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Physicochemical properties and mineral elements in Honey from various species of Malaysian Stingless Bee

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Flower constancy by variety of stingless bee species influence the quality of honey. This study subjected to compare the physicochemical features, antioxidant activity and minerals elements in stingless bee honey produced by *Heterotrigona itama*, *Geniotrigona thoracica*, *Lepidotrigona terminata* and *Heterotrigona erythrogastra* domesticated at Banggol Peradong, Manir, Terengganu, Malaysia. The pH of all honey is acidic and significantly differs between stingless bee species. The Brix (ranges from 67.72 to 69.98 °Bx) and electrical conductivity (ranges from 555.2 to 784.8 mS cm-1) exhibited the highest and lowest values both in *L. terminata* and *H. erythrogastra*, respectively. *Heterotrigona erythrogastra* honey possessed significantly highest moisture content (34.51%) compared to the other honey. The tristimulus values of colour indices L*, a*, and b* showed significant difference between four types of honey. The value of L* range from 49.449 to 64.006. *Lepidotrogona terminata* had the darkest honey colour, while *H. erythrogastra* had the lightest honey color. Both total flavonoid content (TFC) and total phenolic content (TPC) were the highest in *L. terminata*. Meanwhile, TPC and TFC were the lowest in *H. erythroga*stra and *G. thoracica* respectively. The highest antioxidant activities were found in *L. terminata* honey which is 80.26% of 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) degradation while the lowest antioxidant activity was found in the *H. erythrogastra* honey which is 73.9% of DPPH degradation. *Heterotrigona itama* had the most abundant of major components of potassium, calcium, magnesium and sodium with value 3634.0, 204.6, 153.3 and 142.0 mg kg^{-1}, respectively. Characterization of honey from the different stingless bee species of same locality showed variabilities that may due to different flower preferences between stingless bee species.

Keywords: stingless bee, honey, physicochemical properties, minerals element

INTRODUCTION

Honey is a sugary-based stuff that produced naturally by bees. The main component of this substances is originated from the plants' nectar, plants' living parts' discharge or plants' sucking insects' excretions. It then processed by the bees by homogenizing it with their own specific substances. Escuredo et al. (2014) detailed that the main components of honey are sugar and it also contained more than 200 of other compounds. Nectar's botanical source, the environmental condition and weather pattern as well as the flower constancy behavior of bees influenced the physicochemical properties of

honey (Fatima et al. 2016). There are various kinds of honey available worldwide, with the most collected honey comes from *Apis* sp. and recently, honey also commercially obtained from stingless bee species (Ya'akob et al. 2019).

Stingless bees or simply called as Meliponines are from Meliponini tribe under Apidae family of Hymenoptera order. Stingless bees' habitats are found in world's area of tropical and subtropical, including Malaysia (Ramli et al. 2017). Stingless bee is smaller in body size compared to honey bee. Still, stingless bee can collect nectar from most of the floral plants, yet, with lesser volume compared to honeybees (Do Nascimento et al. 2015). Their tiny measurements give them access to approach many different kinds of flowers with various opening sizes, including too narrow openings while this being limited for other flower visitors, such as *Apis* species. In Malaysia, 35 available species of stingless bees were identified (Jaapar et al. 2016), beyond 30 species of those stingless bees are from the genus *Trigona* (Ya'akob et al. 2019). Among those species, *Heterotrigona itama* and *Geniotrigona thoracica* bees have been successfully domesticated for their honey (Omar et al. 2019). Honey made by these stingless bees has discrete flavor and unique sour taste compared to the honey of *Apis mellifera*; make it as great interest among consumers.

Multiple biochemical and mineral components are available in honey including amino acids, carbohydrates and vitamin (Fechner et al. 2016), volatile components, esters, organic acids, hydroxymethylfurfural, phytochemicals and enzymes (Siddiqui et al. 2017), a few elements that has antioxidants function inclusive of vitamin C and vitamin E, phenolic contents and also enzymes (catalase and peroxidase) (Aljadi & Kamaruddin, 2004). However, the availability and amount of these components can vary according to environment, apiculture techniques and factors of honey processing (Fechner et al. 2016), its maturation process, climatic changes and types of floral foraged by bees (Rebiai & Lanez, 2014). Nordin et al. (2018) reviewed that the difference of bee species gives out significant variations on the phytochemical properties of honey.

The physicochemical, antioxidants and minerals characteristics of stingless bee honey has recently become a topic of interest for developing stingless bee honey regulatory standard. A standard, MS 2683: 2017 regulated specially for Malaysian stingless bee honey by Malaysian Standard (2017) on the stingless bee honey's different grade demand. Based on the standard, the demands are as following: sucrose < 7.5%; moisture < 35%; hydroxymethylfurfural (HMF) < 30 mg/kg; ash < 1.0%; pH from 2.5 to 3.8; and the presence of plant phenolic. However, due to the broad variation in composition of honey, the physicochemical properties, antioxidant properties and minerals contents exhibited by the honey are varied. Thus, the purpose of this study is to determine the physicochemical, antioxidant properties and minerals content of honey produced by four different species of stingless bees (*H. itama, G. thoracica, L. terminata and H. erythrogastra*) from one locality (Banggol Peradong, Manir, Terengganu, Malaysia).

MATERIALS AND METHODS

Honey Samples

Honey of four stingless bee species obtained from the apiary at Banggol Peradong, Manir, Terengganu, Malaysia (5.306667648963178, 103.04720030316003). The species of stingless bee are *H. itama, G. thoracica, L. terminata* and *H. erythrogastra.* The honey samples were tightly sealed in glass bottles of sterile airtight after harvested to avoid moisture absorption throughout sampling, storage and analytical test. The honeys were stored in the laboratory refrigerator (Sun-tech LC-213LD, Taiwan) at 4 ºC in less than 24 hours after harvested until further analysis.

pH

A pH meter from Thermo Scientific Orion 2-Star Benchtop was used to quantify the pH of honey solution. The method to determine pH of the honey was explained by Belay et al. (2013) with some adjustment. The sample was prepared by diluting 3 g of honey in 22.5 mL distilled water. A calibrated pH meter used with proper buffers (pH solution of 4.0 and 7.0) for every sample of honey. After calibration, the electrode of the pH meter was dip into the honey solution for measuring purpose. The procedure was repeated three times in order to achieve accurate result and the readings were recorded.

Electrical Conductivity

Electrical conductivity was measured by using Horiba *Laquatwin* Compact Conductivity Meter (EC33, Japan). Two grams of honey was dissolved in 10 mL of distilled water to prepare approximately 20% honey solution. Then, the prepared honey solution was dropped onto

conductivity meter for measuring its electrical conductivity. The electrical conductivity of every sample was analyzed in three replicates, and the mean values were recorded in µS/cm (Fatima et al. 2016).

Moisture Content

Moisture content was determined based on a standard method described by AOAC (2000). Approximately 2 mL of honey samples in triplicates were put in pre- weighed dried crucibles, left in an oven for overnight at 110 °C and weighed. Six replicates of moisture content reading were recorded. The loss in weight of the sample was taken to measure the moisture content as calculated based on the following formula:

$$Moisture\ (\%) = ((Weight\ of\ Fresh\ Sample - Weight\ of\ Dry\ Sample) / (Weight\ of\ Fresh\ Sample)) \times 100$$

Colour

Colour parameters (L*, a*, b*) of the honey samples was quantified by a reflectance spectrometry, Minolta Chroma Meter CR-400 colorimeter (Konica Minolta, Tokyo, Japan). This instrument was calibrated with a white background calibration plate prior to measuring the samples. The honey samples in petri dishes were measured on a white background. Six replicate values of the coordinates were record. L* referred to lightness (indicates black to white), a* referred to redness (indicates green to red) and b* referred to yellowness (indicates blue to yellow)

Total Soluble Solid

A refractometer (HANNA instruments, Italy) was used. On the prism surface of the refractometer, approximately 0.3 mL of each honey sample was applied. The values in Brix° were read in six replicates. Prior to measure the samples, the refractometer was zeroed with distilled water (Colucci et al. 2016).

Total Phenolic Content

Gallic acid standard solutions (y = 0.0132x + 0.0388 R^2 = 0.9932) was used to determine total phenolic content in honey based on Folin–Ciocalteu method (Moniruzzaman et al. 2013). Concisely, 1 mL of honey was added to 1 mL of Folin Ciocalteu's phenol reagent, and waited for 3 min. Next, 1 mL of 10% Na_2CO_3 solution was added to the mixture and distilled water used to adjust the final volume to 10 mL. The reaction solution was hold in dark, and after 90 minutes, the absorbance was read at wavelength 725 nm using a UV-1280 Multipurpose UV-Visible Spectrophotometer. Standard curve was calculated based on Gallic acid (2.5, 5.0, 12.5, 25.0, 37.5, 50.0, 100.0 and 150.0 mg/L; R2 = 0.9932). The phenolic compounds' concentration was measured and recorded in triplicate. The results were reported in mean ± standard error and was expressed as mg of gallic acid equivalents (GAEs) per gram of honey.

Total Flavonoid Content

The total flavonoid content in each honey sample was measured based on Moniruzzaman et al. (2013) by using the colorimetric assay. Approximately 1 mL of honey was mixed into 4 mL of distilled water. After that, 0.3 mL of $NaNO_2$ (5%) was added. After five min, 0.3 mL of $AlCl_3$ (10%) was added, and after the following 6 min, 2 mL of NaOH (1 M) was added. The final volume was top up until 10 mL by the addition of 2.4 mL distilled water. To ensure adequate mixing, the mixture was energetically shaken, and the absorbance was read at wavelength of 510 nm using UV-1280 Multipurpose UV-Visible Spectrophotometer. By using a standard solution of catechin (0.02, 0.04, 0.06, 0.08, 0.10 and 0.12 mg/mL; R^2 = 0.992), a calibration curve was created. The results were expressed as mg catechin equivalents (CE) per gram of honey.

Radical Scavenging Activity (DPPH)

Based on the procedure from Ferreira et al. (2009), the activity of scavenging of DPPH radical in honey was conducted. Approximately 0.5 mL of honey (0.2 g mL^{-1}) extract was mixed with 2.7 mL of methanolic DPPH radical solution (0.024 mg mL^{-1}). The mixture was shaken in a vigorous way, incubated for 15 min and then the absorbance was measured at wavelength 517 nm. From the following formula, the radical-scavenging activity (RSA) was calculated:

$$\%RSA = \frac{A_{DPPH} - A_S}{A_{DPPH}} \times 100$$

Where, A_S is the absorbance of the solution when the sample extract has been added at a particular level and A_{DPPH} is the absorbance of the DPPH solution.

Mineral Analysis

Microwave digestion method was applied on all honey samples. Approximately 0.5 g of each honey sample was digested with 9 mL of 65% HNO_3 in a microwave digestion system. The digested honey was diluted by marking up the solution up to 25 mL with ultrapure water. A blank digest was implemented in the same protocol. Approximately 10 mL of the sample solution was poured into a test tube for analyzing using ICP-OES (Thermo Scientific iCAP 7000 Series, UK). The obtained results from the instrument were express in mgL^{-l}.

Data analysis

The obtained data were assessed by analyses in R statistical software version 4.0.3 (R Core Team, 2020). The analyses were put through to one-way ANOVA (Analysis of Variance) for significant difference at 95% confidence level. P-value ≤ 0.05 was viewed to be significant. LS means was used to determine the significance differences between means values, and stingless bee species as explanatory variables were used for separation through post-hoc Tukey tests.

RESULTS AND DISCUSSION

The data from physicochemical analyses of honey based on pH, °Brix, electrical conductivity, moisture content and colors produced by four stingless bee species are represent in Table 1. The values of pH of all honey samples are significantly differs between stingless bee species; and ranged between 2.98 to 3.44 with the lowest value was found in *H. erythrogastra* honey and the highest value was found in *H. itama* honey. This may suggest that different stingless bee species even from same locality bring about variation in pH value. These values were close and likewise to those disclosed for Malaysian *Apis* sp. honeys (3.53- 4.03) (Moniruzzaman et al. 2013), Malaysian stingless bee *H. itama* honey of 3.26 (Kek et al. 2018), Brazilian stingless bee honeys (2.90- 3.83) (Silva et al. 2013), and Thai stingless bee honeys (3.10- 3.90) (Chuttong et al. 2016). However, all of the honey types had acidic character; which is naturally common for stingless bee honey and this contributed to its sour taste.

The total soluble solid of *H. erythrogastra* was the lowest with value 67.72 ° Brix and the highest was in *L. terminata* with the value of 69.98 ° Brix (Table 1). There was significant difference among the species, yet, between *G. thoracica* and *H. erythrogastra*, there was no significant difference recorded. Total soluble solid (°Brix) in stingless bee honey in contrast to honey produce by *Apis mellifera* is always described as lower in relation to the higher water content and accordingly lower sugar content (Biluca et al. 2016). Usually, honey with higher moisture content emerged from tropical countries. According to Bogdanov et al. (1999), high annual rain volume and unripening honey samples most probably influenced the high moisture contents in honey samples. Organic acids sugars, and minerals serve as the indicator parameter of solution solids rate, which however directly related to sugars and moisture in the samples. Honey adulteration is associated with sugar levels in honey; thus, it could be determined based on its Brixº value (Guerrini et al. 2009).

Result obtained shows that the highest moisture content of honey was determined from *H. erythrogastra* (34.51%) and the lowest moisture content was determined from *H. itama* of 33.13% (Table 1). These values were close to those reported in *Trigona* honeys which the moisture content reported ranging from 30.8% to 36.45% (Wong et al. 2019). According to Omar et al. (2019), moisture content of stingless bee honey from four species (*H. itama, G. thoracica, L. terminata* and *Tetragonula laeviceps*) range from 23.93% to 27.63%, with the average value was 25.78%, in which, was lower than our findings. European Union (EU Council, 2001) and international standards (Codex Alimentarius, 2001) stipulated that honey should only contains the maximum of 20% moisture to be classified as good for usage and storage. Even so, the obtained results were exceeded the limit as the limit was intentionally aims for the moisture of *Apis mellifera* honey. Marinus (2006) reported stingless bees honey's moisture can reach up to 42%. As mentioned by Ramón-Sierra et al. (2015), high moisture content in stingless bee honey may be influenced by tropical weather humidity, where it is actually almost impossible to extract low water content nectar. The main factor that possibly influence the moisture content is regional humidity of nectar collection areas; which subsequently might be able to explain the reason of no significant difference among the honeys produced by the different species of stingless bee in this study.

Electrical conductivity of all honeys ranges from 555.2 µS/cm to 784.8 µS/cm (Table 1) through which, the lowest and highest value was displayed in *H. erythrogastra* and *L. terminata* respectively.

Table 1: Physicochemical criteria of honey produced by the four species of stingless bee

Types of Analysis	*H. itama*	*G. thoracica*	*L. terminata*	*H. erythrogastra*
pH	3.44 ± 0.009^{a}	3.06 ± 0.005^{b}	3.18 ± 0.00 ^{c}	2.98 ± 0.006^{d}
° Brix	69.52 ± 0.124^{a}	67.94 ± 0.024^{bd}	69.98 ± 0.058^{c}	67.72 ± 0.073^{bd}
Electrical Conductivity (µS/cm)	636.2 ± 2.177^{a}	559.2 ± 0.583^{bd}	784.8 ± 0.970^{c}	555.2 ± 0.583^{bd}
Moisture Content (%)	33.13 ± 0.087^{abc}	33.44 ± 0.098^{abc}	33.26 ± 0.086^{abc}	34.51 ± 0.084^{d}
Color L*	52.38 ± 0.117^{a}	64.01 ± 0.076^{b}	49.45 ± 0.150^{c}	61.00 ± 0.148^{d}
Color a*	0.682 ± 0.014^{a}	-4.287 ± 0.009^{b}	1.890 ± 0.073^{c}	-3.887 ± 0.012^{d}
Color b*	10.12 ± 0.243^{a}	12.00 ± 0.809^{b}	35.93 ± 0.177^{c}	14.07 ± 0.123^{d}

Values conveyed as mean ± standard error. Different superscript letters in the same row designate significant differences (ANOVA, $p < 0.005$).

This indicates that honey floral origin exhibited high variabilities, thus, it is important for determining floral origin of honey, especially when it comes from various bee species. The values were in accordance with standard stipulated in EU Directive (EU Council, 2001) which stated that electrical conductivity value of honey should be lower than 800 µS/cm. Nonetheless, the values were far greater than the electrical conductivity values reported by Omar et al. (2019) in different species of stingless bee honey from Kelantan, that ranged between 180 µS/cm to 290 µS/cm. Besides that, based on finding from Fatima et al. (2016), the electrical conductivity of honey from *Trigona* sp. range from 470 µS/cm to 550 µS/cm which were closer to our findings. The great variation of electrical conductivity may cause by the difference in organic acids, mineral concentration and protein content of extracted nectar from the floral source.

Visual colour of stingless bee honey were examined in three tristimulus values namely L*, referred as lightness (0= black to 99= white), a*, referred as redness (towards +60 direction red, towards -60 direction green) and the b*, referred as yellowness (towards +60 direction yellow, towards -60 direction blue) (Wilczyńska, 2014). The colour characteristics of the analyzed honeys are presents in Table 1. The value of L* range from 49.449 to 64.006, a* values range from -4.287 to 1.890 and b* values range from 10.116 to 35.920. *Lepidotrogona terminata* had the darkest honey colour, while *H. erythrogastra* had the lightest honey colour. According to González-Miret et al. (2007), honey samples with L value greater than 50 are lighter colored honeys, whereas, samples with L value lower than 50 are dark honeys. In this study, *L. terminata* was the only honey that had L value lower than 50 (49.449), therefore, from this classification it can be considered as dark coloured honey, while the other three honey species were considered as light honey. The positive a* and b* value in *L. terminata* indicated the presence of red and yellow components respectively in the honey. *H. erythrogastra* honey was the light-coloured honey with L* tristimulus value of 61. Meanwhile, negative a* value and positive b* value indicated it made up of red and blue components. According to González - Miret et al. (2007), Da Silva et al. (2016) and Colucci et al. (2016), the rate of darkening has been correlated to the composition of honey and the storage temperature and time. The methods that used to process honey can also altered the colour of honey (Ramly et al. 2021). Furthermore, research by Habib et al. (2014), Czipa et al. (2015) and Da Silva et al. (2016) had found close correlation between colour and pollen content, mineral content, geographical origin, plant origin and also between colour and physical properties of the honey, for example electrical conductivity. Regardless of that, based on United States Standards for Grades of Extracted Honey (USDA, 1985), the colour of extracted honey is not a factor of quality honey for the purpose of colour grade designations of extracted honey.

Plant phenolic form one of the main groups of compounds working as primary antioxidants or free radical scavengers. According to Kroyer & Hegedus (2001), one of the main classes' polyphenols, flavonoids are present in pollen collected by honeybees. Silva et al. (2013) reported that phenolics and flavonoids in honey resulted in superior antioxidant activity. The

results attained for Total Phenolics Content (TPC) and Total Flavonoids Content are presented in Figure 1.

Stingless bee honey from different species expressed variations in their TPC and TFC. According to Rosidi Sujanto et al. (2021), TPC value surfaced the variations maybe because of different types of phenolic acids present in stingless bee honey. Result shows that the TPC and TFC values of the honey made by those four stingless be species are varied between 1.395-6.801 mg GAE/g and 0.909-2.298 mg CE/g respectively. The value of TPC and TFC are highest in *L. terminata* honey, while *H. erythrogastra* and *G. thoracica* honey had the lowest value. The TPC and TFC values resulted in our study were higher than Malaysian honey (*Apis* sp.) with the value of 0.412 mg GAE/g and 0.074 mg CE/g respectively (Khalil et al. 2011).

However, our results were slightly in harmony with the finding by Olievera et al. (2017) which ranges between 3.07- 85.5 mg GAE/g and 3.02-28.0 mg QE/g respectively. The differences between the honeys could be due to the flower preferences of the discrete stingless bee species regardless they reside the same environment. The phenolic compounds of honey are dependent directly on the botanical resources, namely as nectars, pollens, oils and resins that are provided to the bees (Aljadi & Kamaruddin, 2004), bee species, foraging area and floral origin of honey (Saxena et al. 2010; Olievera et al. 2017). As a consequent, honeys from distinct floral origins have different bioactive properties (Aljadi & Kamaruddin, 2004) and responsible for the aroma and antioxidant potentiality (Saxena et al. 2010; Olievera et al. 2017). According to Lachman et al. (2010) and Escuredo et al. (2019), the content of polyphenol and flavonoid are highly related to the honey colour. Honey of dark color has been reported to possess high antioxidant activity (Aljadi & Kamaruddin, 2004). Radical Scavenging Activity (DPPH) assay was done to measures a substance's ability to scavenge the radical activity of DPPH, thus, it was reduced into hydrazine. Hydrazine is produced (color change from violet to pale yellow) when a hydrogen atoms donor substance is added to DPPH solution. The flower sources used to gather nectar, have effect on the composition and antioxidant capacity of honey (Baltrusaityte et al. 2005).

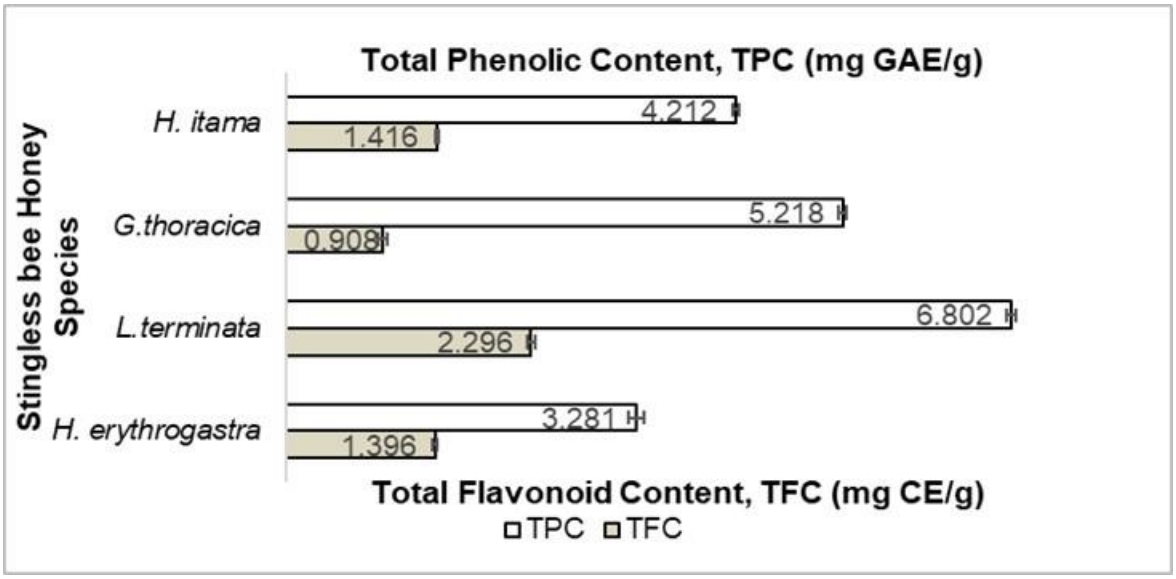

Figure 1: Total phenolic content (TPC) and total flavonoid content (TFC) of four stingless bee species

Figure 2 presents the DPPH scavenging activities of honey produced by four stingless bee species. In determining the activity of scavenging the free radical, positive control used was ascorbic acid. Ascorbic acid as standard proclaimed the highest in the inhibition of DPPH radical. Its inhibition on DPPH was almost flawless which ranges from 98.97% to 99.50% when the concentration of honey was between 20-40 mg/mL.

Ascorbic acid had been used as standard as its availability in various food sources especially in

citrus fruit, and it is a low cost of antioxidant source. Result shows that the highest percentage of DPPH inhibition of all honey collected from all stingless bee species was observed at the highest concentration; which at 40 mg/mL of honey. Meanwhile, significant differences ($p < 0.05$) were recorded between *H. itama, G. thoracica, L. terminata* and *H. erythorgastra* with percent of inhibition was 78.81%, 75.64%, 80.26% and 75.35% respectively. However, there was no significant difference being observed between *G. thoracica* and *H. erythrogastra* honey. The results were significantly higher than those reported from stingless bee from five different regions; south (Johor), eastern (Kelantan), central (Selangor), northern (Kedah) and also east coast region of Sabah and Sarawak in Malaysia that only ranged between 1.98% to 44.05% of DPPH inhibition (Maringgal et al. 2019).

In this study, nine minerals were identified and quantified in honey sample which are; potassium, calcium, sodium, magnesium, phosphorus, copper, manganese iron, and zinc (Table 2). Potassium is the major metal detected in the honey samples, followed by sodium, calcium, phosphorus and magnesium. The trace elements include copper, iron, manganese and zinc were also detected and quantified (Table 2).

Mineral with highest concentration that present in the honeys was potassium that ranged between 3486.2 to 3645.6 mg kg-1. Potassium exhibited the highest amount in *L. terminata* honey (3645.6 mg kg-1), while *H. itama* honey contains the higher amount of sodium, calcium, magnesium and phosphorus (142.0, 204.6, 153.3 and 46.8 mg kg-1 respectively). In accordance with our finding, Abu Bakar et al. (2017) reported that the highest quantity of potassium in stingless bee honey was ranging from 236.33 to 701.33 mg kg-1. On the other hand, Yücel & Sultanoğlu (2013) quantified the value of potassium in Turkey *Apis* sp. honey was ranging from 104.40 to 895.50 mg kg-1, while Dourado et al. (2019) reported that Brazil stingless bee honey contains approximately 30.75 to 412.22 mg kg-1 potassium. It can be concluded that the potassium content of the honeys in this study was far higher when compared to the other previous study as stated in the literature. Based on WHO, the recommended dietary allowance, RDA for potassium intake is at least 3510 mg/day for adults (WHO, 2009). Thus, the daily intake of these stingless bee honeys is sufficient for our body.

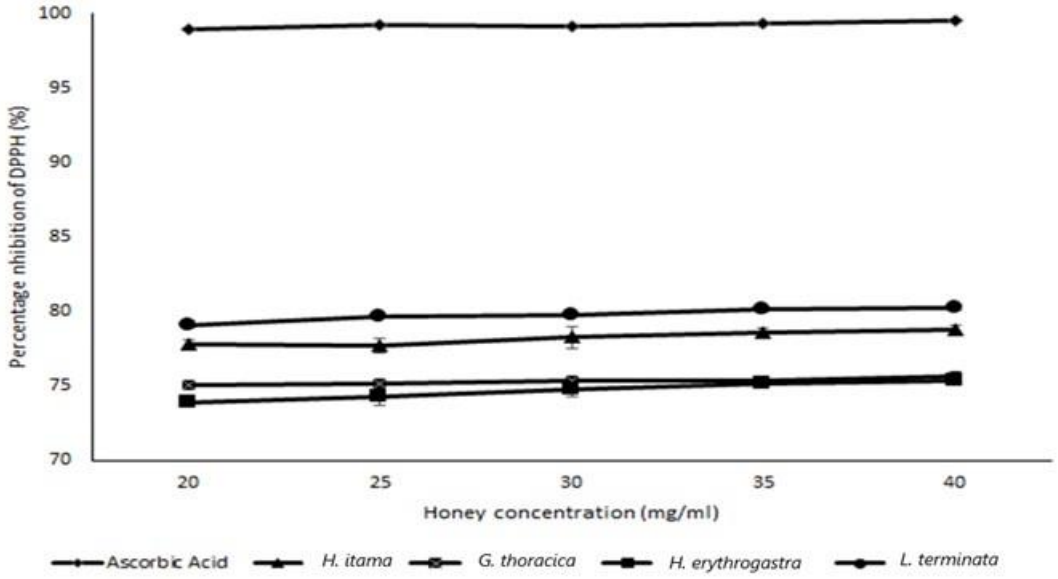

Figure 2: Percentage of DPPH radical scavenging activity inhibition at different concentrations of four different stingless bee species

Table 2: Mineral concentrations in honey from four different stingless bees' species (mg kg$^{-1)}$

Elements	*H. itama*	*G. thoracica*	*L. terminata*	*H. erythrogastra*
Potassium (K)	3634.0 ± 11.62^{ac}	3486.2 ± 29.53^{bd}	3645.6 ± 19.04^{ac}	3509.8 ± 15.26^{bd}
Sodium (Na)	142.0 ± 5.21^{a}	71.9 ± 5.34^{bcd}	94.4 ± 12.53^{bcd}	78.7 ± 4.50^{bcd}
Calcium (Ca)	204.6 ± 10.57^{a}	3.8 ± 0.20^{bcd}	14.0 ± 2.90^{bcd}	5.2 ± 0.46^{bcd}
Magnesium (Mg)	153.3 ± 13.13^{a}	0.6 ± 0.00^{bcd}	10.2 ± 0.77^{bcd}	3.9 ± 0.51^{bcd}
Manganese (Mn)	9.7 ± 0.03^{a}	0.1 ± 0.12^{bcd}	0.2 ± 0.06^{bcd}	0.1 ± 0.03^{bcd}
Phosphorus (P)	46.8 ± 5.07^{ac}	1.4 ± 0.04^{bd}	38.6 ± 0.78^{ac}	12.6 ± 1.21^{bd}
Iron (Fe)	16.0 ± 0.22^{a}	5.9 ± 0.06^{bcd}	3.6 ± 0.50^{bcd}	3.3 ± 1.85^{bcd}
Copper (Cu)	1.2 ± 0.04^{a}	1.5 ± 0.01^{a}	2.5 ± 1.13^{a}	1.2 ± 0.01^{a}
Zinc (Z)	3.0 ± 0.43^{a}	2.8 ± 1.05^{abd}	0.1 ± 0.12^{cd}	0.4 ±0.04 ^{bcd}

Values conveyed as mean ± standard error. Different superscript letters in the same row designate significant differences (ANOVA, $p < 0.005$).

CONCLUSION

Honey harvested from *H. itama, G. thoracica, L. terminata,* and *H. erythroastra* showed significant variability on physicochemical characteristics when compared to each other except for the values of Brix°, moisture content and electrical conductivity. Moisture content doesn't demonstrate significant difference in between most of the species except for the *H. erythrogastra*. Meanwhile, Brix° and electrical conductivity demonstrated significant difference among almost all the species except for between *G. thoracica* and *H. erythrogastra*. Honey produced by those stingless bee species are significantly differ in colour with the darkest can be observed in *L. terminata*, otherwise the brightest is in *H. erythrogastra*. In both TPC and TFC, the highest result was recorded in *L. terminata*, while the lowest was recorded in *H. erythrogastra* and *G. thoracica* respectively. TPC result showed great significant differences among the species, though in TFC result, there was no significant difference recorded between *H. itama* (1.416 mg CE/g) and *H. erythrogastra* (1.396 mg CE/g). The percentage of DPPH inhibition from this analysis was exceptionally higher than previous literature with range 75.35% to 80.26%. *Lepitrogona terminata* honey shows the highest DPPH inhibitor, meanwhile *H. erythrogastra* was the lowest DPPH inhibitor recorded. This result was in character with

Brix°, electrical conductivity and colours of the honey by means of the highest and the lowest value was recorded in *L. terminata* and *H. erythrogastra* respectively. According to the analysis on mineral content, potassium was the most abundant mineral detected in all honey species with greatly higher than the other elements. In addition, potassium was the highest in *L. terminata* honey when compared to other bee species. However, elements of sodium, calcium, magnesium and phosphorus concentrated the highest value all in *H. itama*. As trace elements, copper, iron, manganese and zinc available in a lowly amount.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

NSR planned and managed the study, performed the experiment, collecting and analysis the data, write the manuscript; ISRS performed the experiment, collecting and analysis the data, write the manuscript; JTYH, AAG and NA conceived the idea, interpreted data and write the manuscript; SM and AJZ interpreted the data and write the manuscript; NN conceived the idea, designed the research methodology planned the study, supervised the project, performed and interpreted the data analysis, write the manuscript. All authors read and approved the final version.

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