
Geniotrigona thoracica
Heterotrigona itama and *Heterotrigona erythrogastra*
Anti-oxidant activity
Anti-microbial activity

ABSTRACT

Stingless honey bees form a large group of bees that lack of a sting and are found among Meliponinae species indigenous to various tropical and subtropical regions. They are able to produce "stingless bee honey" that contains divergent categories of phenolic and flavonoid compounds and have been associated with antioxidant and antibacterial activity. This study examines the physicochemical properties, antioxidant-activity and anti-microbial activity of stingless bee honey from Malaysia that was produced by *Geniotrigona thoracica, Heterotrigona itama* and *Heterotrigona erythrogastra*. The results show that *G. thoracica* honey has the highest concentration of the total phenolic context (99.04 ± 5.14 mg/ml) and the greatest reducing power (19.05 ± 0.79%), while flavonoids (17.67 ± 0.75 mg/ml), reducing power (18.10 ± 0.35%), DPPH (47.40 ± 3.18%) and FRAP (50.66 ± 5.77 mM of Fe^{2+}/100 g) of *H. itama* honey is significantly higher than those of the other honeys. In addition, *G. thoracica* honey has the highest antibacterial activity against *Staphylococcus xylosus* (2.10 ± 0.10 cm), which is Gram-positive bacterium, and against *Pseudomonas aeruginosa* (1.60 ± 0.10 cm) and *Vibrio parahaemolyticus* (2.03 ± 0.06 cm), which are Gram-negative bacteria. These results suggest that stingless bee honeys possess useful amounts of phenolic and flavonoid compounds that are able to act as natural anti-oxidants and also have significant anti-microbial activity.

Introduction

Stingless bees are a huge and diverse monophyletic group of native eusocial bees that are abundant in tropical and subtropical regions throughout the planet, including Australia, Africa, Southeast Asia, and tropical America. They have a sting that has undergone evolutionary decline and is unlikely to be able to cause harm or injury to a human. Stingless bees are among the most highly developed bees and have existed for > 90 million years. They belong to the order Hymenoptera and comprise the tribe Meliponini in the family Apidae (Chuttong et al., 2016a). Moreover, they are closely related to the eusocial honey bees, to bumblebees and to orchid bees (Bradbear, 2009). They are active all year round worldwide and are usually found in colonies under the earth, in a fissure in rock, inside a hole in a tree or within the bough of a tree. Around 500 different species of stingless bees have been discovered altogether and these include > 300 species in the Americas, 50 species in Africa, 60 species in Asia, 10 species in Australia and four species in Madagascar (Bradbear, 2009).

Stingless bees are able to produce "stingless bee honey", which is a blonde sugary liquid with a glorious taste and aroma. It can be separated into different categories based on the honey's physical and chemical constituents, which are related to the physiology of production of the raw material, the territorial location of the floral source, the species of bee and the conditions of the ecosystem in which the bees live. Stingless bee honey consists mostly of carbohydrates, water, amino acids, vitamins and minerals (Chuttong et al., 2016b). Additionally, it also contains unique and distinct phenolic and flavonoid compounds that seem to play a critical role in its antibacterial, anti-inflammatory and antioxidant activities of the Western honey bee *Apis mellifera* in previous studied (Liu et al., 2013).

When compared to *Apis mellifera* which is the world superior in honey production, Stingless bees produce and keep still less honey on a per hive basis (Chuttong et al., 2016a). The limited stingless bee honey yield, particularly as an international commodity, results in a little

Abbreviations: DPPH-1, 1-diphenyl-2-picryl-hydrazyl; MIC, minimum inhibitory concentration; MBC, minimum bactericidal concentration; TSS, total soluble solids; FRAP, Ferric reducing antioxidant power; PMS, phenazine methosulfate; NADH, nicotinamide adenine dinucleotide; NBT, nitroblue tetrazolium chloride; TPTZ, tripyridyl triazine; TSA, tryptic soy agar

* Corresponding author.
E-mail address: bocky@nfu.edu.tw (C.-C. Peng).

https://doi.org/10.1016/j.aspen.2018.03.007
Received 8 January 2018; Received in revised form 27 February 2018; Accepted 14 March 2018
Available online 20 March 2018

knowledge about antioxidant and antibacterial property of stingless bee honey. Therefore, this study is perhaps the first observation to study of its kind.

Honey is an energy rich medical product produced by different species of honey bees including stinged and stingless bees. It has had a valued place in traditional medicine since ancient times, with many functional applications, which effect from its chemical and physical composition. The ancient Egyptians, Chinese, Greeks, and Romans utilized honey cure their affliction as diseases of the intestine and skin wounds (Rao et al., 2016). Since a few decades ago, honey recently became the focus of attention by several research groups. The most remarkable disclosure was their antioxidant and antibacterial activity which has been revealed in several researches.

Free radicals are natural byproducts that are created by oxidation and include hydroxyl radicals, superoxide anion radicals and hydrogen peroxide. They are created by sunlight, pollution, stress, processed food and also environmental toxins. They may lead to chain reactions in the body and are able to destroy cells. Many diseases have been linked to a body's burden of free radicals, including heart disease, diabetes, cancers, aging and other events. Antioxidants are molecules that inhibit the oxidation of other molecules. They are able to donate an electron to the unpaired valence electron within a free radical, thus boosting the immune system and preventing cell damage.

Honey is a huge source of antioxidants. It can be helpful at preventing damage or injury of cells by acting as a natural antioxidant against such reactive oxygen species. Different varieties of honey from various countries and geographical regions exhibited different antioxidant properties. The identified mechanisms by which honey does this are associated with honey's chemical composition, specifically the presence of phenolic and flavonoid compounds to reduce oxidative reactions or free radicals within the food systems and human health (Gismondi et al., 2017; Di Marco et al., 2016). The potential of it depends on the number and arrangement of the hydroxyl groups in the molecules of interest. Alvarez-Suarez et al. (2010), documented that five monofloral Cuban types of honey show a high correlation between total phenolic content and the results of the ferric reducing antioxidant power assay. Furthermore, using three honey samples from Malaysia, Tualang, Gelam, and Acacia honey, Chua et al. (2013), found that total flavonoid content is well correlated with three antioxidant assays that use different mechanisms, namely free radical scavenging activity by the 1,1-diphenyl-2-picrylhydrazyl (DPPH), the ferric reducing antioxidant power assay and the β-carotene bleaching assay.

The antibacterial properties of honey have been well known in folk medicine for many years. These healing powers are directly due to the honey's chemical composition, including the presence of hydrogen peroxide, as well as other non-peroxide factors. There are many non-peroxide constituents that form part of the antibacterial activity of honey and these include phenolic compounds, flavonoids and a number of other components Zainol et al. (2013), and Weston et al. (1999), reported that the phenolic compounds present in Manuka honey as well as in Manuka nectar, pollen and propolis, form a powerful mixture that creates the non-peroxide antibacterial activity of New Zealand Manuka honey. Estevinho et al. (2008), found that phenolic compounds extracted from honey from Northeast Portugal have antibacterial activity against Gram-negative and Gram-positive bacteria.

There are > 30 species of stingless bees found in Borneo (Sarawak) and the properties of the honeys produced by these bees have not been explored. This study examines the physicochemical properties, the antioxidant-activity and the anti-microbial activity of stingless bee honey, where past research seem to have been minimal effort. Moreover, some species are commonly used in domesticated in the agro ecosystem for meliponiculture as pollinating agents for many important crops in Malaysia and the remainder are species, mainly for reasons of the environment tolerance group since they are present in most locations (Jaapar et al., 2016). Therefore, the basic aim of this research was to study antioxidant-activity and the anti-microbial activity of stingless bee honey produced by *Geniotrigona thoracica*, *Heterotrigona itama* and *Heterotrigona erythrogastra*, which are species of bee indigenous to Borneo (Sarawak).

Materials and methods

Honey samples

Honey samples from three species of stingless bee, namely *Geniotrigona thoracica, Heterotrigona itama* and *Heterotrigona erythrogastra*, were obtained by Kie-Yiong Wong in his bee farm from *BEE EXC SCI TECK SDN. BHD* in Sibu, Sarawak, Malaysia. The harvesting took in September 2016, honey pots were penetrated with a keen tool and laboured through syringe extraction from independent and co-operative honey pots (Chuttong et al., 2016a). All honey samples were two times diluted and filtered through a 0.2 μm fliter (Millipore) in the laboratory to eliminate contaminating micro-organisms. All samples were adjusted to 35% moisture content and were stored at 4 °C before being examined. All tests were carried out in triplicate ($n = 3$) and the outcome averaged to give the mean ± SD.

Determination of total soluble solids (TSS), protein content and total phenolic content

Measurement of the total soluble solids (TSS) present in the honey samples was carried out by refractometry. The protein content of the honey samples was measured by the Bradford method (Bradford, 1976) using bovine serum albumin as the standard (Liu et al., 2013). The total phenolic content of the honey samples was determined by the Folin–Ciocalteu colorimetric method (Liu et al., 2013). Briefly, 100 μl of honey sample was diluted in 5 ml of water, then 500 μl of Folin–Ciocalteu reagent was added and sample mixed for 3 min. After that, 1 ml of Na_2CO_3 solution (3.5%, *w/v*) was added and mixed in. After 1 h of incubation at room temperature, the absorbance was measured at 725 nm.

Determination of sugars

Honey sugar contents including fructose, glucose, maltose and sucrose were assayed according to the modified method of Chuttong et al. (2016a), by high performance liquid chromatography (HPLC) with refractive Index detector (RID). A 5% (*w/v*) solution of stingless bee honey in distilled water and filtered through 0.45 mum filter paper and injected into HPLC system (Milford, Massachusetts, United States), which was equipped with a waters 1525 Binary HPLC pump, 717 plus Auto samplers, Waters 2414 Refractive index detector coupled to a computer with Empower Build 1154 Software. For the determination of sugars an Supercosil LC-NH2 column (25 cm × 4.6 mm, 5 μm), mobile phase with HPLC acetonitrile/water (72,25) was used at a flow rate 1 ml/min, with an oven temperature of 40 °C.

Determination of total flavonoid content

The total flavonoid content of each honey sample was measured by the aluminum chloride colorimetric method. Quercetin (≥95% (HPLC), Sigma-Aldrich, St. Louis, MO) (0–100 μg/ml) was used as a reference material (Liu et al., 2008). First, 500 μl of the honey sample was mixed with 1.5 ml of 95% alcohol, 100 μl of 10% aluminum chloride hexahydrate, 100 μl of 1 M potassium acetate, and 2.8 ml of deionized water, then the mixture was incubated in the dark at room temperature for 30 min. Finally, the absorbance at 415 nm was measured.

Radical-scavenging effect on DPPH

The free radical scavenging activity of the three honeys on DPPH• was assessed using1, 1-diphenyl-2-picryl-hydrazyl by the method of Liu