



Chemical and Biological Investigation of Apiculture Products from Stingless Bees *Heterotrigona itama*

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ABSTRACT

Heterotrigona itama is one of the common stingless bee species in Malaysia. Similar to sting bee, stingless bee also produce honey, propolis and beebread in their hive. Propolis and beebread of Malaysian stingless bee were less explored compared to honey. This work examine nutritional content, antioxidant activity and chemical profiling of honey, propolis and beebread produced by stingless bee species *Heterotrigona itama*. Nutritional contents such as moisture content, ash, protein, fat and carbohydrate were measured. Antioxidant activity was determined using DPPH method; Folin-Ciocalteu method for total phenolic content (TPC) and Aluminium Chloride method for total flavonoid content (TFC). Chemical profiling was carried out by using thin layer chromatography (TLC) in solvent system of toluene: ethyl acetate: formic acid with ratio 8 : 2 : 0.1 v/v/v. The results revealed that honey possessed the highest moisture content and total carbohydrate with values of $30.67\% \pm 1.30$ and $65.88\% \pm 1.70$, respectively. Meanwhile, propolis contained the highest total fat ($49.2\% \pm 5.31$) which due to its compositions of wax and resin. Total protein and ash contents were abundant in beebread with values of $30.43\% \pm 0.40$ and $3.28\% \pm 0.13$, respectively. The highest total phenolic ($70.64\% \pm 0.81 \mu\text{g/mL}$) and flavonoid contents ($31.75\% \pm 0.05 \mu\text{g/mL}$) were found in propolis. Propolis possessed the highest antioxidant properties with IC_{50} of $5 \mu\text{g/mL}$ and followed by beebread with $300 \mu\text{g/mL}$. Honey possessed the least antioxidant activity (IC_{50} $500 \mu\text{g/mL}$). Antioxidant activity of propolis is comparable to positive control, Trolox (IC_{50} value: $3 \mu\text{g/mL}$). These results revealed that propolis is a good source of antioxidant as compared to honey and beebread. TLC analysis revealed that propolis also contained higher chemical compositions as compared to honey and beebread. This finding shows that stingless bee products have potential in therapeutic as strong natural antioxidant (propolis) and high in their nutritional content (honey and beebread).

Keywords: propolis, honey, stingless bee, *Heterotrigona itama*, apiculture

INTRODUCTION

Stingless bee is widely distributed in the Indo-Malay/Australasian region, stretches from India to Solomon Island and from China to Australia (Mohd Norowi et al, 2008). It has been suggested that the great abundance of stingless bees in Thailand and Malaysia including Borneo is due to the abundance of resin-secreting trees (Dipterocarpaceae) and humid tropical climate (Rasmussen, 2006 Schwartz, 1937). Stingless bees are biologically different from honeybees, especially in feeding behaviour and hive architectures. Stingless bees brood cells are arranged in clustered or latered combs, normally in a progression of a spiral. Their nest can be found in hollowed cavities in tree trunk or big branches. The nest is made of a waxy material called cerumen, collected from resinous trees and covered in a tough wax known as batumen.

Heterotrigona itama (Fig. 1A) is a common stingless bee species in Malaysia and function as an important pollinator for crops (Siok et al., 2014). *H. itama* belongs to family *Apidae* and has no sting (Roubik, 2006). Stingless bee produces bee products such as honey, propolis and beebread in their hive. Figure 1B shows the interior of a typical stingless beehive, where beebread, honey and propolis can be seen. Honey is a sweet and sticky caramel-like liquid that formed from nectar of the flowers collected by bees (Bankova et al., 2014). Honey consists of different types of colour, odour and taste. Nowadays, honey is widely used in culinary, medicinal, cosmetic and becoming a major product in apiculture industry. Propolis is a mixture of resin of leaf bud and other botanical sources collected by bees from tree exudates (Millind et al., 2013) and have been used in their hive as building materials as well as defensive substances from insects or microorganism (Bankova et al., 2014). Propolis is lipophilic materials that become sticky, soft and flexible in warm condition and become hard in cold temperature (Umthong et al., 2011). The composition of propolis depends on its botanical sources and geographical origin (Bogdanov, 2012). It also depends on season and vegetation (Araújo et al., 2016). Nowadays, propolis is still used in folk medicine. There is an increasing growth of propolis consumption as nutraceutical and pharmaceutical products that makes propolis a highly valued apiculture material. Beebread (bee pollen) comprised of floral pollen that mixed with nectar and bee secretion, which was collected by bees (Chantarudee et al., 2012). It is used as a protein source for bees (Silva et al., 2014). The chemical composition of beebread depends on plant sources, geographic origin, climatic condition, soil types, and beekeepers activities (Xesus et al., 2012; Chantarudee et al., 2012). The major compounds in beebread are protein, amino acid, lipid and carbohydrate (Varsha et al., 2014).

Honey has been consumed and established among consumers as it contains high nutritional content such as vitamin and minerals, protein and micronutrients and acts as a therapeutic agent. Besides it has a high commercial value both in the local and international market. The economic value of propolis and beebread is not as high as that of honey. The reason behind this could be the limitation of the knowledge on their potential by general public. Substantial publications have appeared in the last 50 years reporting therapeutic and biological enhancement properties of bee products. Most of the studies carried out using honeybee products. Fewer studies report the activities of stingless bee product and much less of Malaysian stingless bees. In order to compare the potential for those three apiculture products, we investigate chemical and biological activities of honey, propolis and beebread of stingless bee, *Heterotrigona itama*. The scientific data obtained from this study unleashed the potential of bee products; honey, propolis and beebread for health benefits.

MATERIALS AND METHODS

Sampling

Honey, propolis and beebread of *Heterotrigona itama* were harvested from University's Apiary, Universiti Sultan Zainal Abidin Besut Campus. Honey sample was collected using 200 mL battery pump and was kept in 250 mL

glass bottle. After harvesting, honey samples were stored at 4°C until further analysis, while Propolis and beebread were directly collected from stingless bee colonies and stored at -20°C prior analysis.

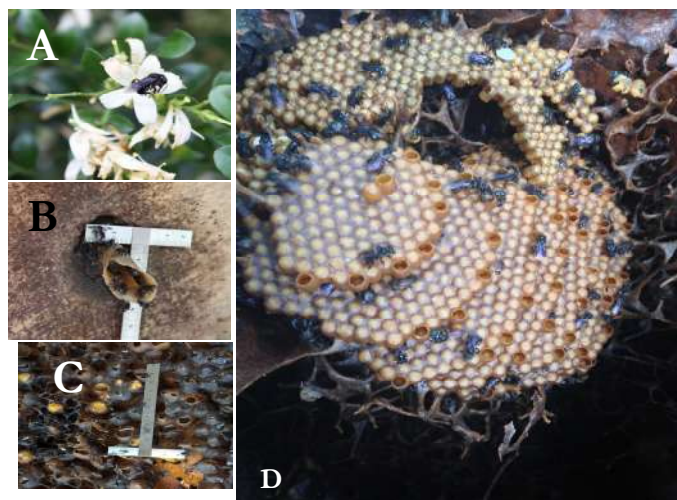


Fig. 1. Stingless bee *Heterotrigona itama* (A) the bee, (b) the entrance shape (c) honey pots and beebread (d) bee brood

Preparation of extraction

Honey extraction

Fifty grams of honey was eluted with 250 mL of acidified water pH 2.0 and 100 grams of Amberlite XAD-2 resin. The solution was mixed well into conical flask and was then aliquot into ten units of 50 mL of centrifuge tube. Those ten centrifuge tubes were mixed on the roller for 1-2 hours. Next, all solution in the centrifuge tubes were poured into the column with sintered glass. The mixture of resin in the column was then allowed to settle down for 10 minutes. The liquid solution was then drained and resin was remained (Note: the solution drained are the removal sugars). The mixture of resin and honey sample in the column was then washed with 250 mL of acidified water (pH 2) and subsequently with 300 mL of distilled water. Then, the elutions were obtained by eluting those mixtures of resin and honey sample with 300 mL of methanol. The methanol extract of honey was then concentrated under vacuum pressure at 45°C.

Propolis and Beebread extraction

Twenty grams of each propolis and beebread samples were macerated in 500 mL of 95% ethanol for three days. The ethanol extract of both propolis and beebread were filtered and concentrated under reduce vacuum pressure at 45°C. The crude extracts of bee products samples were coded as HIM (honey), PIE (propolis) and BBIE (bee bread).

Measurement of nutritional content

Moisture content

The samples were ground if necessary. The crucibles were heated in oven at 105°C for 1 hour. Then, the cool empty crucibles were cooled in desiccator. The empty crucibles were weighed after reaching room temperature and recorded as W1. The samples were weighed approximately 3 grams into crucible and recorded as W2. The samples was spread evenly across the crucible and weighed as rapidly as possible. The crucibles containing samples were dried at 105°C per 1 hour. The crucibles containing samples were transferred to desiccator and

weighed soon after reaching room temperature and noted as W3. Formula below was used to calculate the moisture content:

$$\text{Moisture (\%)} = (W2-W3)/(W2-W1) \times 100$$

Ash Content

The gravimetric method was used to determine the ash content. Three grams of the samples (honey, propolis and bee bread) were transferred to the crucible. The samples were incinerated in preheat furnace at 550 °C, for at least 5 hours. Ash contents was determined as follows:

$$\text{Ash content (\%)} = \text{Difference of crucible's weight} / \text{Total weight of the samples}$$

Protein Content

The nitrogen content was determined using micro Kjeldahl method. One gram of samples weighted into the digestion tube. Two tablets of catalyst Kjeldahl Cu 3.5 were added into the digestion tube. Then, 12 mL of concentrated H₂SO₄ was added, mixed and placed into digestion unit. Twenty-five millilitres of receiver solution (25 mL of boric acid with 10 drops of bromocresol green) was added into the conical flask. The receiver solution turned to green indicating the presence of alkali-ammonia. The distillate with standardized HCl 0.1N was titrated until the color of mixture turn to red color. The percentage of protein was calculated using the following formula:

$$\text{Protein (\%)} = 0.1 \times (\text{mL acid for sample} - \text{blank}) \times 14 \times 100 / \text{Weight of the samples} \times 1000$$

Fat Content

The extraction cups were dried in the oven for 105°C for 6 hours a day before experiment and cooled in desiccator. Pre-dried of extraction cups were weighed and recorded as W2. The samples were weighed (W1) and wrapped with the filter paper and put into extraction thimble. By using the volumetric cylinder, 110 mL petroleum ether was measured and placed into the extraction cup. The extraction cups were attached to the Automated Soxhlet Fat Extractor. When the extraction completed, the extraction cups were detached from the extractor. The extraction cups were dried in the oven 105°C for 2 hours. Next, the extraction cups were cooled in the desiccator and weighed (W3).

$$\text{Fat (\%)} = W3 - W2 / W1 \times 100$$

Carbohydrate

Total carbohydrate contents was obtained by following equation:

$$\text{Total carbohydrate (\%)} = 100 - (\text{ashes} + \text{protein} + \text{fat} + \text{moisture})$$

DPPH Free Radical Scavenging Antioxidant Assay

The antioxidant activity, 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay was performed according to Park et al. (2011) with slightly modification. Five milligrams of the stock solutions of samples extracts and standards (controls) were prepared in 1 mL of DMSO. All samples and standards were made serial

dilutions in 96-well micro litre plate from the stock solutions to get final concentration of 500, 250, 125, 62.5, 31.25, 15.625 and 7.8125 µg/ mL in each respectively wells. Trolox standard was used as positive controls. The samples and standards were then mixed with 0.125 mM DPPH in methanol, to produce a final DPPH concentration of 0.1 mM in final volume of 250 µL each well. The mixture of samples and DPPH solutions were incubated in the dark for 30 minutes at room temperature. The reaction was measured by absorbance at 517 nm. The percentage of inhibition was calculated using the following formula:

Percentage of inhibition:

$$[1 - (A_{517\text{nm, sample}} / A_{517\text{nm, control}}) \times 100]$$

Determination of Flavonoid Compound

Total flavonoid content was determined using colorimetric assay methods (Ibrahim et al., 2016). Quercetin used as positive control at different concentration. Four milligrams of samples extract were diluted in 1 mL of methanol. Then, 0.3 mL of NaNO₂ (5% w/v) was added. After 5 minutes, 0.3 mL AlCl₃ (10% W/V) was added and followed by the addition of 2 mL of NaOH (1M), 6 minutes later. It was continued with the addition of 2.4 mL of distilled water. The mixture was shaken and incubated in the dark at room temperature for 30 minutes. After that, the absorbance was read at 510 nm using microplate reader. Estimation of the flavonoid content was carried out in triplicate as the results were expressed in mean ± standard deviation.

Determination of Phenolic Compound

The total phenolic contents were estimated by using Folin-Ciocalteu method in micro litre plate as described by Ainsworth and Gillespie (2007) with some modifications. The total phenolic contents in the extracts were expressed as Gallic Acid equivalents (GAE) which mg of gallic acid per g of samples extracts. The standard stock solution of gallic acid (1 mg/mL) were prepared with serial dilutions to produce the final concentration of 200, 160, 120, 80 and 40 µg/ mL in micro tubes. For samples extracts, 60 µL of stock solution of each samples extracts (5 mg/mL) were added to get 1 mg/mL of final concentration of samples extracts. The gallic acid and samples extracts solutions were then make up to 100 µL with methanol. Two hundred microliters of Folin-Ciocalteu (F-C) reagent were pipette into all micro tubes and were vortexes thoroughly to complete the final volume of 300 µL each micro tubes. For the last step of the reaction, 800 µL of 7.5 % sodium carbonate (Na₂CO₃) were added into each micro tubes in order to complete the reaction and turn the solution into blue colour (Note: sodium carbonate is excluded in final volume of micro tube). All micro tubes then were incubated at room temperature for two hours. This two hours incubation at room temperature are the critical step which allowed the homogenously colour development as well as resulting in low standard error of mean (s.e.m) between samples replicates. Two hundred microliters of gallic acid standards and sample extracts were transferred into 96-well micro plate from assay tubes and the absorbance of each well was measured at 765 nm. The total phenolic content of samples extracts were calculated using linear regression equation obtained from Gallic Acid Equivalent (GAE) calibration curve and following formula:

$$C = cV/m$$

where,

C: total phenolic content (mg of GAE / g of samples extract)

c: the concentration of gallic acid (mg/mL) established from the calibration curve

V: final volume of sample extract

m: the weight of samples extract

Chemical Profiling by High Performance Thin Layer Chromatography (TLC)

An amount of 8 µL of HIM, PIE and BBIE were spotted on the silica gel F₂₅₄-precoated TLC plates (20 cm x 10 cm) by using Automatic TLC sampler 4 (ATS4, Camag, Muttens, Switzerland). Band was adjusted at 8 mm

between the samples. Plate was developed in the saturated (20 min) twin through chamber using Automated Developing Chamber 2 (ADC2, Camag) with a mixture of toluene: ethyl acetate: formic acid (8: 2: 0.1 v/v/v) as a mobile phase. Formic acid is used as to improve the shape of band formed. The samples were developed in the chamber until it reached the distance at 85 mm. After development of band, the plate was taken out and captured for the image under UV light at short wavelength (254 nm) and long wavelength (366 nm) capturing performed by using TLC Visualizer (Camag, Muttens, Switzerland). The plate was post-chromatogram derivatized using Anisaldehyde-Sulphuric acid reagent (AS). The plate sheet was sprayed manually with AS reagent and was heat using hot air gun. The color formation was visualized under 366 nm and white light by using TLC Visualizer.

Data Analysis

The samples were performed in triplicate (n=3). The results were expressed as mean $\pm$ standard deviation. One-way ANOVA and Post Hoc Turkey were used for comparing significant different between the samples for nutritional contents, antioxidant activity, total phenolic contents and total flavonoid contents. Results for chemical profiling were observed by naked eye.

RESULTS AND DISCUSSION

Nutritional Assessment

Stingless bee products are becoming valuable due to the believed that stingless bee products have more therapeutic value than that of honeybee products. The access to the information of the quality of stingless bee products such as honey, propolis and beebread is important to facilitate the development of these products. In this study, we analysed and compare the nutritional, phytochemical and antioxidant property of *H. itama* honey, propolis and beebread. We also screened the chemical profile of these materials to correlate with their phytochemical content and antioxidant activity. Nutritional content was determined in order to get the information regarding the benefit, hygienic and sources of nourishment.

In this study, five parameters of nutritional contents, (moisture contents, ash contents, protein, fat and carbohydrate) were measured. Based on data in Table 1, HIM possessed the highest value of moisture content, which is $30.67\% \pm 1.30$ followed by BBIE ($30.45\% \pm 0.10$) and PIE ($3.41\% \pm 0.30$). Higher moisture contents in honey can causes fermentation to occur (Iglesias et al., 2014). The moisture content of each honey, propolis and beebread depended principally on the production season and the meteorological conditions in the area where the honey was produced. Moisture content affects honey the most especially the physical properties such as viscosity, colour, palatability, taste, specific gravity, solubility and conservation (Escuredo et al., 2013). Higher moisture content could lead to undesirable honey fermentation during storage, caused by osmotolerant action from yeast upon the sugars fructose and glucose and resulting in the formation of ethyl alcohol and carbon dioxide. In the present of oxygen, the alcohol may then be transformed into acetic acid and water (Chirife et al 2006). It also could possibility encourage microbial growth in beebread. The moisture content affects the commercial value of bee products. According to Honey Commision (Codex Alimentarius Commission, 2001), the moisture content should be lower than 20%. Thus, honey and beebread used in this study must undergo drying treatment before it can be used.

BBIE showed the highest percentages of ash contents with the value of $3.28\% \pm 0.13$ due to more inorganic residues after water and organic matter were burnt away. HIM contains $1.45\% \pm 0.22$ of ash and PIE recorded the least ash contents with $0.23\% \pm 0.10$. On the other hand, PIE showed the highest value of fat ($49.2\% \pm 5.3$) due to the mixture of resin balm and wax (Aroujo et al., 2016). It was followed by BBIE ($13.43\% \pm 0.48$) and HIM, which gave the least value ($0.51\% \pm 0.24$). Besides, HIM gave major total carbohydrate for stingless

bee products ($65.88\% \pm 1.7$), BBIE gave minor total carbohydrate value ($22.36\% \pm 0.88$) and PIE recorded $57.40\% \pm 5.2$ for its total carbohydrate.

Honey is the oldest and most traditional sweetening food across countries, cultures and generations. It has been reported to contain about 200 substances. This material basically consists of concentrated fructose, glucose, maltose, sucrose and other oligo- and polysaccharides. As shown in this work, these carbohydrates comprise more than 65% of honey total dry weight. This value could be higher if the moisture content been reduced by centrifugation or heating methods. In this study, the type of sugars in *H. itama* honey was not determined but was reported that fructose and glucose are the primary sugars in honey (Wintersteen et al., 2005; Gheldof et al., 2002). This make honey is the most suitable product (compare to bee bread and propolis) to be consumed as energy source. In the process of digestion after consuming honey, those principal carbohydrates are transported into the blood and can be utilized for energy requirement. According to Ajibola et al (2012), honey can be potent source of carbohydrate and a better replacement to glucose fundamental of different classes of sugar.

Propolis contains more than 50% of carbohydrate. This could be from the honey remnant in the honey pots. However, propolis has the highest fat content, which could be from the plant lipid collected by the bees in form of resin, balsam or wax. In this study, beebread gave the major protein content with the value of $30.43\% \pm 0.40$, PIE was $2.77\% \pm 0.28$ and HIM was the least value ($1.50\% \pm 0.37$) in stingless bee products. Mutsaers et al (2005) reported that beebread is the source of proteins with essential amino acids, fats, minerals, vitamins, and flavonoids.

Table 1. Nutritional contents of HIM, PIE and BBIE of *H. itama*

Samples	Moisture (Mean $\pm$ SD)	Ash (Mean $\pm$ SD)	Protein (Mean $\pm$ SD)	Fat (Mean $\pm$ SD)	Carbohydrate (Mean $\pm$ SD)
HIM	30.67 ± 1.3	1.45 ± 0.22	1.5 ± 0.37	0.51 ± 0.24	65.88 ± 1.70
PIE	3.41 ± 0.30	0.23 ± 0.10	2.77 ± 0.28	49.2 ± 5.31	57.40 ± 5.20
BBIE	30.45 ± 0.10	3.28 ± 0.13	30.43 ± 0.4	13.43 ± 0.48	22.36 ± 0.88

Antioxidant Activity

The antioxidant activity of each sample was evaluated using DPPH method. Antioxidants are elements molecules in our bodies that eliminate of harmful by products of normal metabolic functions by suppressing destructive chemical reactions in our bodies (Masoud, 2014). DPPH method is broadly used to examine free radical scavenging activity in various samples. Based on Figure 2 the highest antioxidant was found in PIE followed by BBIE and the lowest was HIM. Antioxidant activity was compared by the value of their inhibition concentration at 50% (IC_{50}) for each sample. IC_{50} indicates the concentration of antioxidant needed to scavenging 50% of the DPPH radical. Lower IC_{50} revealed the highest scavenging activity of free radicals in the samples (Khalil et al., 2012). PIE possessed the highest antioxidant properties when the values IC_{50} is $5 \mu\text{g/mL}$ and followed by BBIE with $300 \mu\text{g/mL}$. HIM possessed the least antioxidant activity because even at higher concentration ($500 \mu\text{g/mL}$), HIM do not reach 50% of the DPPH radicals.

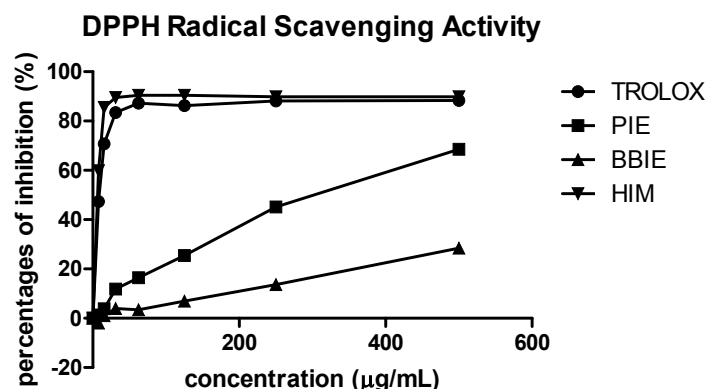


Fig. 2. Graph demonstrates DPPH radical scavenging activity of HIM, PIE, BBIE and trolox is used as positive control. HIM- Honey methanol extract; PIE- Propolis ethanol extract; BBIE- Beebread ethanol extract

Table 2: Concentration of each samples needed to scavenging 50% of the DPPH radical.

Samples	Concentration at IC ₅₀ (µg/mL)
HIM	>500
PIE	5
BBIE	300
TROLOX	3

Total Flavonoid and Phenolic Content

Total flavonoid and phenolic contents were determined using Aluminium Chloride (AlCl_3) and Folin-Ciocalteu methods, respectively. The principle of aluminium chloride colorimetric method is aluminium chloride forms acid stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols. In addition, aluminium chloride forms acid labile complexes with the orthodihydroxyl groups in the A- or B-ring of flavonoids (Bag and Devi, 2015). The principle of Folin-Ciocalteu methods is based on the oxidation and reduction in alkaline condition, which the phenolate ion is oxidized. The MO^{6+} and W^+ complex ion in follin –ciocalteu is reduced and change the reaction turn to blue color (Medic- Saric et al., 2013). Figure 3(b), showed that PIE has the highest flavonoid content ($31.75 \pm 0.05 \mu\text{g/mL}$), followed by BBIE ($5.05 \pm 0.02 \mu\text{g/mL}$) and the lowest was HIM ($2.56 \pm 0.004 \mu\text{g/mL}$). Ibrahim et al. (2016) reported that different components of flavonoids in propolis were due to different favoured area of stingless bee gathering the plants. Meanwhile, current study showed that flavonoid derived from the pollen of different botanical origin and geographical compound have different nutritional significance (Aličić et al., 2014). Flavonoid contents in honey has been reported to relate with environmental condition and geographic area (Masaoud, 2014).

PIE possessed the highest phenolic contents with $70.64 \pm 0.81 \mu\text{g/mL}$, followed by BBIE $56.33 \pm 3.37 \mu\text{g/mL}$ and HIM ($20.24 \pm 0.59 \mu\text{g/mL}$), shown in Figure 3(d). Region of origin and plant sources of propolis affected the phenolic content in propolis (Niken et al., 2014). According to Juszczak et al. (2015), propolis gave the great

antioxidant capacity and phenolic contents between bee products and this is similar to this study when propolis showed the highest result related to antioxidant capacity and phenolic contents. Phenolic contents in beebread were affected by geographical area and botanical origin (Aličić et al., 2014). Different total phenolic contents of honey is due to different sources of honey that produced from the nectar of the flowers, pollen and propolis that composed of benzoic acid, ester and flavonoids (Silva et al., 2014). Phenolic and flavonoid content in stingless bee's product mostly depended on botanical sources. In University's Apiary, *Melaleuca sp.* is the main source for stingless bee to gather flower nectar, pollen or trees exudate. The chemical compounds contain in the trees possibly can affect the total phenolic and flavonoid content in stingless bee's products. As for the concentrations of total phenols and flavonoids, the graph showed that an increase in phenols leads to an increase in flavonoids in all samples. Based on the result of phenolic and flavonoid contents in the samples, these results can be related to the antioxidant activity in the samples. Higher phenolic and flavonoid contents in the samples lead to the increase in antioxidant activity.

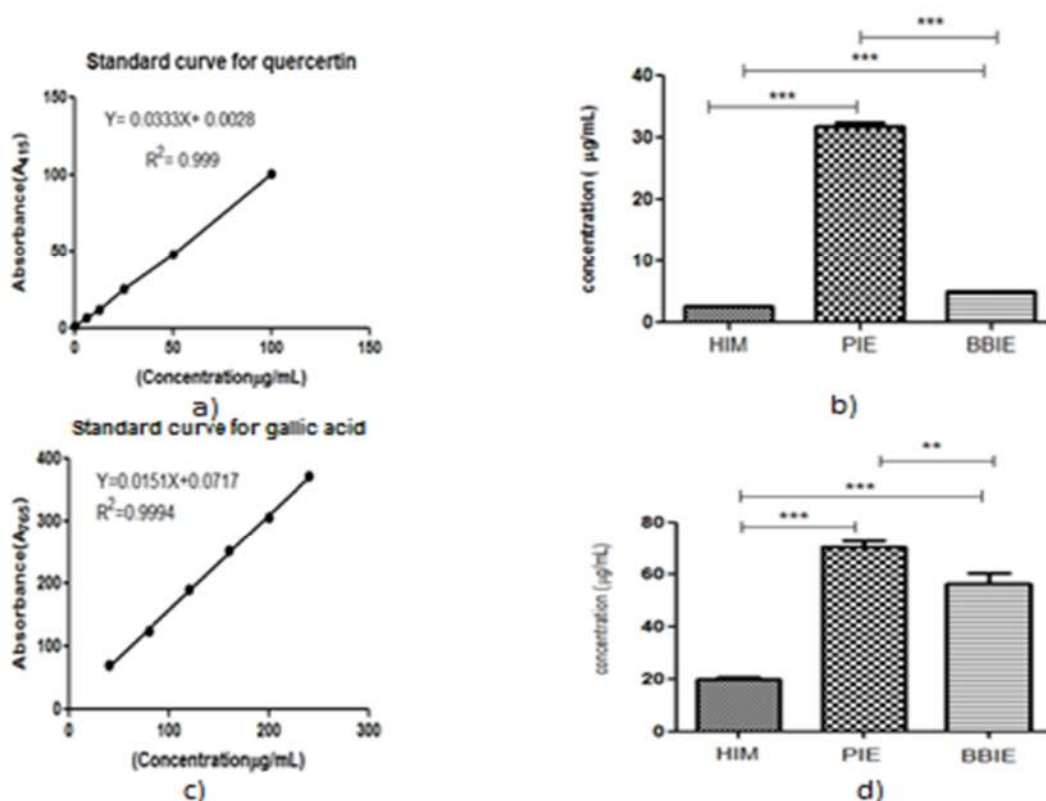


Fig. 3. Standard curves of a) Quercetin (for TFC), c) Gallic acid (for TPC). Bar chart of b) Total flavonoid content with Quercetin Equivalent and (d) Total phenolic content with Gallic Acid Equivalent. TFC and TPC showed that were significant different between all samples. The [***] indicate the samples are highly significant different which $p < 0.0001$. HIM- Honey methanol extract; PIE- Propolis ethanol extract; BBIE- Beebread ethanol extract.

Chemical Profiling by High Performance Thin Layer Chromatography (TLC)

HPTLC is the enhancement of thin layer chromatography. Qualitative HPTLC chromatogram of HIM, PIE and BBIE are presented in e Figure 4 and R_f values of all samples are shown in Table 3. Separation of chromatogram formed on the plate based on R_f value of HIM, PIE and BBIE. The chromatogram revealed different chemical compound in the stingless bee products. Based on the chromatogram in Figure 4(a) and 4(b), PIE showed complex chemical composition compared to HIM and BBIE under 254 nm and 366 nm. Most of the compounds visible under UV 366 nm are native in fluorescence and UV 254 nm is used for detection of

compound that aromatic rings and conjugated double bond. Compounds that absorb UV light can be observed on a layer contains indicator (phosphor) that fluoresces when excited with 254 nm and 366 nm. When irradiated, absorbing compound quench the uniform layer fluorescence and are visualized as dark zones on bright background usually green colour (Sherma and Fried, 2003). After sprayed with anisaldehyde- sulphuric acid, there were several colour detected. Based on the Figure 4(c) and 4(d), HIM revealed the least spot compare to PIE and BBIE under 366 nm and white light. Only one spot presented in HIM, 14 spots in PIE and 12 spots in BBIE detected under white light. The purple colour formed on the plate showed the present of phenols, sugar, steroids and terpenes in stingless bee products. Most of the samples contained purple colour at different R_f value. According to John et al (2016), yellow colour formed after sprayed with anisaldehyde-sulphuric acid is detection of 2-monoenamide structures. This structure only presented in BBIE at R_f 0.21. In general, chemical compounds in PIE are more complex as compared to HIM and BBIE. This result also can be related to antioxidant activity in PIE. Higher chemical compound in PIE might leads to increase in antioxidant activity, phenolic contents and flavonoid contents.

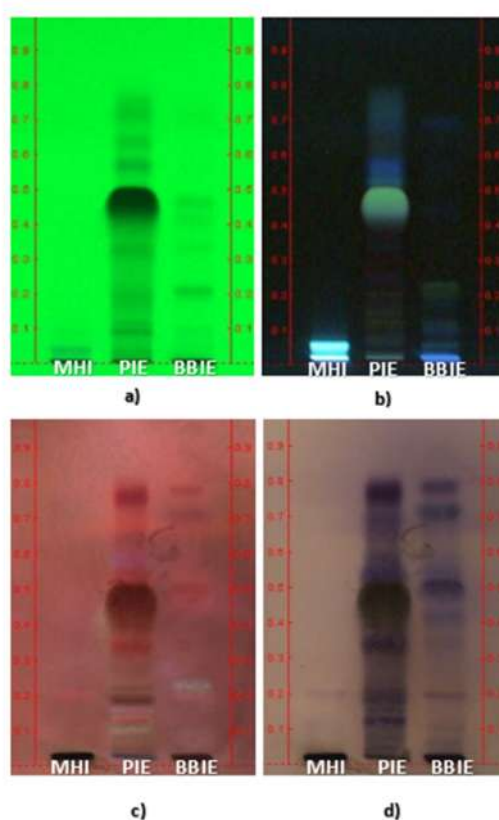


Fig. 4. HPTLC chromatogram of HIM, PIE and BBIE at (a) 254 nm b) 366 nm c) Anisaldehyde-Sulphuric acid derivatization under 366 nm and d) Anisaldehyde-Sulphuric acid derivatization under white light. All the samples were spotted in the form of narrow band on silica gel F254-precoated TLC plates. The mobile phase used for developing the chromatogram is toluene: ethyl acetate: formic acid (8:2:0.1 v/v/v). MHI- Honey methanol extract; PIE- Propolis ethanol extract; BBIE- Beebread ethanol extract.

Table 3. R_f value for HIM, PIE and BBIE of HPTLC analysis.

VISUALIZATION / R _f VALUE				
UV light			Derivatives with sulphuric acid.	anisaldehyde-
SAMPLES	254 NM	366 NM	366 NM	WHITE R
HIM	0.04	0.04	0.19	0.19
PIE	0.04, 0.09, 0.12, 0.17, 0.20, 0.32, 0.47, 0.58, 0.65, 0.71	0.04, 0.09, 0.12, 0.20, 0.27, 0.32, 0.47, 0.53, 0.58, 0.65, 0.71	0.04, 0.05, 0.07, 0.12, 0.15, 0.19, 0.20, 0.32, 0.39, 0.47, 0.53, 0.58, 0.68, 0.78	0.04, 0.05, 0.07, 0.12, 0.15, 0.19, 0.20, 0.32, 0.39, 0.47, 0.53, 0.58, 0.68, 0.78
BBIE	0.09, 0.22, 0.34, 0.42, 0.47, 0.71	0.06, 0.09, 0.13, 0.46, 0.70	0.19, 0.21, 0.50, 0.66, 0.71, 0.78	0.06, 0.13, 0.19, 0.21, 0.34, 0.40, 0.50, 0.54, 0.58, 0.71, 0.78

HIM- Honey methanol extract; PIE- Propolis ethanol extract; BBIE- Beebread ethanol extract.

CONCLUSION

In this study, nutritional contents, antioxidant activities, phytochemical screening and chemical profile of honey, propolis and beebread from Malaysian stingless bee species *Heterotrigona itama* were evaluated. Based on the findings, propolis has the highest antioxidant activity compared to honey and beebread. Honey, propolis and beebread have different chemical profile, phytochemical composition and nutritional contents. Therapeutic and nutritional contents shown in propolis, honey and beebread of stingless bee of *Heterotrigona itama* make them an excellent potential for food supplement.

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