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Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

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Short Communication
Physicochemical and Antioxidant Potential of Raw Unprocessed Honey From Malaysian Stingless Bees

[1]Mohd Fadzelly Abu Bakar, [1]Shuaibu Babaji Sanusi, [1]Fazleen Izzany Abu Bakar, [1]Ong Jin Cong and [2]Zakbah Mian

[1]Faculty of Applied Sciences and Technology, Universiti Tun Hussein Onn Malaysia (UTHM), Pagoh Campus, KM1 Panchor Road, 84000 Muar, Johor, Malaysia
[2]National Apiary Centre, Malaysian Department of Agriculture, Parit Botak, 83200 Senggarang, Batu Pahat, Johor, Malaysia

Abstract

Objective: The current study investigates the physicochemical, phytochemical, nutritional composition and antioxidant activity of raw unprocessed honey produced by two Malaysian stingless bees, *Heterotrigona itama* and *Geniotrigona thoracica* collected from different places in the Southern part of Peninsular Malaysia. **Materials and Methods:** Physicochemical (pH, color) and nutritional compositions (moisture, ash, protein, fat, carbohydrates, minerals) were determined and evaluated using standard methods. Phytochemical contents (flavonoid and phenolic compounds) and antioxidant activites were determined spectrophotometrically. **Results:** pH of collected honey samples were found to be acidic, ranging from 3.24-3.42, while the colors ranged from 85.34-490.37 mm Pfund (golden to dark amber). The moisture, ash, protein, fat and carbohydrate contents of honey samples ranged from 26.5±0.00 to 31.8±0.00 g/100 g, 0.15±0.01 to 0.67±0.00 g/100 g, 0.016t 0.54 g/100 g, 0.02 to 0.15 g/100 g and 67.12 to 73.26 g/100 g, respectively. The total flavonoid content of honey samples dissolved in distilled water and methanol ranged from 53.81±4.12 mg rutin equivalents kg^{-1} to 549.05±9.74 mg rutin equivalents kg^{-1} while total phenolic content in both solvents ranged from 357.14±3.57 mg gallic acid equivalents kg^{-1} to 520.83±4.49 mg gallic acid equivalents kg^{-1}. The antioxidant activity of this honey displayed superior antioxidant potential and was higher than Manuka honey. **Conclusion:** Clearly, honey produced by these two stingless bee types in the Southern part of Peninsular Malaysia is a budding functional food and possible nutraceuticals with great potential for use in complementary and alternative medicines.

Key words: Stingless bee honey, physicochemical composition, phytochemical composition, nutritional composition, antioxidant activity

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Corresponding Author: Mohd Fadzelly Abu Bakar, Faculty of Applied Sciences and Technology, Universiti Tun Hussein Onn Malaysia (UTHM), Pagoh Campus, KM1 Panchor Road, 84000 Muar, Johor, Malaysia Tel: +606-9742021, Fax: +606-9742191

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Honey is a natural food product produced by honey bees after consumption of the floral nectar of the plants[1]. It is a supersaturated solution of multiple sugars, including fructose (38.3%), glucose (30.3%), maltose (7.1%) and sucrose (1.3%). Other substances found in honey include acids (0.5%), proteins (0.3%), minerals (0.2%) and trace compounds, such as flavonoids, phenolic compounds, ascorbic acid, enzymes (catalase and peroxidase), carotenoids and Maillard reaction products, all of which are considered major contributors to the antioxidant potential of honey[2-4]. However, the specific composition of each honey is primarily dependent on source plant and nectar composition, bee species and seasonal and environmental factors[2,5]. Other factors, such as processing method, handling and storage conditions, also play an important role on the chemical composition of each honey[6-8].

The *Trigona* sp. of stingless bee, known as kelulut, is a stingless bee species found in Malaysia. Two common kelulut species, *Heterotrigona itama* and *Geniotrigona thoracica*, are also the main pollinators in this region[5]. These bees produce kelulut honey, a multiflora honey stored in clusters of small resin pots near the extremities of kelulut bee nests, while honey from *Apis* sp. bees is stored in hexagonal-shaped honey combs. Kelulut honey reportedly has many medicinal and therapeutic uses and excellent potency[9]. According to Biluca *et al.*[10] honey from stingless bees has a distinct aroma and taste, more fluid texture and undergoes slow crystallization. Stingless bee honey is very different from that produced by bees of other genera. Recently, demand for this honey has increased in the world market, being of higher commercial value than *Apis mellifera* (manuka) honey. However, the lack of complete studies regarding the physicochemical characteristics of stingless bee honey hampers the definition of quality patterns and standards[6,11].

In Malaysia, research on stingless bee honey is scarce[5]. The composition of honey produced by stingless bees may boost immune defenses and promote cell functions in erythrocytes associated with antiseptic, antimicrobial, anticancer, anti-inflammatory and wound-healing properties[10,12]. The present study investigated the physicochemical and nutritional composition and antioxidant activity of raw unprocessed honey produced by two types of Malaysian stingless bees.

MATERIALS AND METHODS

Honey collection: Honey from *Heterotrigona itama* (*H. itama*) and *Geniotrigona thoracica* (*G. thoracica*) stingless honey bees (identified by Zakbah Mian from National Apiary Centre) was sampled at four different locations in Peninsular Malaysia, Parit Botak and Peserai (Johor), as well as Jasin and Ayer Molek (Malacca). Honey samples were collected into sterilized plastic containers and transported to the laboratory on ice to keep them at 4°C. The samples were kept in the same container at 4°C in the laboratory for a few days prior to analysis.

Physicochemical analysis: Physicochemical parameters were analyzed according to the methods recommended by the European Honey Commission[13]. These parameters included pH and color. Color was measured using optical comparison while pH meter was used to determine pH of honey samples by direct insertion into 10% w/v honey suspension.

Nutritional composition analysis: Nutritional composition was analyzed using the Association of Official Analytical Chemists official methods[14]. The protein content was determined using the Kjeldahl method, while the Soxhlet extraction method was used to determine crude fat content. Total carbohydrate content was determined according to the following Eq.:

$$\text{Carbohydrate (\%)} = 100\%-(\text{Moisture \%}+\text{Crude fat \%}+\text{Crude protein \%}+\text{Ash \%})$$

Mineral analysis was conducted by atomic absorption spectrophotometer.

Phytochemical analysis: The content of phytochemicals, such as flavonoids and phenolic compounds, in honey samples were determined using a UV-Vis spectrophotometer and reported as mg equivalents of rutin (mg rutin) per kg of honey and mg equivalents of gallic acid (mg gallic acid) per kg of honey, respectively[15].

Antioxidant activities: The antioxidant activity of honey samples was measured using a 2,2-Azinobis-(3-ethylbenzo-thiazoline-6-sulfonic acid) [ABTS] decolorization assay and 1,1-Diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay. Absorbance was measured for each assay type (734 nm for ABTS; 518 nm for DPPH)[16,17].

RESULTS AND DISCUSSION

Physicochemical parameters: The physicochemical properties of *H. itama* and *G. thoracica* honey are shown in Table 1. All honey samples were found to be acidic, ranging in pH from 3.24-3.42 which exhibited significant differences

Table 1: Physicochemical properties of stingless bee honey samples

	H.I Ayer Molek	G.T Ayer Molek	H.I Jasin	H.I Peserai	H.I Parit Botak
pH	3.27±0.01	3.36±0.01	3.36±0.01	3.24±0.01	3.42±0.01
Color (mm Pfund)	225.48±0.43	85.59±0.21	449.87±3.50	99.58±0.21	163.59±0.21

H.I: *Heterotrigona itama*, G.T: *Geniotrigona thoracica*. Data are expressed as the mean±standard deviation (SD). Mean values are significantly different ($p<0.05$)

($p<0.05$) between all samples. This pH range was found to be much lower compared to other honeys, such as from *A. cerana*, *A. dorsata* and *A. mellifera* (pH, 3.53- 4.03)[7]. Yap and Abu Bakar[18] reported a tropical honey with the highest known pH at 4.26±0.08, while manuka honey has the lowest recorded pH at 4.02±0.24, both of which are higher than the honey measured in the current study. Oddo *et al.*[19] also reported that *T. carbonaria* honey had a high pH (4.01±0.10) compared to *H. itama* and *G. thoracica* honey. The high acidity of the stingless bee honey analyzed in the present study was due to the presence of high levels of organic acids and hydrogen peroxide produced by the enzymatic fermentation of sugars present and is responsible for two important characteristics of honey, flavor and stability against microbial spoilage[20,21].

Honey color is the primary characteristic for its classification according to US Department of Agriculture-approved standards[22]. The color of honey is an important parameter used to determine the presence of certain compounds, such as flavonoids or carotenoids[23]. The color of honey varies naturally, ranging from light yellow to amber to dark amber and black in extreme cases and sometimes even green or red hues[24]. The color value for all honey samples measured in the present study ranged from 85.59±0.21 to 449.87±35.07 mm Pfund, with *H. itama* honey collected in Jasin being the darkest (449.87±35.07 mm Pfund), followed by *H. itama* honey collected in Ayer Molek (225.48±0.43 mm Pfund), *H. itama* honey collected in Parit Botak (163.59±0.21 mm Pfund), *H. itama* honey collected in Peserai (99.58±0.21 mm Pfund) and *G. thoracica* honey collected in Ayer Molek (85.59±0.21 mm Pfund). These color values lead to classification of *G. thoracica* honey as amber, while *H. itama* honey was classified as dark amber, according to US Department of Agricultural-approved standards. There was significant difference between all honey samples for the color ($p<0.05$).

Nutritional composition: The ash content of stingless bee honey was found to be between 0.15±0.01 and 0.67±0.00 g/100 g[25]. *H. itama* honey collected in Parit Botak showed the highest ash content with the value of 0.67±0.00 g/100 g which was also higher than that of stingless bee honey from Thailand (0.53±0.63 g/100 g)[26] and *T. carbonaria* honey (0.48±0.06 g/100 g)[19]. The lowest ash content was found in *H. itama* honey collected in Peserai (0.15±0.01 g/100 g). The ash contents of all honey samples exhibited significant differences ($p<0.05$). Variability in ash content is associated qualitatively with different botanical and geographical origins which may affect the amount of trace mineral in the honey[6,27].

The concentration of proteins and amino acids in honeys are also based on their botanical and geographical origins, as well as storage time. Enzymes are the main protein constituents present in honey. Various enzymes are also added by the bees during the honey ripening process, which increases the protein levels[7,27]. Current results showed that the protein content of honey produced by *H. itama* and *G. thoracica* bees varied from 0.096±0.08 and 0.31±0.11 g/100 g. The highest protein content was found in *H. itama* honey collected in Parit Botak (0.31±0.11 g/100 g) which almost similar to that of *A. mellifera* honey (0.28±0.01 g/100 g)[6] followed by *H. itama* honey collected in Jasin, Peserai, Ayer Molek and *G. thoracica* honey collected in Ayer Molek with the values of 0.28±0.21, 0.28±0.37, 0.25±0.17 and 0.096±0.08 g/100 g, respectively. No significant difference ($p>0.05$) existed in the protein contents among the honey samples. In addition, the fat content of honey samples analyzed in the present study ranged from 0.025±0.00 to 0.73±0.00 g/100 g. *G. thoracica* honey collected in Ayer Molek contained the highest value of fat with 0.73±0.00 g/100 g which was higher than that of *A. mellifera* honey (0.37-0.39 g/100 g)[6] while *H. itama* honey collected in Parit Botak showed the lowest ones (0.025±0.00 g/100 g). The fat contents of different honey samples exhibited significant differences ($p<0.05$). Fat content can also be used in determining the botanical origins of the honey[6].

Carbohydrate analysis showed values ranging from 67.20±0.11 to 73.01±0.35 g/100 g. The highest value of carbohydrate content was found in *H. itama* honey collected in Peserai (73.01±0.35 g/100 g) which was lower than that of Nigerian honey (82.30±2.03 g/100 g)[23] but much higher than that of stingless bee honey from Thailand (52±21 g/100 g)[26] followed by *H. itama* honey collected in Jasin (72.52±0.52 g/100 g), *G. thoracica* and *H. itama* honey collected in Ayer Molek (71.52±0.52, 70.23±0.18 g/100 g) and *H. itama* honey collected in Parit Botak (67.20±0.11 g/100 g).

Table 2: Nutritional composition of stingless bee honey samples

	H.I. Ayer Molek	G.T. Ayer Molek	H.I. Jasin	H.I. Peserai	H.I. Parit Botak
Water content (g/100 g)	28.87±0.06	28.17±0.06	26.53±0.60	26.50±0.00	31.80±0.00
Ash (g/100 g)	0.47±0.01	0.18±0.01	0.46±0.01	0.15±0.01	0.67±0.00
Carbohydrate (g/100 g)	70.23±0.18	71.52±0.52	72.52±0.52	73.01±0.35	67.20±0.11
Protein (g/100 g)	0.25±0.17	0.096±0.08	0.28±0.21	0.28±0.37	0.31±0.11
Fat content (g/100 g)	0.15±0.00	0.73±0.00	0.043±0.00	0.065±0.00	0.025±0.00
Potassium (mg kg^{-1} of sample)	680.73±33.79	352.53±26.51	701.33±26.27	236.33±1.29	NIL
Calcium (mg kg^{-1} of sample)	292.67±1.17	84.12±1.58	62.85±1.55	51.83±1.40	NIL
Magnesium (mg kg^{-1} of sample)	50.30±2.83	51.61±0.08	18.53±0.08	26.00±0.20	NIL
Zinc (mg kg^{-1} of sample)	4.37±0.29	3.61±0.28	5.33±0.36	4.45±0.21	NIL

H.I: *Heterotrigona itama*, G.T: *Geniotrigona thoracica*, NIL: Not in list. Data are expressed as the mean±standard deviation (SD). Mean values are significantly different ($p<0.05$) except for protein ($p>0.05$). Samples measured in dry weight

Table 3: Phytochemical analysis of stingless bee honey samples

	H.I. Ayer Molek	G.T. Ayer Molek	H.I. Jasin	H.I. Peserai	H.I. Parit Botak
Total flavonoid content (mg rutin kg^{-1} honey) in distilled water	82.38±4.12	60.95±4.12	308.57±7.14	53.81±4.12	72.85±12.37
Total flavonoid content (mg rutin kg^{-1} honey) in methanol	168.10±2.88	72.86±4.68	549.05±9.74	91.91±3.30	222.86±6.54
Total phenolic content (mg gallic acid kg^{-1} honey) in distilled water	380.36±3.57	371.95±11.61	519.64±5.36	520.83±4.49	357.14±3.57
Total phenolic content (mg gallic acid kg^{-1} honey) in methanol	439.59±5.35	435.69±8.96	516.07±5.36	498.81±2.54	489.29±3.28

H.I: *Heterotrigona itama*, G.T: *Geniotrigona thoracica*. Data are expressed as the mean±standard deviation (SD). Total phenolic content is expressed as mg equivalents of gallic acid in 1 kg of dry sample (mg gallic acid kg^{-1}). Total flavonoid content is expressed as mg equivalents of rutin in 1 kg of dry sample (mg rutin kg^{-1}). Mean values are significantly different ($p<0.05$)

There was significant difference observed between the samples for carbohydrate content ($p<0.05$). The high percentage of carbohydrate indicates that it was a main constituent of the honey, making up about 90% of the honey's dry weight[27].

Mineral analysis in the present study showed that Malaysian stingless bee honey was highest in potassium (701.33±26.27 mg kg^{-1}), followed by calcium (292.67±1.17 mg kg^{-1}), magnesium (51.61±0.08 mg kg^{-1}) and zinc (5.33±0.36 mg kg^{-1}). A previous study showed that the mineral content of Colombian stingless bee honeys was lower compared to *H. itama* and *G. thoracica* honey, with values of 576.6±177.69 and 99.6±63.4 for potassium and calcium, respectively[28]. However, the magnesium (56.0±27.59) and zinc (19.6±8.3) contents of Colombian stingless bee honey were higher than Malaysian stingless bee honey[28]. The results indicated there were significant differences between examined samples ($p<0.05$) (Table 2). The optimal level of several minerals such as zinc, iron and copper together with natural and synthetic antioxidant are believed to maintain the efficient levels of endogenous antioxidants in the tissues[29]. In fact, few minerals such as copper and iron possess antioxidant properties and responsible for the redox properties of honey[30]. In addition, the differences in mineral content in honey samples collected from different places (i.e. Ayer Molek, Jasin, Peserai and Parit Botak) might be due to the type of soil in which the original nectar bearing was located[31] and the percentage of mineral content normally represents as a quality criterion indicating the possible botanical origin of honey[32].

Phytochemicals: Flavonoids are a group of low molecular weight phenolic compounds responsible for the aroma and antioxidant potential of honey. In this study, flavonoid content produced by *H. itama* and *G. thoracica* bees varied from 53.81±4.12 and 549.05±9.74 mg rutin kg^{-1} when dissolved in two solvents, distilled water and methanol. Significant difference was observed between all samples ($p<0.05$). Both *H. itama* and *G. thoracica* honey samples displayed higher flavonoid content in methanol compared to distilled water. The highest flavonoid content was found in *H. itama* collected in Jasin for both extracts (distilled water and methanol) with the values of 308.57±7.14 and 549.05±9.74 mg rutin kg^{-1}, respectively. These values were higher than that of Australian stingless bee honey (10.02±1.59 mg quercetin equivalents/100 g)[19] and *A. mellifera* honeys, such as Tualang, Gelam and Borneo tropical honey (17.10-227.57 mg catechin kg^{-1})[7]. Flavonoids are antioxidants known to scavenge free radicals; their oxidation by free radicals results in a more stable, less reactive radical species[33]. Therefore, honey with a high flavonoid concentration is more desirable due to its antioxidant potential.

Polyphenol content in honey is represented by the total phenolic content. This group of important compounds contributes to the appearance and functional properties of honey. In the present study, phenolic content of stingless bee honey dissolved in distilled water and methanol showed values ranging from 357.14±3.57 to 520.83±4.49 mg gallic acid kg^{-1}, both of which were much higher than that of *A. mellifera* honey (243.01±74.91 mg gallic acid kg^{-1})[7]. Significant difference was observed between all samples ($p<0.05$) (Table 3). The

Table 4: Antioxidant analysis of stingless bee honey samples

	Radical scavenging activity (%) in 100 mg mL^{-1} (DPPH)	Antioxidant activity (%) in 100 mg mL^{-1} (ABTS)	EC_{50} (mg mL^{-1})
H.I. Ayer Molek	78.51±2.37	93.98±1.15	51.08
G.T. Ayer Molek	52.33±0.07	48.90±0.29	101.88
H.I. Jasin	97.30±0.84	95.99±0.31	30.17
H.I. Peserai	88.53±0.07	96.40±0.08	37.85
H.I. Parit Botak	60.07±0.13	69.99±0.34	101.35
A.M. (Manuka)	83.74±0.27	87.13±0.31	29.70

H.I: *Heterotrigona itama*, G.T: *Geniotrigona thoracica*, A.M: *Apis mellifera*, EC_{50}, half maximal effective concentration, DPPH: 1,1-Diphenyl-2-picrylhydrazyl, ABTS: 2,2-Azinobis-(3-ethylbenzo-thiazoline-6-sulfonic acid). Data are expressed as the mean±standard deviation (SD). Mean values are significantly different ($p<0.05$)

Table 5: Correlation matrix between the results of employed assays for 5 stingless bee honey samples

	Flavonoids	Phenolic compounds	DPPH	ABTS	Color
Flavonoids	1	0.3374/0.3845	0.3678	0.1833	0.9362
Phenolic compounds	0.3374/0.3845	1	0.3440	0.1279	0.2054
DPPH	0.3678	0.3440	1	0.8368	0.4400
ABTS	0.1831	0.1276	0.8368	1	0.2797
Color	0.9362	0.2054	0.4400	0.2797	1

H.I: *Heterotrigona itama*, G.T: *Geniotrigona thoracica*, DPPH: 1,1-Diphenyl-2-picrylhydrazyl, ABTS: 2,2-Azinobis-(3-ethylbenzo-thiazoline-6-sulfonic acid). Data are expressed as the mean±standard deviation (SD). Correlation between flavonoids and phenolic compounds r = 0.3374 (dissolved in distilled water), r = 0.3845 (dissolved in methanol)

variation in total flavonoid and phenolic content of honey is due to many factors, including the type of honey, floral sources, geographical location, collection seasons, mode of storage and harvesting technology and conditions[11,34]. Differences in the phenolic contents of *H. itama* and *G. thoracica* honey herein are most likely related to the different sampling localities (i.e., Malacca and Johor).

Antioxidant activities: Herein, the antioxidant activity of *H. itama*, *G. thoracica* and *A. mellifera* honeys was investigated using two different antioxidant assays: DPPH free radical scavenging activity and ABTS radical cation decolorization. The antioxidant activity of honey depends on several factors, such as floral sources, botanical origins, season, environment, processing method and the presence of the pigments (i.e., flavonoids and phenolic compounds)[16]. Current results showed that most of the honeys displayed high antioxidant activity, with *H. itama* honey collected in Jasin exhibiting the highest levels by DPPH assay (97.30±0.84%) and *H. itama* honey collected in Peserai showing the highest levels in the ABTS assay (96.40±0.08%). This high radical scavenging activity was most likely due to the high phenolic and flavonoid content, as the antioxidant potential of honey has been reported to be directly proportional to the quantity of these compounds present[35]. There was significant difference remarked between samples in both DPPH and ABTS assays. Interestingly, manuka honey displayed higher free radical scavenging activity (83.74±0.27%) in the current study than in a previous report (81.53±0.25%)[36]. Moreover, the radical scavenging activity of all *H. itama* honey samples measured herein was higher than that of other honeys (56.78%)[17]. ABTS assay results for *H. itama* honey was also higher compared to polish honey and Italian honeys (2.29-31.51 and 59.02±1.86%, respectively)[33]. The high antioxidant activity of Malaysian stingless bee honey indicates that it possesses potential health benefits (Table 4).

Correlation: The correlation matrix between physicochemical and antioxidant activity is shown in Table 5. A strong positive correlation was observed between the color intensity and total flavonoid content of the stingless bee honey samples (correlation coefficient r = 0.9362). These results are in agreement with those reported by Yap and Abu Bakar[18], who showed a strong positive correlation between color intensity and total flavonoid content (r = 0.884). The color intensity of stingless bee honey usually increases with the increase in flavonoids and phenolic compounds[7]. DPPH free radical scavenging activity also showed a strong positive correlation with ABTS radical cation decolorization (r = 0.924). Meanwhile, low positive correlations were observed between the total phenolic and flavonoid contents dissolved in distilled water and methanol (r = 0.3374 and 0.3845), total flavonoid content and DPPH free radical scavenging activity (r = 0.3678), total flavonoid content and ABTS radical cation decolorization (r = 0.1831), total phenolic content and DPPH free radical scavenging activity (r = 0.3440) and total phenolic content and ABTS radical cation decolorization (r = 0.1276). Moniruzzaman *et al.*[37] also reported that phenolic and flavonoid content positively correlated with DPPH values, indicating that their presence mainly contributed to the

antioxidant activity of the honey. In contrast, Aralas *et al*.[38] reported the antioxidant activities of the honey extracts (by DPPH assay) were highly correlated with total phenolic content and only moderately correlated with flavonoid content.

CONCLUSION

The results obtained from the current physicochemical analysis of *H. itama* and *G. thoracica* honey were similar to previous studies. However, Malaysian stingless bee honey has a much lower pH and higher moisture content compared to *A. mellifera* honey. The difference in composition or quality of this honey might be due to many factors such as floral sources, botanical origin, environmental condition, geographical and method of harvesting and processing. Other parameters, such as ash, protein and total carbohydrate contents were similar to that of *A. mellifera* honey and some of mineral contents were higher than that of Colombian stingless bee, while the flavonoid and phenolic contents were higher, indicating high antioxidant properties. These high antioxidant properties of both *H. itama* and *G. thoracica* honeys may be useful for nutraceutical and pharmaceutical applications. Further research is needed to unlock and understand the potential health benefits of the stingless bee honey.

SIGNIFICANCE STATEMENT

Honey produced by stingless bees has been reported to have ethno-medicinal properties. This study discovers the physicochemical and antioxidant potential of honey produced by stingless bees collected in Malaysia. This paper will provide the insight and fundamental of stingless bees honey for future research and also for the development of functional food and nutraceutical.

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REFERENCES

1. Idris, Y.M.A., A.A. Mariod and S.I. Hamad, 2011. Physicochemical properties, phenolic contents and antioxidant activity of Sudanese honey. Int. J. Food Properties, 14: 450-458.
2. Manyi-Loh, C.E., A.M. Clarke and N. Ndip, 2011. An overview of honey: Therapeutic properties and contribution in nutrition and human health. Afr. J. Microbiol. Res., 5: 844-852.
3. Silva, T.M.S., F.P. dos Santos, A. Evangelista-Rodrigues, E.M.S. da Silva and G.S. da Silva *et al*., 2013. Phenolic compounds, melissopalynological, physicochemical analysis and antioxidant activity of jandaira (*Melipona subnitida*) honey. J. Food Composit. Anal., 29: 10-18.
4. Jahan, N., M.A. Islam, F. Alam, S.H. Gan and M.I. Khalil, 2015. Prolonged heating of honey increases its antioxidant potential but decreases its antimicrobial activity. Afr. J. Trad. Complement. Altern. Med., 12: 134-144.
5. Ibrahim, N., A.J. Zakaria, Z. Ismail and K.S. Mohd, 2016. Antibacterial and phenolic content of propolis produced by two Malaysian stingless bees, *Heterotrigona itama* and *Geniotrigona thoracica*. Int. J. Pharmacog. Phytochem. Res., 8: 156-161.
6. Bertoncelj, J., U. Dobersek, M. Jamnik and T. Golob, 2007. Evaluation of the phenolic content, antioxidant activity and colour of Slovenian honey. Food Chem., 105: 822-828.
7. Almeida Muradian, L.B., K.M. Stramm, A. Horita, O.M. Barth, A. Silva de Freitas and L.M. Estevinho, 2013. Comparative study of the physicochemical and palynological characteristics of honey from *Melipona subnitida* and *Apis mellifera*. Int. J. Food Sci. Technol., 48: 1698-1706.
8. Moniruzzaman, M., M. Khalil, S.A. Sulaiman and S.H. Gan, 2013. Physicochemical and antioxidant properties of Malaysian honeys produced by *Apis cerana*, *Apis dorsata* and *Apis mellifera*. BMC Complement. Altern. Med., Vol. 13. 10.1186/1472-6882-13-43.
9. Zainol, M.I., K.M. Yusoff and M.Y.M. Yusof, 2013. Antibacterial activity of selected Malaysian honey. BMC Complement. Altern. Med., Vol. 13. 10.1186/1472-6882-13-129.
10. Biluca, F.C., F. Della Betta, G.P. de Oliveira, L.M. Pereira, L.V. Gonzaga, A.C.O. Costa and R. Fett, 2014. 5-HMF and carbohydrates content in stingless bee honey by CE before and after thermal treatment. Food Chem., 159: 244-249.
11. Kek, S.P., N.L. Chin, Y.A. Yusof, S.W. Tan and L.S. Chua, 2014. Total phenolic contents and colour intensity of Malaysian honeys from the *Apis* spp. and *Trigona* spp. Bees. Agric. Agric. Sci. Procedia, 2: 150-155.
12. Da Silva, I.A.A., T.M.S. da Silva, C.A. Camara, N. Queiroz and M. Magnani *et al*., 2013. Phenolic profile, antioxidant activity and palynological analysis of stingless bee honey from Amazonas, Northern Brazil. Food Chem., 141: 3552-3558.
13. Bogdanov, S., P. Martin and C. Lullmann, 2002. Harmonised methods of the international honey commission. Swiss Bee Research Centre, FAM., Liebefeld.
14. Helrich, K.C., 1990. Official methods of Analysis of the AOAC. Vol. 2, Association of Official Analytical Chemists Inc., Washington, D.C., USA.
15. Ozkok, A., B. D'arcy and K. Sorkun, 2010. Total phenolic acid and total flavonoid content of Turkish pine honeydew honey. J. ApiProd. ApiMed. Sci., 2: 65-71.

16. Perna, A., I. Intaglietta, A. Simonetti and E. Gambacorta, 2013. A comparative study on phenolic profile, vitamin C content and antioxidant activity of Italian honeys of different botanical origin. Int. J. Food Sci. Technol., 48: 1899-1908.
17. Khalil, M.I., M. Mahaneem, S.M.S. Jamalullail, N. Alam and S.A. Sulaiman, 2011. Evaluation of radical scavenging activity and colour intensity of nine Malaysian honeys of different origin. J. ApiProd. ApiMed. Sci., 3: 4-11.
18. Yap, P. and M.F. Abu Bakar, 2014. Physicochemical, phytochemical and antimicrobial properties of wild honey collected at mangrove and mountain areas in Sabah, Malaysian Borneo. Int. J. Pharm. Pharm. Sci., 6: 287-292.
19. Oddo, L.P., T.A. Heard, A. Rodriguez-Malaver, R.A. Perez and M. Fernandez-Muino *et al*., 2008. Composition and antioxidant activity of *Trigona carbonaria* honey from Australia. J. Med. Food, 11: 789-794.
20. Olaitan, P.B., O.E. Adeleke and O.E.I.O. Ola, 2007. Honey: A reservoir for microorganisms and an inhibitory agent for microbes. Afr. Health Sci., 7: 159-165.
21. Bogdanov, S., T. Jurendic, R. Sieber and P. Gallmann, 2008. Honey for nutrition and health: A review. J. Am. Coll. Nutr., 27: 677-689.
22. Oshomah, M.U. and E.B. Agbaji, 2015. Physico-chemical assessment of commercial honey from EDO state, Nigeria. Int. J. Applied Sci. Eng. Res., 4: 151-160.
23. Duarte, A.W.F., M.R. dos Santos Vasconcelos, A.P.D. de Menezes, S.C. da Silva, M. Oda-souza and A.M.Q. Lopez, 2012. Composition and antioxidant activity of honey from Africanized and stingless bees in Alagoas (Brazil): A multivariate analysis. J. Apicult. Res., 51: 23-35.
24. Diez, M.J., C. Andres and A. Terrab, 2004. Physicochemical parameters and pollen analysis of Moroccan honeydew honeys. Int. J. Food Sci. Technol., 39: 167-176.
25. Bijlsma, L., L.L. de Bruijn, E.P. Martens and M.J. Sommeijer, 2006. Water content of stingless bee honeys (*Apidae*, *Meliponini*): Interspecific variation and comparison with honey of *Apis mellifera*. Apidologie, 37: 480-486.
26. Chuttong, B., Y. Chanbang, K. Sringarm and M. Burgett, 2016. Physicochemical profiles of stingless bee (Apidae: Meliponini) honey from South East Asia (Thailand). Food Chem., 192: 149-155.
27. Buba, F., A. Gidado and A. Shugaba, 2013. Analysis of biochemical composition of honey samples from North-East Nigeria. Biochem. Anal. Biochem., Vol. 2. 10.4172/2161-1009.1000139.
28. Fuenmayor, C.A., C.M. Zuluaga-Dominguez, A.C. Diaz-Moreno and M.C. Quicazan, 2012. Nutritional composition and physicochemical properties of *Tetragoanisca angustula* honey. Interciencia, 37: 143-147.
29. McDowell, L.R., N. Wilkinson, R. Madison and T. Felix, 2007. Vitamins and minerals functioning as antioxidants with supplementation considerations. Proceedings of the Florida Ruminant Nutrition Symposium, January 30-31, 2007, Best Western Gateway Grand, Gainesville, FL., pp: 30-31.
30. Ahmed, M., M.I. Shafiq, A. Khaleeq, R. Huma and M. Abdul Qadir *et al*., 2016. Physiochemical, biochemical, minerals content analysis and antioxidant potential of national and international honeys in Pakistan. J. Chem., 10.1155/2016/8072305.
31. Vanhanen, L.P., A. Emmertz and G.P. Savage, 2011. Mineral analysis of mono-floral New Zealand honey. Food Chem., 128: 236-240.
32. Alvarez-Suarez, J.M., S. Tulipani, S. Romandini, E. Bertoli and M. Battino, 2010. Contribution of honey in nutrition and human health: A review. Mediterranean J. Nutr. Metab., 3: 15-23.
33. Nijveldt, R.J., E. van Nood, D.E.C. van Hoorn, P.G. Boelens, K. van Norren and P.A.M. van Leeuwen, 2001. Flavonoids: A review of probable mechanisms of action and potential applications. Am. J. Clin. Nutr., 74: 418-425.
34. Ayaad, T.H., G.H. Shaker and A.M. Almuhnaa, 2012. Isolation of antimicrobial peptides from *Apis florae* and *Apis carnica* in Saudi Arabia and investigation of the antimicrobial properties of natural honey samples. J. King Saud Univ. Sci., 24: 193-200.
35. Beretta, G., P. Granata, M. Ferrero, M. Orioli and R.M. Facino, 2005. Standardization of antioxidant properties of honey by a combination of spectrophotometric/fluorimetric assays and chemometrics. Analytica Chimica Acta, 533: 185-191.
36. Philip, Y. and A.B.M. Fadzelly, 2015. Antioxidative and acetylcholinesterase inhibitor potential of selected honey of Sabah, Malaysian Borneo. Int. Food Res. J., 22: 1953-1960.
37. Moniruzzaman, M., S.A. Sulaiman, M.I. Khalil and S.H. Gan, 2013. Evaluation of physicochemical and antioxidant properties of sourwood and other Malaysian honeys: A comparison with manuka honey. Chem. Central J., Vol. 7. 10.1186/1752-153X-7-138.
38. Aralas, S., M. Mohamed and M.F. Abu Bakar, 2009. Antioxidant properties of selected salak (*Salacca zalacca*) varieties in Sabah, Malaysia. Nutr. Food Sci., 39: 243-250.