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A COMPARISON OF CHEMICAL COMPOSITIONS IN *KELULUT* HONEY FROM DIFFERENT REGIONS

(Perbandingan Komposisi Kimia dalam Madu Kelulut dari Kawasan Berbeza)

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Abstract

Kelulut honey (KH) is honey produced by stingless bees (Trigona spp.) found in Malaysia. This study investigated the difference inherent in the chemical composition of kelulut honey collected from the east coast, and the central and northern regions of Peninsular Malaysia. Total sugar content, individual sugar content, total phenolics, total flavonoids, ascorbic acid, ascorbic acid equivalent antioxidant content (AEAC), and proline content were determined. Sugar analysis revealed that kelulut honey contained 62.33-79.53 g/100g of total sugar, with maltose as the predominant sugar (15.85-37.74 g/100g), followed by fructose (9.91-53.64 g/100g), glucose (10.96-25.04 g/100g), and sucrose (0.54-3.48 g/100g). The results indicate that total flavonoids (78.95±0.70 mg QE/kg) and phenolics (1149.48±40.52 mg GAE/kg) were the highest in honey from the east coast region. The proline and ascorbic acid content were less likely to be affected by geographical factors. Kelulut honey possesses a unique sugar profile that may contribute to its unique taste. In conclusion, the geographical and floral origin of honey are the two most important factors that fundamentally affect the physical-chemical properties of honey samples.

Keywords: chemical, compositions, kelulut, honey, region

Abstrak

Madu kelulut (KH) ialah madu yang dihasilkan oleh lebah tidak bersengat (Trigona spp.) yang terdapat di Malaysia. Kajian ini menyelidik perbezaan komposisi madu kelulut yang dikumpul dari pantai timur, tengah dan utara Semenanjung Malaysia. Jumlah dan gula individu, jumlah fenolik, jumlah flavonoid, asid askorbik, asid askorbik kandungan antioksidan setara (AEAC) dan kandungan prolin telah ditentukan. Analisis kandungan gula menunjukkan bahawa madu kelulut mengandungi 62.33-79.53 g/100g jumlah gula dengan maltosa sebagai gula utama (15.85-37.74 g/100g), diikuti oleh fruktosa (9.91-53.64 g/100g), glukosa (10.96-25. 100g) dan sukrosa (0.54-3.48 g/100g). Jumlah flavonoid (78.95±0.70 mg QE/kg) dan fenolik (1149.48±40.52 mg GAE/kg) adalah tertinggi dalam madu dari pantai timur. Prolin dan asid askorbik adalah kurang berkemungkinan dipengaruhi oleh faktor geografi. Madu kelulut mempunyai profil gula yang unik yang mungkin menyumbang kepada rasa unik madu

kelulut. Secara kesimpulannya, geografi dan floral madu adalah dua parameter kualiti penting yang pada asasnya mempengaruhi sifat fizikal-kimia sampel madu.

Kata kunci: kimia, komposisi, kelulut, madu, kawasan

Introduction

Honey is a natural food consisting of about 200 different compounds including sugars, phenolics, amino acids, enzymes, organic acids, and aromatic compounds. Honey composition varies by bee species, nectar source, and geographic origin [1]. Honey is not only known for its nutritional benefit, but also as traditional medicine due to its healing properties. It has been proven that honey exert antioxidant, anti-inflammatory, anti-bacterial and anti-mutagenic properties. The antioxidant activity assay for any product is based on the capability of the compounds to inhibit oxidation. The DPPH and FRAP assays are typically chosen because they are simple and rapid methods for assessing a honey sample's antioxidant capacity. These two assays are based on different principles, and thus may be used to screen compounds that have different antioxidant mechanisms. Recently, there is an increase in the Malaysian consumer demand for honey, especially for health maintenance and promotion purposes. There has been a surge of interest in honey's capacity as an antioxidant and its protective effects on the brain and other organs [2]. *Kelulut* honey is produced by the stingless bees that are indigenous to Malaysia. A local study reported that a low dose of *Kelulut* honey taken orally could improve wound healing rate in epidermal keratinocyte *in vitro* wound healing model [3]. It has also been reported to have a potential in preventing damage to sperm and testes in diabetic rats [4]. *Kelulut* honey is a multifloral honey which does not have any predominant pollen present in it. The nutritional profile and composition of this honey is still understudied. Over the decades, research on Malaysian honey production has been focused on *Apis* spp. which left a gap to be bridged by research work on *kelulut* honey. The effect of different botanical and geographical environment on honey chemical composition could be studied by comparing the honey of a particular type produced in different localities. Previous studies indicated that the nectar source can affect the antioxidant content and the antibacterial and radical scavenging activities of the honey [5]. Bioactive compounds from the plant are secreted into the nectar, thereby impacting the quality of honey and its medicinal properties that are very dependent on its bioactive composition. The present study was undertaken to study the compositional variations of *kelulut* honey collected from different locations in peninsular Malaysia.

Materials and Methods

Sample collection

Kelulut honey from *Heterotrigona itama* species were collected from three peninsular Malaysia regions, namely the east coast region, the northern region, and the central region. The honey samples were collected from each region in two different batches. Each batch were analyzed in triplicates, and the results were expressed as an average ± standard deviation of six replicates from samples collected in two batches.

Individual and total sugar analysis using HPLC

The composition of individual sugars in *kelulut* honey samples was determined using high performance liquid chromatography (HPLC) [6]. About 1 g of honey sample was weighed and loaded into a 50 mL centrifuge tube. 25 mL of 50:50 acetonitrile/deionized water was added into the tube. The sample was then filtered using a 0.45 μM nylon filter. The purified sugar extract was analyzed using a HPLC Waters model 2707 with an XBridge Amide column (250 x 4.6mm, 3.5μM) coupled with Water RI-2414 refractive index detector. The mobile phase was acetonitrile/deionized water (75:25). The injection volume was 20 μL with flow rate of 1.0 mL/min. The retention time for each sugar was determined by comparing with a known standard sugar solution consisting of fructose, glucose, sucrose, and maltose with a concentration of 0.5 g/10 mL. The composition of the individual sugar was calculated using Empower 2 software.

Total sugar content using chemical analysis

Total sugar content in *kelulut* honey were also determined using the phenol-sulphuric acid method [7]. This analysis was performed in this study because honey also contains a small amount of oligosaccharides that the HPLC method could not detect. For this analysis, 0.1 g of honey sample was diluted 100-fold and the solution was homogenized, 1 mL of the new solution was 20-fold diluted. A 2 mL aliquot of the honey solution was combined with 1 mL of 5% aqueous phenol solution in a test tube. 5 mL of concentrated sulphuric acid was then added rapidly to the mixture. The mixture was allowed to stand for 10 min and subsequently vortexed for 30 seconds. The test tube was then placed in a water bath at room temperature for 20 minutes for color development. The absorbance was then recorded on a spectrophotometer (Secomam, France) at 490 nm. Glucose solutions 0f 0.0-0.1 g/L were used as standard.

Total phenolic content

The Folin-Ciocalteu assay [8] was used to determine the total phenolic content in *kelulut* honey. 0.5 mL of the 10% honey solution was mixed with 2.5 mL of 0.2 N Folin-Ciocalteu reagent, and the mixture was left to stand for 5 minutes. Then, 2 mL of 7.5% sodium carbonate solution was added. The mixture was allowed to stand at room temperature for 2 hours. The absorbance of the mixture was then measured at 760 nm using spectrophotometer (Secomam, France) against methanol as blank. Gallic acid (50-200 mg/L) was used to produce standard curve (R^2 = 0.987). The total phenolic content was expressed in mg of gallic acid equivalent (GAE)/kg honey.

Total flavonoid content

The total flavonoid content in the honey samples was analyzed using the method described by Zhishen et al. [9]. A 1 mL volume of 20% honey was mixed with 4 mL of distilled water and 0.3 mL of sodium nitrite. After 5 minutes, 0.3 mL of 10% aluminum chloride was added followed by 2 mL of 1 M sodium hydroxide six minutes later. The volume was made up to 10 mL by distilled water, and the mixture was shaken thoroughly. The absorbance was read using spectrophotometer (Secomam, France) at 510 nm wavelength. A standard curve was plotted using a quercetin solution with concentrations ranging between 25 - 100 µg/mL (R^2 = 0.987). The results were expressed as mg quercetin equivalent (QE)/kg honey.

Proline content

The proline content in the honey sample was determined using the method described by Bogdanov et al. [10]. A 0.5 mL sample of honey (0.05 g/mL) was mixed with 1 mL of formic acid (80%) and 1 mL of ninhydrin solution (3% in ethylene glycol monomethylether). The mixture was shaken vigorously for 15 minutes. The mixture was incubated in a boiling water bath for 14 minutes before being transferred to a 70 ºC water bath for 10 minutes. A 5 mL 50% propanol-water solution was added, and the mixture was allowed to cool for 45 minutes. Its absorbance at 510 nm was measured. Water was used as the blank and a 0.032 mg/mL proline solution was used as the standard. Proline content (mg/kg) was calculated as follow:

$$Proline = \frac{E_s}{E_a} X \frac{E_1}{E_2} X80 \quad (1)$$

where E_s is the absorbance of the sample solution; E_a is the absorbance of the proline solution; E_1 is the concentration of proline in mg used for the standard; E_2 is the weight of honey in g; 80 is the dilution factor.

Ascorbic acid content determination

The honey sample (100 mg) was extracted with 10 mL of metaphosphoric acid for 45 minutes at room temperature and the mixture was filtered through a filter paper. About 1 mL of the filtrate was mixed with 9 mL of 2,6-dichlorophenolindophenol and the absorbance was determined at 515 nm within 30 min. L-ascorbic acid (0.02-0.12 mg/mL) was used as standard [11].

Ascorbic acid equivalent antioxidant content

AEAC was performed according to the procedure described by Meda et al. [12]. A 0.75 mL samples of honey in methanol (0.04 g/mL) was mixed with 1.5 mL of 0.02 mg/mL methanolic DPPH radical solution. The mixture was left for 15 minutes at room temperature before its absorbance was measured using a spectrophotometer (Secomam, France) at 517 nm. The

blank sample was prepared from a mixture of 0.75 mL of honey solution and 1.5 mL of methanol.

Carotenoid content

The measurement of carotenoid in *kelulut* honey was performed based on the method described by Ferreira et al. [11]. A 100 mg honey sample was shaken with a 4:6 ratio mixture of acetone and hexane for 1 minute. The mixture was filtered with filter paper and the absorbance of the filtrate was measured using a spectrophotometer (Secomam, France) at 454 nm, 505 nm, and 663 nm. The content of β-carotene inside the sample was calculated using the equation $0.216A_{663} - 0.304A_{505} + 0.452A_{453}$ while the lycopene content was calculated using the equation $-0.0458A_{663} + 0.372\ A_{505} - 0.0806\ A_{453}$. The total carotenoid content is defined as the sum of β-carotene and lycopene, and all measurements were expressed in mg/100mL.

Anti-oxidative activities

The DPPH radical scavenging capacity of *kelulut* honey was investigated using the procedure described Ferreira et al. [11]. Approximately 0.5 mL of the 0.2 g/mL honey extract was mixed with 2.7 mL of methanolic 0.024 mg/mL DPPH radical solution. The mixture was vigorously shaken and incubated for 15 minutes, then its absorbance was measured at 517 nm. IC_{50} was defined as the concentration of a substance that would scavenge 50% of DPPH radicals, which means that a lower IC_{50} value corresponds to a stronger antioxidant property. The FRAP assay was performed using the procedure described by Benzie & Strain [13]. A 200 µL sample of 0.1 g/mL honey was mixed with 1.5 mL of the FRAP reagent prepared from acetate buffer (300 mM/L), 2,4,6-tris(2-pyridyl)-*s*-triazine (TPTZ) solution (10 mM in 40 mM/L HCl), and ferric chloride ($FeCl_3.6H_2O$) (20mM) in the ratio of 10:1:1. The mixture was pre-warmed at 37 °C for 4 minutes before its absorbance was measured at 593 nm using a spectrophotometer (Secomam, France). Ferrous sulfate ($FeSO_4.7H_2O$) solutions with concentrations between 250-1000 µM/L was used to construct the standard curve (R^2=0.991). The FRAP values were expressed as µM Fe (II) per kg honey.

Data analysis

IBM SPSS version 23 was employed for the statistical analyses. ANOVA with Tukey's Honest Significant Difference (HSD) test was used for the comparisons of the chemical compositions of *kelulut* honey from three different zones. Pearson correlation was used to establish correlation between bioactive compounds and anti-oxidative activities. The significant level was set as α=0.05.

Results and Discussion

The compositional analysis revealed that the *kelulut* honey samples from *Trigona* spp. Collected in this study contained fructose and glucose at the concentrations of 9.91-27.33 g/100g and 10.96-25.04 g/100g, respectively (Table 1). Maltose was the predominant sugar of *kelulut* honey from the east coast and central regions of Peninsular Malaysia. The total sugar content of *kelulut* honey was 62.3-79.53 g/100g. The honey also contained oligosaccharides as the minor sugar constituents. In this study, the oligosaccharide content of *kelulut* honey was estimated by subtracting the individual sugars analyzed using HPLC from the total sugars analyzed using the phenol-sulphuric acid method. The results showed that *kelulut* honey contained 3.84-14.68 g/100g of oligosaccharides.

The total phenolic content of the *kelulut* honey samples collected in the present study was in the range of 530.28-1166.77 mg GAE/kg while the total flavonoid content was in the range of 48.82-79.02 mg QE/kg (Table 2). *Kelulu*t honey samples collected from the east coast region contained the highest level of carotenoids at 1.52 ± 0.17 mg lycopene/kg and 3.09 ± 0.25 mg β-carotene/kg. The total carotenoid content of *kelulut* honey samples in this study ranged between 2.22-4.61 mg carotenoids/kg. The proline content of *kelulut* honey in the present study was 277.76-291.89 mg/kg. The ascorbic acid content of the *kelulut* honey was within the range of 75.71-95.85 mg/kg. The central region *kelulut* honey had a significantly higher (p<0.05) ascorbic acid content compared to the east coast and northern *kelulut* honey. Meanwhile, the AEAC of *kelulut* honey was 78.10-146.48 mg/kg. Ascorbic acid in the *kelulut* honey contributed 65.44-96.94% to AEAC.

Table 1. Types of sugar in *kelulut* honey

Sugars	**East Coast Region Honey (n=6)**	**Northern Region Honey (n=6)**	**Central Region Honey (n=6)**
Total sugars (g/ 100g)	62.33 ± 10.33^{a}	79.53 ± 13.22^{b}	64.28 ± 19.63^{ab}
Maltose (g/100g)	22.80 ± 1.74^{a}	15.85 ± 0.05^{b}	37.74 ± 8.57^{c}
Fructose (g/100g)	10.41 ± 0.18^{a}	27.33 ± 0.15^{b}	9.91 ± 3.99^{a}
Glucose (g/100g)	10.96 ± 0.18^{a}	25.04 ± 0.14^{b}	11.98 ± 1.68^{a}
Sucrose (g/100g)	3.48 ± 0.65^{a}	0.54 ± 0.01^{b}	0.81 ± 0.22^{c}

Total sugar was obtained from phenol-sulphuric acid method while individual sugars were analyzed using HPLC. Different superscripts across the same row indicates significant difference (p <0.05).

Table 2. Phenolic, flavonoid, proline, ascorbic acid content and AEAC of *kelulut* honey

Parameters	**East Coast Region Honey (n=6)**	**Northern Region Honey (n=6)**	**Central Region Honey (n=6)**
TPC (mg GAE/kg)	1166.77 ± 47.85^{a}	728.42 ± 58.06^{b}	530.28 ± 30.31^{c}
TFC (mg QE/kg)	7902 ± 0.59^{a}	48.82 ± 0.67^{b}	55.38 ± 0.37^{b}
AEAC (mg/kg)	146.48 ± 6.43^{a}	78.10 ± 3.75^{b}	96.92 ± 1.31^{c}
Ascorbic acid content (mg/kg)	95.85 ± 13.48^{a}	75.71 ± 12.69^{a}	92.79 ± 0.75^{a}
Proline (mg/kg)	277.76 ± 19.75^{ab}	291.89 ± 3.46^{a}	284.82 ± 10.72^{b}
Lycopene (mg/kg)	1.52 ± 0.17^{a}	0.64 ± 0.08^{b}	0.76 ± 0.08^{c}
β-carotene (mg/kg)	3.09 ± 0.25^{a}	1.58 ± 0.14^{b}	1.57 ± 0.05^{b}
IC_{50} (DPPH radical scavenging) (mg/mL)	15.17 ± 1.02^{a}	28.47 ± 2.03^{b}	24.27 ± 2.68^{c}
FRAP (μM Fe (II)/ kg)	7481.08 ± 57.57^{a}	4122.97 ± 424.44^{b}	3633.78 ± 127.21^{c}

Different superscripts across the same row indicates significant difference (p <0.05)

The DPPH and FRAP assays were chosen because they are simple and rapid methods for assessing the antioxidant capacity of honey. The FRAP assay measures the ferric-reducing capacity of antioxidants, whereas the DPPH assay measures the ability of antioxidants to scavenge the DPPH radical. These two assays are based on different principles, and thus may be used to screen compounds that have different antioxidant mechanisms. *Kelulut* honey exhibited IC_{50} values in the range of 15.17-28.46 mg/mL. The honey collected from the east coast region showed the strongest DPPH radical scavenging activity with the lowest IC_{50} value of 15.17 ±1.02 mg/mL. The reducing power of *kelulut* honey assessed using FRAP was outlined in the order of east coast region *kelulut* honey > northern region *kelulut* honey > central region *kelulut* honey. The FRAP values were in the range of 3633.78-7481.08 μM Fe (II)/ kg.

The IC_{50} of the honey samples had a significant inverse correlation (p<0.01) with their bioactive compound compositions, namely total phenolics, total flavonoids, lycopene, β-carotene, ascorbic acid, and AEAC (Table 3). Their FRAP also had a significant and inverse

correlation ($p<0.01$) with their bioactive compound compositions, namely total phenolics, total flavonoids, lycopene, β-carotene and AEAC. The IC_{50} and FRAP had a strong inverse correlation ($r=-0.868$, $p<0.01$). The correlation matrix indicated the importance of the bioactive constituents of honey to its anti-oxidative capacity. The total phenolic content of a honey sample was negatively correlated with its IC_{50} value ($r^2 = -0.789$; $p<0.05$) and positively correlated with the FRAP value ($r^2 = 0.970$; $p<0.05$). In addition, a significant negative correlation was found between the honey sample's ascorbic acid composition and its IC_{50} value ($r^2 = -0.545$; $p<0.05$) and a positive correlation was found between its ascorbic acid composition and its lycopene value ($r^2 = 0.634$; $p<0.01$). These correlations between the bioactive compound content and the antioxidant activity of the extracts suggest that phenolic compounds are primarily responsible for the antioxidant activity of the honey samples as measured in the DPPH and FRAP assays.

Table 3. Correlation matrix of antioxidant content and anti-oxidative activities

	TPC	**TFC**	**IC_{50}**	**FRAP**	**Lycopene**	**β-carotene**	**Ascorbic Acid**	**AEAC**
TPC	1							
TFC	0.989**	1						
IC_{50}	-0.789**	-0.751**	1					
FRAP	0.970**	0.965**	-0.868**	1				
Lycopene	0.883**	0.861**	-0.895**	0.911**	1			
β-carotene	0.932**	0.924**	-0.868**	0.951**	0.987**	1		
Ascorbic Acid	0.228	0.172	-0.545**	0.323	0.634*	0.527	1	
AEAC	0.812**	0.813**	-0.948**	0.909**	0.932**	0.926**	0.557	1

TPC- total phenolic content; TFC- total flavonoid content; **Significant correlation at $p<0.05$; *Significant correlation at $p<0.01$

The fructose and glucose content of the *kelulut* honey samples gathered in this study were lower than those reported in the literature. Honey produced by stingless bees in the semi-arid region of Northeastern Brazil [14] has fructose as its predominant sugar (50.0-59.2 g/100g), followed by glucose (37.7-45.7 g/100g) and sucrose (0.7-3.9 g/100g). Ecuadorian stingless bee honey contained similar amounts of glucose (25.5 g/100g) and fructose (25.2 g/100g) with a smaller amount of sucrose (3.72 g/100g) [15]. Biluca et al. [16] also reported that the stingless bee honey from Santa Catarina, Brazil, contained 8.21-31.3 g glucose/100g and 30.4-46.1 g fructose/100g, while the honey from *Trigona fuscipennis* contained 36.22 g fructose /100g and 20.4 g glucose /100g. The amount of glucose and fructose measured were in accordance with the Malaysian Standard specification on kelulut stingless honey (MS 2683:2017), which dictates that it contains less than 7.5% sucrose and less than 85% fructose. The high concentration of sugar in honey combined with an acidic pH (3-5) do retard the growth of bacteria in honey [17].

The maltose content of the honey samples collected in the current study exceeded the limit set by the Malaysian Standard on kelulut stingless honey (MS 2683:2017), which dictated that the maltose content should be less than 9.5%. However, the current findings were in line with a recent study conducted by Chuttong et al. [18] which reported that Thai stingless bee honey contained 41 g maltose /100g, 17 g fructose/100g, 14 g glucose/100g and 1.2 g

sucrose/100g. An earlier study [19] also reported the *Trigona* honey, specifically of *Frieseomelita aff varia,* contained maltose as the predominant sugar (32.3%), followed by fructose (24.2%), glucose (18.1%), and sucrose (0.2%). The total sugar in our study were higher than those reported for Thai stingless bee honey (55±21 g/100g) [18]. The total sugar content of the *kelulut* honey in the current study was within the range of total sugars reported for Brazilian stingless monofloral honey (62.7-71.2 g/100g) [14]. The sucrose content (0.54-3.48 g/100g) obtained in this study was within the limit set by Codex Alimentarius (2001) [20] which is less than 5g/100g. *Kelulut* honey has a different sugar profile compared to the typical *Apis* spp. honey that contains fructose and glucose as the predominant sugar. This sugar profile in kelulut honey may contribute to its distinct flavor. Previous studies indicated that honey contains the oligosaccharides nigerose (1.11±2.81%), turanose (0.78±2.03%), maltotriose (0.24±1.03%), melezitose (0.21±0.37%) and raffinose (0.10±0.25%) that may also be present in *kelulut* honey.

Phenolic compounds play a role in countering the damage that oxidative stress incurs on the body. Having these active compounds makes the stingless bee honey valuable for medicinal purpose [21]. The vast difference in the phenolic content of the honey samples collected from the three different regions may be attributed to the differing bee species that produce those honey. A study showed the honey samples collected from three stingless bee species, namely *Geniotrigona thoracica*, *Heterotrigona itama,* and *Heterotrigona erythrogastra,* had significant differences in their phenolic content, which were 99.04 ± 5.14, 67.86 ± 7.40, 44.72 ± 6.50 mg/mL, respectively [22]. Apart from the different species of bees, geographical and botanical origins could explain the variation of phenolics content in stingless bee honey. Biluca et al. [16] reported that various stingless bee honey samples contained 10.3-98.0 mg GAE/100g phenolics while Silva et al. [23] reported a range of 1.1-1.3 mg GAE/g of phenolics found in stingless bee honey.

Furthermore, the total flavonoid content of *kelulut honey* were in fair agreement with results reported by Chan et al. [24], in which *Trigona* spp. honey from Malaysia contained 44.60-79.13 mg QE/kg. Tuksitha et al. [22] reported a higher flavonoids range (12.41-17.67 mg/mL) for stingless bee from Borneo. Previous study reported that various kinds of Hungarian honey contained 252-2283 mg proline /kg [23]. The value obtained in this study was within the range. The protein content of floral honey is 1.0-1.5% and the amino acid content is 1.0% of the total protein in honey. Proline is the main amino acid present in honey, constituting 50-85% of the total amino acids [23]. The proline content of honey decreases at a constant rate during storage; thus, it acts as an indicator of ripeness [23]. The minimum value of 180 mg/kg of proline is accepted for pure honey [26]. The value of ascorbic acid obtained in this study was lower than those obtained in Portuguese honey (140.01-145.80 mg/kg [11] and Algerian honey (159.70±0.78 mg/kg). A previous study [27] reported that Algerian honey contained 0.30-1.01 mg carotenoids/ 100 g.

A more recent study reported that floral honey from Tunisia contained 1.16-4.72 mg carotenoids/kg [28]. The carotenoid content of the *kelulut* honey samples collected from the three regions were significantly different (p <0.05), indicating the influence of geography on carotenoid content. Environmental factors and the changing seasons also contribute to the carotenoid content in honey [28]. The ascorbic acid content measured in this study was lower than those measured in Portuguese honey (140.01-145.80 mg/kg) [11] and Algerian honey (159.70±0.78 mg/kg). The value of AEAC in the present study was lower than those reported from other countries such as India (15.1-29.5 mg AEAC/100g) [29] and Bangladesh (18.4-34.1 mg AEAC/100g) [30].

The values of IC_{50} obtained in this study agreed well with the results reported for Tunisian floral honey which had a range of IC_{50} values from 11.08 mg/mL to 93.26 mg/mL [28]. Omani honey had higher IC_{50} values which were 25.1 mg/mL, 49.3 mg/mL, 144.5 mg/mL as the average values for Sumer, Sidr, and multifloral honey, respectively [31]. Our results were

higher than those of Brazilian honey (3.17-8.79 mg/mL) [32]. Despite the differences, we should acknowledge the inter-laboratory differences of these studies. Silva et al. [23] and Biluca et al. [16] reported that stingless bee honey had between 10.6-12.9 mg ascorbic acid equivalent/100g and 1.46-18.5 mg ascorbic acid equivalent/100g, respectively as the concentration needed to scavenge DPPH radicals. Recently, Tuksitha et al. [22] reported that stingless bee honey from Sarawak, Malaysia, contained 25.78-50.66 μM Fe (II)/100g. Compared to that, the samples collected in this study had stronger reducing activities. Our results were in fair agreement with the FRAP values of Polish honey 0.6-5.7 mM Fe (II)/kg honey [33] and stingless bee honey reported by Biluca et al. [16] in the ranged of 61.1-624 μM Fe (II)/100g.

Conclusion

Kelulut honey contains maltose as its predominant sugar and a relatively low amount of sucrose. *Kelulut* honey contains bioactive compounds namely phenolics and flavonoids, the compositions of which were significantly affected by the honey's botanical and geographical origin. Honey from the east coast region contained the highest amount of phenolics and flavonoids. Proline and ascorbic acid were not correlated with geographical factor.

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