ORIGINAL ARTICLE

Comparison of Sugar Content, Mineral Elements and Antioxidant Properties of *Heterotrigona Itama* Honey from Suburban and Forest in Malaysia

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ABSTRACT

Introduction: In Malaysia, *Heterotrigona itama* sp. (stingless bee) industries start to grow rapidly since 2015 but the study on its health benefit is still lacking. This study was aimed to analyse and compare the sugar content, minerals and antioxidant properties of stingless bee honey collected from forest and suburban area in Malaysia. **Methods:** Sugar content was determined by HPLC, minerals and heavy metals was determined by Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) and Atomic Absorption Spectrometer (AAS), total phenolic content (TPC) and total flavonoid content (TFC) by Folin-Ciocalteu and aluminium chloride colorimetry method, respectively. For determining the antioxidant activity of the samples, 2,2-diphenyl-1-(2,4,6-trinitrophenyl) hydrazyl (DPPH), 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and ferric reducing antioxidant power (FRAP) assay were used. **Results:** Fructose, glucose and sucrose are found in all samples in range of 16.03-33.13 g/100g, 8.63-20.72 g/100g and 2.68-34.04 g/100g, respectively. Potassium and sodium were major minerals in all stingless bee honey with an average of 622.36 and 496.01 mg/kg, respectively. Sample from the forest (Sibu, F2) has the highest TPC and TFC with value 520.663±8.119µg GAE/g and 443.25±18.194µg RE/g, respectively. The higher antioxidant activities (DPPH, ABTS, FRAP) also found in samples collected from the forest (F2) with 602.15±12.7 µg TE/g, 575.18±9.38 µg TE/g and 641.36±42.11µg TE/g, respectively. **Conclusion:** All stingless bee honey studied shown a significant amount of important minerals and antioxidant properties with samples from forest clearly shown significantly higher TPC and TFC as well as the antioxidant activity than samples collected from the suburban area.

Keywords: Stingless bee honey, *Heterotrigona itama* sp., Forest, Suburban, Antioxidant

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INTRODUCTION

Free radicals are unstable and reactive as they have a high tendency to pair up with other molecule to produce a stable compound that causes negative functional alterations on cell components such as protein and the nucleic acid that lead to various pathological diseases (1). Antioxidant protecting the body against free radical by converting the free radical into less harmful molecule thus stops the radical chain reaction that caused oxidation (2).

Heterotrigona itama sp (Stingless bee) is among the group of eusocial insect belonging to the family of Apidae in the tribe Meliponini (3). The compositions of honey produced by stingless bee are closely related with bee species, geographical and floral source (4). Honey contains compounds which responsible for antioxidant and redox activities such as phenolic acids, flavonoids and minerals (5). As reported, stingless bee honey has higher phenolic content compared to Apis spp. honey (6). This is due to the size of stingless bee which is much smaller compared to Apis spp. that enable them to collect a nectar from various type of flowers thus increase the content of the bioactive compound in the honey itself (7).

In Malaysia, stingless bee honey been reared in two major area which are forest and suburban. Although there are no solid evident which reared area better in term of quality, people tends to consume honey that been reared at forest compared to the honey that been reared in suburban area. Therefore, this study aimed to analyse and compare the sugar content, mineral elements and antioxidant properties of stingless bee honey from suburban area and forest.

MATERIALS AND METHODS

Honey samples collection

Eight honey samples from *Heterotrigona itama* sp were harvested from the suburban area (Kuala Nerang S1, Universiti Putra Malaysia S2, Muar S3 and Kuala Terengganu S4) and from the forest (Tumpat F1, Sibu F2, Kota Belud F3 and MARDI F4) during July 2017. All the samples were collected fresh and stored in a sterile glass bottle at 4°C until further analysed. All the analyses were conducted in triplicate and according to the procedures explained by the International Honey Commission (8).

Chemicals and reagents

Ultrapure water, methanol, acetonitrile, acetic acid (HPLC grade) were purchased from Sigma Chemical Co (St. Louis, Missouri, United States), saccharide standards (fructose, glucose and sucrose) from Systerm (Selangor, Malaysia); sodium carbonate, aluminium chloride ($AlCl_3$), 2,2-diphenyl-1-hydrazyl-hydrate (DPPH), potassium ferricyanide, sodium dodecyl sulphate (SDS) and ferric chloride ($FeCl_3$) were from Sigma Aldrich Co (St. Louis, Missouri, United States). Gallic acid, rutin and Trolox standard were supplied by Merck (Darmstadt, Germany) and all chemicals and reagents used were of analytical grade.

Moisture content

The moisture content of the honey samples was determined using the method by the Association of Analytical Communities (AOAC) (9). The samples were placed in the crucible for 4 hours in 105 °C oven before being transferred to a desiccator for 15 minutes to cool before weighing. The percentage of moisture content was calculated using the formula:

$$\text{Moisture content (\%)} = \frac{\text{pre-dry weight (g)} - \text{after drying weight (g)}}{\text{Pre-dry weight (g)}} \times 100\%$$

Determination of sugars by HPLC–Refractive Index

High Performance Liquid Chromatography (HPLC) equipped with a refractive index detector (RID) (Agilent 1100) was performed to determine the sugar content of the stingless bee honey (AOAC Official Method 977.20). A 5% (w/v) of a honey solution was injected on a Phenomenex NH2 column (5 µm, 250 mm x 4.6 mm). The column was kept at 30 °C and the injection volume was 20 µL. The mobile phase consisted of acetonitrile and the separation was performed by isocratic elution with water (75:25) at a flow rate of 1.2mL/min. Standard used were glucose, sucrose and fructose with high purity (> 99.9%) (Systerm, Selangor, Malaysia). All solutions of samples were filtered through 0.46 µM filter membrane before used.

Mineral analysis

The mineral of the honey samples was analysed according to the methods by Kek et al. (6). Three millilitres of nitric acid (65% v/v) and 2 mL of hydrogen peroxide (30% v/v) were added to 0.5 g of stingless bee honey and then the digestion process was conducted in a microwave system (MLS 1200 Mega, Italy). The digested sample was diluted to 50 mL with deionized water. The contents of potassium, sodium, calcium, magnesium, iron, zinc and heavy metals of cadmium, antimony, lead, arsenic was determined by using an Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) (Perkin Elmer 5300 DV, Waltham, Massachusetts, USA) followed by Atomic Absorption Spectrometer (AAS), (AAS) (Perkin Elmer Pinaacle 500) for heavy metal mercury (Hg). The results for mineral content were expressed as mg/kg of stingless bee honey.

Total phenolic content (TPC)

TPC of stingless bee honey was quantified using Folin-Ciocalteu reagent followed the method by Ooi et al. (10) with slight modification. For this assay, an aliquot of samples (100 µL) was mixed together with 500 µL of 10% Folin-Ciocalteu reagent and vortexed for 10 seconds. Then, 400 µL of 7.5% sodium bicarbonate solution was added and further vortexed for 10 seconds to make a homogenous solution. After incubation for 30 min in dark environment at 40 C, the absorbance of the reaction mixtures was measured at 760 nm by using Biotek Synergy H1 Multi-Mode Reader (Winooski, USA). Gallic acid served as a standard solution and TPC of the samples was expressed in microgram gallic acid equivalent per gram sample (µg GAE)/g sample).

Total flavonoid content (TFC)

Aluminium chloride colorimetric assay (10) was used to evaluate TFC of stingless bee honey, with minor modification. In brief, 100 µL of diluted sample (1 g in 10 mL) was reacted with 2% $AlCl_3$ for 10 min at room temperature in a 96-well plate. Rutin was used as standard and TFC of the samples were expressed in microgram rutin equivalent per gram sample (µg RE)/g sample).

Antioxidant assays

DPPH free radical scavenging activity

This procedure was conducted according to Chan et al. (11) with some modifications. An aliquot of 50 µL of diluted samples was mixed with 200 µL of 0.1 mM DPPH methanolic solution in a 96-well plate, gently swirled and left to stand at room temperature for 1 hr in a dark room. The mixture was then measured spectrophotometry at 540 nm. Trolox was used as a

standard with a series of concentration. The DPPH free radical scavenging activity was expressed in microgram Trolox equivalent per gram sample (µg TE)/g sample).

ABTS radical cation scavenging activity

The free radical scavenging activity of the samples was determined by ABTS radical cation decolourization assay according to the methods by Chan et al. (12). ABTS radical cation was produced by mixing 1 mL of ABTS stock solution (7 mmol/L) to 1 mL of potassium persulfate (2.45 mmol/L) for 12 hrs in the dark at room temperature. The mixture was then diluted with deionized water to form a solution with the final absorbance of 0.70 ± 0.02 at 734 nm. The scavenging activity was determined by the addition of 20 µL diluted sample to 200 µL of the adjusted ABTS radical cation solution. The absorbance of the mixture was recorded at 734 nm after 10 min. Trolox was used as standard and ABTS scavenging activity of the samples was expressed as µg TE/g sample.

Ferric reducing antioxidant power (FRAP) assay

Total reducing capacity of honey samples was determined by using a method described by Khiati et al. (13) with minor modification. Briefly, FRAP assay was performed by mixing 1 mL of diluted sample with 5 mL of deionised water and 1.5 mL of 1 M HCl. Then, 1.5 mL of potassium ferricyanide (1%; w/v) was added to the mixture and subsequently followed by 0.5 mL sodium dodecyl sulphate (1%; w/v) and 0.5 mL of ferric chloride (0.2%; w/v). The solution was incubated for 20 min at 50 C and the absorbance was read at 750 nm against blank. The FRAP values were obtained by comparing the absorbance change in the samples with the linear calibration curve of Fe3+ and expressed as µg Trolox equivalent TE/mg sample.

Statistical analyses

All data were presented as the mean ± standard deviation (SD) from triplicates samples. Analysis of variance (ANOVA) was determined by using GraphPad Prism (Version 7) accompanied with Tukey test to examine the significant difference between samples ($p<0.05$). Pearson correlation test was applied to determine the correlation between phenolic content, flavonoid content and antioxidant activity of the samples.

RESULTS

Moisture and sugar content of honey

The moisture content and sugar analysis of all stingless bee honey samples are presented in Table I. From the analysis, the moisture content of all samples generally was not differed significantly ($p>0.05$) except sample from Tumpat (F1) showed the lowest value of moisture (25.23%) ($p<0.05$). Comparison between honey samples collected from suburban and forest also did not show any significant difference in moisture content ($p>0.05$). The results of sugar analysis of the honey samples were categorised into fructose (F), glucose (G), sucrose (S), summation of fructose and glucose (F+G), ratios of fructose to glucose (F/G) and glucose to water content (G/W) (Table I). Fructose in the samples presents in the range of 16.03-33.13 g/100 g, glucose 8.63-20.72 g/100 g and sucrose 2.68-34.04 g/100 g. For the F+G, the range was 24.81-53.48 g/100 g while for the ratios of F/G and G/W the range was 0.85-2.18 g/100 g and 0.26-0.66 g/100 g, respectively. For sugar content analysis, the comparison between honey samples collected from suburban and forest did not show any significant difference ($p>0.05$).

Mineral analysis

Table II shows the results for potassium (K), sodium (Na), calcium (Ca), magnesium (Mg), iron (Fe) and zinc (Zn) and five heavy metals elements, cadmium (Cd), antimony (Sb), mercury (Hg), lead (Pb) and arsenic (As) in honey samples. The highest potassium was found in F2 (Forest-Sibu) (761.22 mg/kg), sodium and magnesium in F4 (Forest- MARDI) (589.46 mg/kg and 41.06 mg/kg, respectively), calcium in F3 (Forest- Kota Belud) (205.98 mg/kg), iron in F1 (Forest-Tumpat) (781 mg/kg) and lastly zinc was found the highest in S4 (Suburban-Kuala Terengganu) (3.03 mg/kg). Total element content was calculated by summation of all elements tested. F2 honey (Forest-Sibu) from forest show the highest total

Table I: Moisture and sugar content in *H. itama* honey samples

Honey samples	Moisture content %	Sugar content (g/100g)					
		Fructose	Glucose	Sucrose	F+G	F/G	G/W
S1	29.60±1.03^{b}	16.03±1.64^{c}	18.89±2.25^{a}	6.72±2.20^{c}	34.92±3.65^{c}	0.85±0.06^{b}	0.64±0.09^{a}
S2	31.27±0.70^{ab}	28.91±1.58^{ab}	17.06±1.85^{a}	9.30±0.93^{c}	45.97±3.43^{a}	1.70±0.10^{ab}	0.55±0.06^{a}
S3	33.30±1.64^{a}	33.13±1.76^{a}	20.36±2.16^{a}	25.63±1.23^{a}	53.48±2.76^{a}	1.64±0.18^{ab}	0.61±0.08^{a}
S4	33.68±0.70^{a}	16.19±3.08^{c}	8.63±2.30^{b}	6.50±0.56^{c}	24.81±2.50^{d}	2.02±0.91^{a}	0.26±0.07^{b}
F1	25.23±0.74^{c}	25.91±1.49^{b}	16.51±1.63^{a}	7.77±1.99^{c}	42.42±1.83^{bc}	1.58±0.21^{ab}	0.66±0.08^{a}
F2	31.16±0.99^{ab}	27.76±2.13^{ab}	17.01±2.02^{a}	6.91±1.80^{c}	44.77±4.06^{ab}	1.64±0.10^{ab}	0.55±0.08^{a}
F3	32.46±0.05^{a}	26.20±0.64^{b}	16.18±4.20^{a}	2.68±0.73^{d}	42.39±4.82^{b}	1.69±0.44^{ab}	0.50±0.13^{a}
F4	33.37±0.75^{a}	18.58±2.04^{c}	20.72±1.73^{a}	14.48±1.54^{b}	39.31±2.85^{b}	0.90±0.11^{b}	0.62±0.05^{a}

Mean ± standard deviation values in the same column with different superscript letters are significantly different ($P < 0.05$)
F + G summation of fructose and glucose, F/G ratio of fructose to glucose, G/W ratio of glucose to water
Suburban area: (S1) Kuala Nerang, (S2) Universiti Putra Malaysia, (S3) Muar, (S4) Kuala Terengganu
Forest are: (F1) Tumpat, (F2) Sibu, (F3) Kota Belud and (F4) MARDI

Table II: Elements of mineral and heavy metal content in stingless bee honey samples

Samples	Elements (mg/kg)									
	Potassium	Sodium	Calcium	Magnesium	Iron	Zinc	Cadmium	Antimony	Mercury	Lead	Arsenic
S1	484.11± 25.62^{e}	404.54± 27.13^{c}	173.71± 8.24^{ab}	26.26± 4.68^{ab}	6.77± 0.82^{a}	1.16± 0.18^{c}	< 0.01^{a}	< 0.01^{b}	< 0.01^{a}	< 0.10^{a}	< 0.01^{a}
S2	677.2± 38.10^{bc}	581.23± 25.56^{a}	182.48± 14.57^{ab}	33.82± 8.27^{ab}	6.52± 0.59^{a}	2.21± 0.15^{b}	< 0.01^{a}	< 0.01	< 0.01^{a}	< 0.10^{a}	< 0.01^{a}
S3	592.22± 17.78^{d}	505.13± 16.42^{b}	144.22± 9.35^{b}	14.56± 5.67^{b}	6.51± 1.02^{a}	2.11± 0.17^{b}	< 0.01^{a}	< 0.01	< 0.01^{a}	< 0.10^{a}	< 0.01^{a}
S4	556.15± 16.78^{de}	381.47± 37.82^{c}	202.49± 14.77^{ab}	25.96± 11.10^{ab}	6.02± 0.73^{a}	3.03± 0.31^{a}	< 0.01^{a}	< 0.01	< 0.01^{a}	< 0.10^{a}	< 0.01^{a}
F1	609.97± 26.13^{cd}	491.59± 19.84^{b}	141.22± 12.69^{b}	38.45± 6.37^{a}	7.81± 0.87^{a}	1.67± 0.24^{bc}	< 0.01^{a}	< 0.01	< 0.01^{a}	< 0.10^{a}	< 0.01^{a}
F2	761.22± 20.41^{a}	570.07± 17.36^{ab}	171.26± 13.15^{b}	28.66± 7.86^{ab}	7.01± 0.59^{a}	1.02± 0.28^{c}	< 0.01^{a}	< 0.01	< 0.01^{a}	< 0.10^{a}	< 0.01^{a}
F3	599.84± 28.85^{d}	444.59± 17.17^{bc}	205.98± 11.13^{a}	29.48± 11.70^{ab}	5.98± 0.99^{a}	1.51± 0.17^{bc}	< 0.01^{a}	< 0.01	< 0.01^{a}	< 0.10^{a}	< 0.01^{a}
F4	698.20± 30.04^{ab}	589.46± 26.48^{a}	191.89± 12.40^{a}	41.06± 7.85^{a}	7.05± 0.68^{a}	2.09± 0.24^{b}	< 0.01^{a}	< 0.01	< 0.01^{a}	< 0.10^{a}	< 0.01^{a}

Mean ± standard deviation values in the same column with different superscript letters are significantly different (P < 0.05)
Suburban area :(S1)Kuala Nerang, (S2) Universiti Putra Malaysia, (S3) Muar, (S4) Kuala Terengganu
Forest are: (F1) Tumpat, (F2) Sibu, (F3) Kota Belud and (F4) MARDI

element content with 1539.24 mg/kg followed by F4, also from the forest with 1529.75 mg/kg. The average total element content for honey collected from the forest was 1411.77 mg/kg which significantly higher ($p<0.05$) that honey collected from suburban with 1254.97 mg/kg. The content of heavy metals (Cd, Sb, Hg, Pb, and As) in all stingless bee honey samples were less than 0.01 mg/kg with no significant different ($p>0.05$).

Total phenolic content (TPC) and total flavonoid content (TFC)

The results for TPC and TFC of stingless bee honey samples are shown in Figure 1. The mean value of TPC in stingless bee honey samples was 357.06±3.23 µg GAE/g honey. The highest phenolic content was identified in F2 (520.66±8.12 µg GAE/g) whereas the lowest was in S1 (193.056±3.465 µg GAE/g). Among all tested samples, the highest and the lowest level for TFC were in F2 (443.25±9.35 µg RE/g) and S2 (98.28±15.02 µg RE/g), respectively with mean value was 293.47±3.26 µg RE/g honey.

Antioxidant activity

Antioxidant activity of stingless bee honey was evaluated by three antioxidant assays (DPPH, ABTS and FRAP). Results obtained are shown in Figure 2. Results for DPPH and ABTS assays demonstrated that F2 (Forest-Sibu) honey exhibited the highest antioxidant activity against both radicals with 602.15±12.7 µg TE/g, 575.18±9.38 µg TE/g honey, respectively. The antioxidant activity as determined by FRAP assay also showed that F2 (Forest-Sibu) exhibited the highest value whereas the lowest was found in S3 (Suburban-Muar) with the range from 271.26 to 641.36 µg TE/g honey.

Positive correlation were found significant between TPC and antioxidant activity as measured by DPPH, ABTS and FRAP with TPC/DPPH, $r = 0.841$ ($p<0.01$), TPC/ABTS, $r = 0.899$ ($p<0.01$) and TPC/FRAP, $r = 0.830$ ($p<0.01$), respectively as shown in Table III. Similar with TPC, the positive correlations was also found between TFC and antioxidant (TFC/DPPH $r = 0.971$), (TFC/ABTS $r = 0.889$) and (TFC/FRAP $r = 0.819$) with the $p<0.05$.

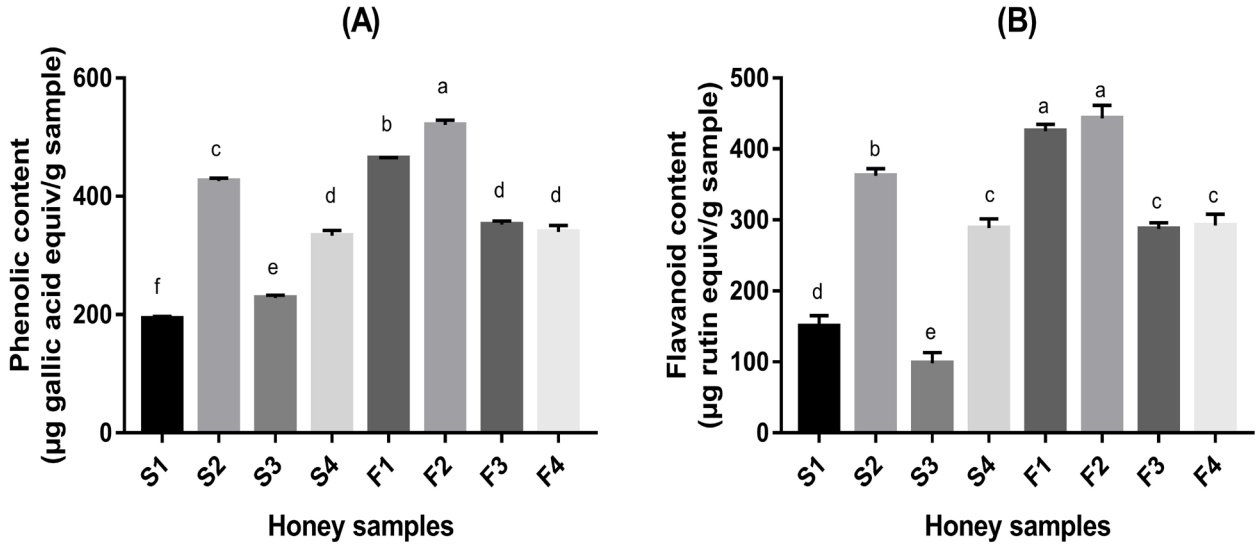

Figure 1: Total phenolic content (A) and total flavonoid content (B) of *H. itama* honey from suburban and forest area. Suburban area :(S1)Kuala Nerang, (S2) Universiti Putra Malaysia, (S3) Muar, (S4) Kuala Terengganu. Forest are: (F1) Tumpat, (F2) Sibu, (F3) Kota Belud and (F4) MARDI. Value with different letters indicate significant differences (P<0.05)

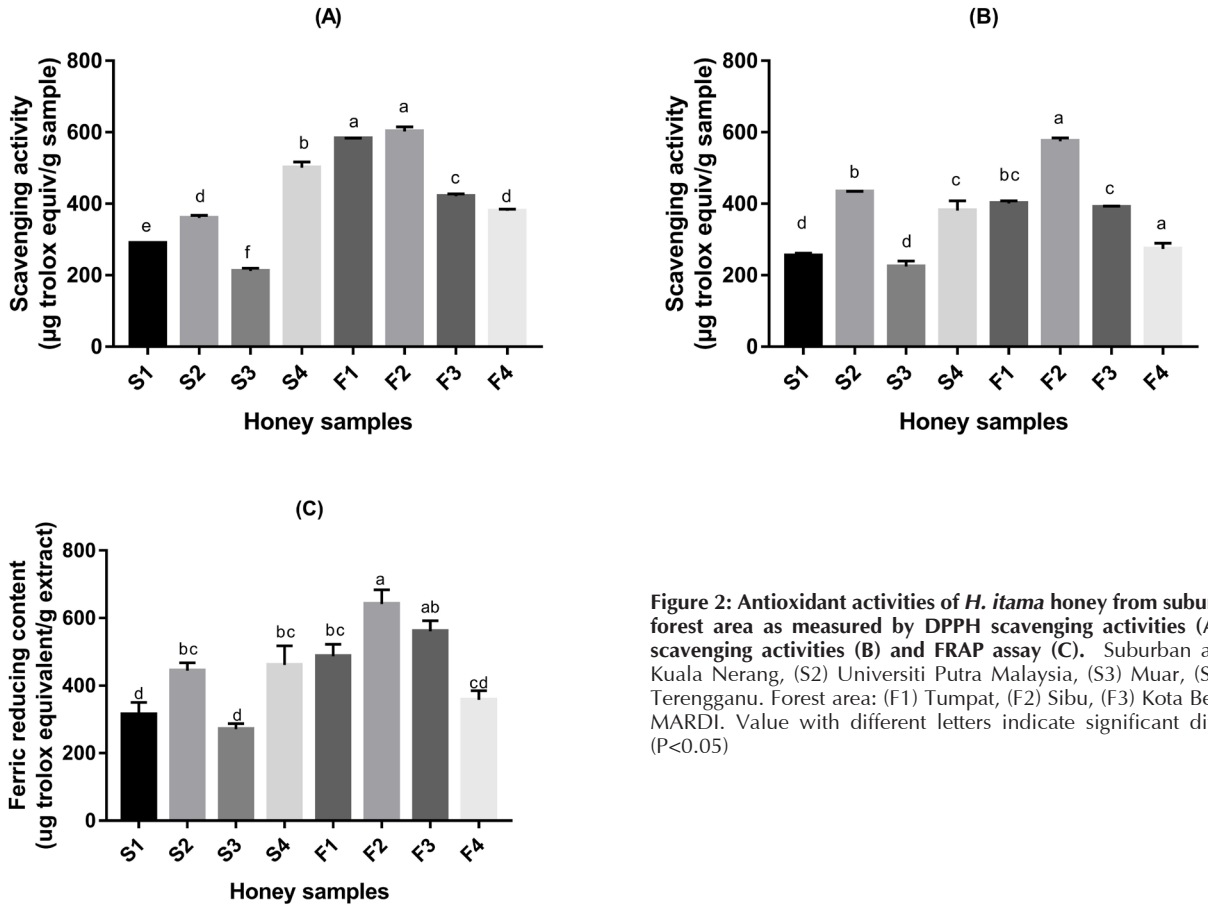

Figure 2: Antioxidant activities of *H. itama* honey from suburban and forest area as measured by DPPH scavenging activities (A), ABTS scavenging activities (B) and FRAP assay (C). Suburban area: (S1) Kuala Nerang, (S2) Universiti Putra Malaysia, (S3) Muar, (S4) Kuala Terengganu. Forest area: (F1) Tumpat, (F2) Sibu, (F3) Kota Belud, (F4) MARDI. Value with different letters indicate significant differences ($P<0.05$)

Table III: Correlation matrix (Pearson correlation coefficients) between TPC, TFC and antioxidant activity

	TPC	TFC	DPPH	ABTS	FRAP
TPC		0.971***	0.841**	0.899*	0.830*
TFC	0.971***		0.889**	0.866**	0.819*
DPPH	0.841**	0.889**		0.809*	0.842**
ABTS	0.899*	0.866**	0.809*		0.930*
FRAP	0.830*	0.819*	0.842**	0.930*	

TPC total phenolic content, TPC total flavonoid content, DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) scavenging assay , ABTS (2,2′-azino-bis 3-ethylbenzothiazoline-6-sulphonic acid) scavenging assay, FRAP (Ferric reducing antioxidant power) assay
(*P < 0.05, **P < 0.01, ***P < 0.001)

DISCUSSION

Moisture content is a significant validation of honey quality with the lower moisture content indicates the better shelf life (14). The average of moisture content in all samples studied was found 30.71%, which was higher than 20% set by Codex standard for honey (15). The significance of moisture in the quality of honey is based on the relationship between the moisture content in honey and yeast spore count. If moisture content (humidity) in honey is under 17%, honey will not ferment, no matter what the amount of yeast is. Thus, honey having a high moisture content as shown in all samples studied is more likely to ferment which can reduce the quality.

Carbohydrates constitute the dominant components (~95%) of the dry matter of all honey. Beside fructose and glucose which are present in the greatest percentage, about 25 different sugars have also been detected in honey (16). Based on the results, S3 (Suburban-Muar) has the highest level of fructose which indicates the higher level of sweetness among all honey samples studied. The interest to analyse the fructose and glucose in honey was also due to the evidence by Soylu et al. (16) who showed that the content of fructose will influence the glycaemic index (GI) of the sample. Food with the low GI is recommended for the diabetic patient as it can reduce the postprandial of blood glucose. This statement was also supported by Atayoğlu et al. (17) who showed a significant negative correlation between the content of fructose and GI which probably related with the ratio of fructose/glucose of the honey. According to the results, all honey samples showed an average fructose concentration of 24.09±1.8 g/100g which was higher than 15.77±2.47 g/100g reported by Kek et al. (18). For glucose, the average concentration for all honey samples studied was 16.92±2.27 g/100g which was slightly higher than previous study by Se et al. (19) (16.4±5.86 g/100g) even though both stingless bee samples (18, 19) are also from Malaysia.

For sucrose concentration, Codex Alimentarius Commissions 2001 (15) set the limit of 5 g/100g in a honey sample to ensure the purity and good quality of the sample. The high amount of sucrose will also directly

increase the blood sugar reading. Results of our present study demonstrated that the concentrations of sucrose in stingless bee honey samples are varied with the range from 2.68 to 34.04 g/100g. This finding is similar with results from Kek et al. (18) who also used stingless bee honey samples from Malaysia. The concentration of sucrose in their sample was 32.3 g/100g which also was higher than the set limit of Codex Alimentarius. However, the study by Se et al. (19) shows that the amount of sucrose in their stingless bee honey samples which also from Malaysia was averaged at 3.46 g/100g which fulfil the Codex Alimentarius, 2001 (15) requirement. The wide ranges of variation among honey in same species are influenced by the process of nectar collection, climate condition and also due to harvesting time and temperature (20). However in our finding, despite the location of the sampling are different in term of sources and geographically, there is no significant difference in sucrose content between samples from suburban area and forest.

As stated earlier, the major types of sugar in honey are fructose (F) and glucose (G). Even though no limits have been fixed for the individual fructose and glucose, the sum of fructose and glucose (F+G) more than 60 g/100 g has been stated as one of the important criterions in the international standard for honey established by Codex Alimentarius Commission Standard. F + G in all honey studied were below than 60 g/100g which is not compliance with the standard. The highest cumulative value recorded in S3 (Suburban- Muar) sample (53.48 g/100g). This finding is similar with studies reported by Se et al. (19) and Kek et al. (18) that the F+G value in their honey samples was 29.8 g/100g and 24.99 g/100g, respectively which also below than 60 g/100g samples. In other Southeast Asia country such as Thailand, Chuttong et al. (21) reported similar results where stingless bee honey from *Tetragonula laeviceps-pagden* show a low F +G (31.0 g/100g) in all their sample studied. The possible explanation of low summation of fructose and glucose could be due to the high moisture content of the samples.

Other important factors that influence the quality of honey include the proportion between the two monosaccharides, fructose and glucose (F/G) and glucose and water content (G/W). The F/G and G/W ratio indicates the ability of honey to crystallize. When its F/G ratio is high honey remains liquids. This is due to the fact that the solubility of glucose in water is lower than fructose, which makes it easier to crystallise (22). Amir et al. (22) stated that honey crystallization is very slow when the ratio of glucose/water is below 1.3, but when the ratio is more than 2.0, the crystallization is rapid and can complete. Results from the current study showed that all the samples have G/W less than 1.3.

Minerals constitute an insignificant portion of the overall composition of honey, so, the contribution of honey to the recommended daily intake (RDI) is marginal. There are many factors that influence the content of minerals in honey which including geographical location, climate, seasons, type of flowers and soil (23). Darker varieties of honey usually are rich in mineral (24) and been compromised by F2 honey sample which also has the same properties. The stingless bee honey collected from the forest area appeared to have more mineral content which also verified by the statement from Kek et al. (18) who reported the botanical and geographical factor to give a significant effect on the mineral content in honey. This is a clear indication that forest's sample is richer in mineral content compared to honey from the suburban sample. As *H. itama* species have an ability to forage 500 meter radius around the hive, the parameter of the bee's foraging site can be monitored well both for the suburban and forest area. For the sample which was collected from the suburban area with human placement, the source of food for the bee to produce honey are limited and also might be polluted by human activity that will lead to loss of mineral content on the soil (25). On the other hand, the honey sample from the forest is far diverse in term of soil, flower, and free from any potential pollutant.

From the nutritional point of view, K, Na, Ca, Mg, Fe and Zn are important minerals for the human body. Generally, potassium functions as enzyme stimulator for cell elongation in plant growth development and also reduces the NADPH oxidase activity in the electron transport chain whereas calcium function to reduce reactive oxygen species by detoxifying oxygen free radical. Both minerals are also good for lowering high blood pressure (26). Iron has been known as an important mineral for the formation of haemoglobin that transports oxygen in the blood. Magnesium will increase the glutathione level in blood which also acts as an antioxidant (27). Similar with magnesium, zinc also will act as a cofactor of superoxide dismutase enzyme by modulating glutathione metabolism (28).

The most abundant mineral element determined in all the samples studied was K, followed by Na and Ca. This finding is in agreement with results from the literature (24). The result was also similar with stingless bee sample studies from Malaysia by Kek et al. (18). The highest amount of K found in F2 which collected from the forest and the lowest was from S1, collected from suburban. The amount of K collected was significantly different between these two samples with 761.22 mg/kg and 484.11 mg/kg, respectively. Then total K content in forest group was 2669.23 mg/kg which are higher compared to the suburban group with 2309.68 mg/kg. F4 and F3 honey samples contain the highest amount of Na and Ca, respectively and both samples were collected from the forest. The amount of Na in all sample was relatively high except for S1 and S4 which both from suburban. Total Mg content in the forest group significantly ($p<0.05$) higher than the suburban group with 137.65 mg/kg compared to 100.6 mg/kg. A study conducted in Brazil by Silva et al. (29) mentioned that the overall average of calcium in honey was used to classify the bee species for *Melipona flavoneata*, and *Apis mellifera*. Mg was found in a moderate amount in the samples whereas Fe and Zn show the least number of element content

and be considered as the minor element in all samples. Fuenmayor et al. (30) reported that the bee species also will affect the mineral content in honey. The differences of mineral content in honey are also due to the material for nest or beehive construction such as resin and wax which differs depending on the natural sources available (31). The honey produced in a hive with different component will produce different mineral contents during the storage period in a hive's propolis with also made from resin and wax.

Honey can also be polluted via different sources of contamination. Environmental contaminants include heavy metal and pesticides which can be occurred during the harvesting and processing period due to the non-hygienic technique as well as the equipment used (steel and aluminium) which not been fully sterile (32). So, honey is a useful indicator of environmental pollution (33). Heavy metals level in all samples analysed, however, were lower than the standard value and can be considered as safe to be consumed.

Folin Ciocalteu is a rapid and simple method to determine the TPC in stingless bee honey. The sensitivity of the TPC test was also sufficient to estimate the number of phenolic in a honey sample (34). The common group of polyphenol that can be found in plants is flavonoids which have been regarded as the most useful and effective antioxidants. From the results obtained the range of TFC between all stingless bee honey sample was widely varied from 98.284) µg RE/g (S2) to 443.25 (F2) µg RE/g sample. The TPC and TFC of stingless bee honey from forest generally are higher than the samples from suburban. The huge biodiversity of the forest might explain the reason why the level of phenolic and flavonoid content of samples collected from the forest were higher than samples collected from suburban. The values of TPC and TFC in our samples were significantly higher ($p<0.05$) than the previous study by Ranneh et al. (35) that used stingless bee honey from Malaysia as their test samples but slightly lower than data from Kek et al. (6) which also used local stingless bee honey. These values were also higher than other types of honey such as Gelam honey (36) and monofloral honey from different sources (37, 38). The differences in the TPC and TFC are expected as the compositions of the honey are affected to the nectar sources and also the harvesting time (39).

DPPH antioxidant assay is one of the rapid yet effective tests to determine the stingless bee samples scavenging activity toward 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical. Upon the reaction, DPPH will reduce to 2, 2-diphenyl-1-picryl hydrazine (yellowish colour) which indicate the ability of the sample to scavenge the stable DPPH radical (39). ABTS antioxidant assay is another radical scavenging based assay based on electron donation performed to evaluate the antioxidant activity of stingless bee honey sample. The oxidant ABTS- was generated by persulfate oxidation of 2, 2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS2-) during the reaction (40). F2 honey sample shows the highest scavenging activity with 602.15 and 575.18 µg TE/g samples for DPPH and ABTS assay, respectively. The third method used to determine the antioxidant activity of honey samples is ferric reducing/antioxidant power assay (FRAP). FRAP provides a direct estimation of antioxidant in the stingless bee honey sample which reduces the Fe^{3+}/Fe^{2+} couple. The values of ferric reducing ability in all stingless bee honey showed statistically significant differences among all samples, with F2 honey sample again recorded to have the highest reducing power of Fe^{3+}. The result for FRAP reducing assay by stingless bee honey F2 (Forest-Sibu) in this study was significantly higher that previous local studies by Kek et al. (41). The value of DPPH scavenging activity and FRAP reducing assay of stingless bee honey in this present study was higher than a study by Moniruzaman et al. (37) but lower than the data by Biluca et al. (42) who extracted Brazil's stingless bee honey by ethyl acetate. The difference could be due to the different solvent used to extract the sample, botanical origin and bee species which also need to be considered.

Correlations test shows that there was a strong and positive correlation between the phenolic content and the antioxidant value in all stingless bee honey samples which complied with other previous studies (16-19). These also indicate that the antioxidant activity in stingless bee honey was partly due to the presence of phenolic content. The high content of phenolic and flavonoid in stingless bee honey sample are due to the polyphenol compound such as gallic acid, chysin, caffeic acid, apigenin, kaempherol, p-coumaric acid and 4-hydrobenzoic acid and quercetin-3-O-rutinosoid which has antioxidant properties (35).

CONCLUSION

Overall, the sugar composition of stingless bee honey (*H. itama*) samples collected from suburban and forest did not show any significant difference. The samples from the forest have higher total mineral content than the samples from the suburban area. Samples collected from the forest (F1, F2, F3 and F4) have significantly higher TPC and TFC as well as antioxidant activity as compared to sample collected from the suburban area (S1, S2, S3, S4). The differences between samples could be due to different locations which will influence the floral source of honey. A further investigation upon quantification of the polyphenol in the stingless bee honey should be done in the future. In addition, due to the high moisture content of all samples studied than the value limit by Codex Alimentarius 2001 standard, this problem should be solved by stingless bee honey industry in Malaysia as it is critical parameters to determine the quality of honey.

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REFERENCES

1. Reuter S, Gupta SC, Chaturvedi MM, Aggarwal BB. Oxidative stress, inflammation, and cancer: how are they linked? Free Radical Biology and Medicine, 2010;49(11):1603-16.
2. Sбnchez-Rodrнguez MA, Ruiz-Ramos M, Correa-Mucoz E, Mendoza-Nьcez VM. Oxidative stress as a risk factor for osteoporosis in elderly Mexicans as characterized by antioxidant enzymes. BMC Musculoskeletal Disorders, 2007;8(1):124.
3. Choudhari MK, Punekar SA, Ranade RV, Paknikar KM. Antimicrobial activity of stingless bee (Trigona sp.) propolis used in the folk medicine of Western Maharashtra, India. Journal of Ethnopharmacology, 2012;141(1):363-7.
4. Karabagias IK, Badeka AV, Kontakos S, Karabournioti S, Kontominas MG. Botanical discrimination of Greek unifloral honeys with physico-chemical and chemometric analyses. Food Chemistry, 2014;165:181-90.
5. Chua LS, Rahaman NL, Adnan NA, Tan E, Tjih T. Antioxidant activity of three honey samples in relation with their biochemical components. Journal of Analytical Methods in Chemistry, 2013;2013.
6. Kek SP, Chin NL, Yusof YA, Tan SW, Chua LS. Total phenolic contents and colour intensity of Malaysian honeys from the Apis spp. and Trigona spp. bees. Agriculture and Agricultural Science Procedia, 2014;2:150-5.
7. Aljadi AM, Kamaruddin MY. Evaluation of the phenolic contents and antioxidant capacities of two Malaysian floral honeys. Food Chemistry, 2004;85(4):513-8.
8. Bogdanov S, Lьllmann C, Martin P, von der Ohe W, Russmann H, Vorwohl G, Oddo LP, Sabatini AG, Marcazzan GL, Piro R, Flamini C. Honey quality and international regulatory standards: review by the international honey commission. Bee World, 1999;80(2):61-9.
9. Horwitz W, Latimer G. Official Methods of Analysis of AOAC International, Gaithersburg MA, USA. Association of Official Analytical Chemist. 2000.
10. Ooi DJ, Iqbal S, Ismail M. Proximate composition, nutritional attributes and mineral composition of Peperomia pellucida L.(Ketumpangan Air) grown in Malaysia. Molecules, 2012;17(9):11139-45.
11. Chan KW, Khong NM, Iqbal S, Mansor SM, Ismail M. Defatted kenaf seed meal (DKSM): Prospective edible flour from agricultural waste with high antioxidant activity. LWT-Food Science and Technology, 2013;53(1):308-13.
12. Chan KW, Khong NM, Iqbal S, Ismail M. Simulated gastrointestinal pH condition improves antioxidant properties of wheat and rice flours. International Journal of Molecular Sciences, 2012;13(6):7496-507.
13. Khiati S, Dalla Rosa I, Sourbier C, Ma X, Rao VA, Neckers LM, Zhang H, Pommier Y. Mitochondrial topoisomerase I (Top1mt) is a novel limiting factor of doxorubicin cardiotoxicity. Clinical Cancer Research, 2014;20(18):4873-81.
14. Fredes C, Montenegro G. Heavy metal and other trace elements contents in honey bee in Chile. Cien. Inv. Agr.(in English) 33 (1): 50-58. Ciencia e Investigaciyn Agraria, 2006;33(1):50-8.
15. Codex Alimentarius. Codex standard, Revised codex standard for honey. Codex Standard, 2001;12:1982.
16. Soylu M, Atayoğlu T, İnanэ N, Silici S. Glycemıc index values of multifloral Turkish honeys and effect of their consumption on glucose metabolism. Journal of Apicultural Research, 2015;54(3):155-62.
17. Atayoğlu At, Soylu M, Silici S, Inanc N. Glycemic index values of monofloral Turkish honeys and the effect of their consumption on glucose metabolism. Turkish Journal of Medical Sciences, 2016;46(2):483-8.
18. Kek SP, Chin NL, Tan SW, Yusof YA, Chua LS. Classification of honey from its bee origin via chemical profiles and mineral content. Food Analytical Methods, 2017; 10(1):19-30.
19. Se KW, Ibrahim RK, Wahab RA, Ghoshal SK. Accurate evaluation of sugar contents in stingless bee (*Heterotrigona itama*) honey using a swift scheme. Journal of Food Composition and Analysis. 2018 Mar 1;66:46-54.
20. Anjos O, Campos MG, Ruiz PC, Antunes P. Application of FTIR-ATR spectroscopy to the quantification of sugar in honey. Food Chemistry, 2015;169:218-23.
21. Chuttong B, Chanbang Y, Sringarm K, Burgett M. Physicochemical profiles of stingless bee (Apidae: Meliponini) honey from South east Asia (Thailand). Food Chemistry, 2016;192:149-55.
22. Amir Y, Yesli A, Bengana M, Sadoudi R, Amrouche T. Physico-chemical and microbiological assessment of honey from Algeria. Journal of Environmental, Agricultural and Food Chemistry, 2010; 9:1485–1494.
23. Alvarez-Suarez JM, Giampieri F, Brenciani A, Mazzoni L, Gasparrini M, Gonzбlez-Paramбs AM, et al. *Apis mellifera* vs *Melipona beecheii* Cuban polifloral honeys: A comparison based on their physicochemical parameters, chemical composition and biological properties. LWT-Food Science and Technology, 2018;87:272-9.
24. Chua LS, Abdul-Rahaman NL, Sarmidi MR, Aziz R. Multi-elemental composition and physical properties of honey samples from Malaysia. Food Chemistry, 2012; 135(3):880-7.
25. Stankovska E, Stafilov T, ajn R. Monitoring of trace elements in honey from the Republic of Macedonia by atomic absorption spectrometry. Environmental Monitoring and Assessment, 2008;

142(1-3):117-26.
26. Siddiqui MH, Al-Whaibi MH, Sakran AM, Basalah MO, Ali HM. Effect of calcium and potassium on antioxidant system of Vicia faba L. under cadmium stress. International Journal of Molecular Sciences, 2012;13(6):6604-19.
27. Zheltova AA, Kharitonova MV, Iezhitsa IN, Spasov AA. Magnesium deficiency and oxidative stress: an update. BioMedicine, 2016;6(4).
28. Cruz KJ, de Oliveira AR, do Nascimento Marreiro D. Antioxidant role of zinc in diabetes mellitus. World Journal of Diabetes, 2015;6(2):333.
29. Silva AD, Alves CN, Fernandes KD, Мьller R. Classification of honeys from Parб State (Amazon region, Brazil) produced by three different species of bees. Journal of the Brazilian Chemical Society, 2013;24(7):1135-45.
30. Fuenmayor CA, Dнaz-Moreno AC, Zuluaga-Domнnguez CM, Quicazбn MC. Honey of Colombian stingless bees: Nutritional characteristics and physicochemical quality indicators. InPot-Honey 2013 (pp. 383-394). Springer New York.
31. Franchin M, da Cunha MG, Denny C, Napimoga MH, Cunha TM, Koo H, et al. Geopropolis from *Melipona scutellaris* decreases the mechanical inflammatory hypernociception by inhibiting the production of IL-1β and TNF-α. Journal of Ethnopharmacology, 2012;143(2):709-15.
32. Pisani A, Protano G, Riccobono F. Minor and trace elements in different honey types produced in Siena County (Italy). Food Chemistry, 2008;107(4):1553-60.
33. Rashed MN, El-Haty MTA, Mohamed SM. Bee honey as environmental indicator for pollution with heavy metals. Journal Toxicological and Environmental Chemistry, 2009;91(3):
34. Ferreira IC, Aires E, Barreira JC, Estevinho LM. Antioxidant activity of Portuguese honey samples: Different contributions of the entire honey and phenolic extract. Food Chemistry, 2009;114(4):1438-43.
35. Ranneh Y, Ali F, Zarei M, Akim AM, Hamid HA, Khazaai H. Malaysian stingless bee and Tualang honeys: A comparative characterization of total antioxidant capacity and phenolic profile using liquid chromatography-mass spectrometry. LWT-Food Science and Technology, 2018;89:1-9.
36. Kishore RK, Halim AS, Syazana MN, Sirajudeen KN. Tualang honey has higher phenolic content and greater radical scavenging activity compared with other honey sources. Nutrition Research, 2011;31(4):322-5.
37. Moniruzzaman M, Yung An C, Rao PV, Hawlader MN, Azlan SA, Sulaiman SA et al. Identification of phenolic acids and flavonoids in monofloral honey from Bangladesh by high performance liquid chromatography: determination of antioxidant capacity. BioMed Research International, 2014;2014.
38. Ka konienė V, Venskutonis PR. Floral markers in honey of various botanical and geographic origins: a review. Comprehensive Reviews in Food Science and Food Safety, 2010;9(6):620-34.
39. Mishra K, Ojha H, Chaudhury NK. Estimation of antiradical properties of antioxidants using DPPH assay: A critical review and results. Food Chemistry, 2012;130(4):1036-43.
40. Lypez-Alarcyn C, Denicola A. Evaluating the antioxidant capacity of natural products: A review on chemical and cellular-based assays. Analytica Chimica Acta, 2013;763:1-0.
41. Kek SP, Chin NL, Yusof YA, Tan SW, Chua LS. Classification of entomological origin of honey based on its physicochemical and antioxidant properties. International Journal of Food Properties. 2017 Dec 31;20(sup3):S2723-38.
42. Biluca FC, Braghini F, Gonzaga LV, Costa AC, Fett R. Physicochemical profiles, minerals and bioactive compounds of stingless bee honey (Meliponinae). Journal of Food Composition and Analysis, 2016;50:61-9.