



Original article

# Analysis of bioactive compounds and chemical composition of Malaysian stingless bee propolis water extracts



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## ABSTRACT

Propolis is a resinous substance collected by stingless bees containing bioactive compounds which exert various biological properties. The present study focused on the evaluation of chemical profiles produced by three Indo-Malayan stingless bee propolis extracted using water. Fresh propolis was collected from the same area and ecosystem conditions in Selangor, Malaysia, namely *Tetrigona apicalis*, *Tetrigona binghami*, and *Heterotrigona fimbriata*. The bioactive compounds and chemical composition of propolis extracts were then analyzed using gas chromatography–mass spectrometry (GC–MS). Results showed that propolis from the three different stingless bee species consisted of major groups such as sugar (31.4%), carboxylic acid (17.1%), terpenoid (14.3%), sugar alcohol (11.4%), hydrocarbon (5.7%), aldehyde (5.7%) amino acid (2.9%) and other constituents (11.4%). *Heterotrigona fimbriata* displayed the highest amount for both total phenolics (13.21 mg/mL) and flavonoids (34.53 mg/mL) compared to other propolis extracts. There is also no significant difference detected between all samples since  $p \leq 0.05$ . In conclusion, this study shows that Malaysian stingless bee propolis contain bioactive components that have great potential to be used for their therapeutic and medicinal benefits. However, more investigations and analysis of stingless bee propolis need to be carried out in order to enhance the understanding and applications of propolis in the future.

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## 1. Introduction

Stingless bees are unique in which they are able to defend themselves despite their lack of a sting by producing a sticky-like substance, known as propolis, which helps to protect their hives and themselves from external intruders and climate change (Ibrahim et al. 2016). Propolis is a natural resinous material made up of leaves, crack barks and flower buds collected from numerous plant sources, mixed with wax and salivary enzymes secreted by the bees (Jayanthi & Kothai 2017; Saricoban & Yerlikaya 2016).

Nowadays, propolis has been extensively studied for its numerous medicinal benefits such as antibacterial, antioxidant, anti-obesity, anti-cancer and many more. Propolis mainly consists of resins (50%), wax (30%), essential oils (10%), phenolics and flavonoids (10%), pollen (5%), including vitamins and minerals (5%) (Özer 2020). A study carried out by Dehghani et al. (2019) in Iran found that several bioactive compounds in propolis such as 3-methyl-2-butenyl caffeate and phenyl-ethyl caffeate can be effectively used to treat orthodontic patients without having the adverse effects such as lesion, unpleasant taste and allergic reaction when using chlorhexidine mouthwash. Furthermore, Cai et al. (2020) also claimed that stingless bee propolis may also be utilized for high-fat diet-induced obesity treatments as well as containing hepatoprotective potentials due to the chemical compounds identified in the propolis.

Propolis is rich in important bioactive compounds that determine its various biological effects. There are approximately 300 different constituents have been identified in propolis so far (Anjum et al., 2018). According to Ramnath et al. (2015), there

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are a significant amount of carboxylic acid (20.4%), terpenoids (15.0%), steroids (11.5%), hydrocarbons (9.6%), sugars (6.4%), alkaloids (6.4%), flavonoids (4.3%), phenols (3.2%), ketones (2.1%), amino acid (2.1%), vitamins (2.1%) and other compounds (15.0%) identified in propolis collected from 6 different Indian regions. Other than that, [Ahangari et al. \(2018\)](#) had also documented that Iranian propolis comprised numerous chemical compounds including amino acids, sugars, vitamins, flavonoids, phenolics, and terpenoids that can be considered as potential sources of therapeutics.

Although studies on chemical composition of stingless bee propolis are being conducted worldwide, the data regarding Malaysian stingless bee propolis, especially using water as solvent, received little attention and are poorly documented. This is because water extracts are deemed to be safer and more biocompatible compared to alcoholic extracts, which had been proven to cause some side effects when utilized ([Rocha et al. 2013](#)). Hence, the aim of this study was to investigate the chemical compositions of water extracted propolis from three different Indo-Malayan stingless bee species known as *Tetrigona apicalis*, *Tetrigona binghami*, and *Homotrigona fimbriata*. The chemical components in the propolis extracts such as phenolics, flavonoids, lipids, alkaloids, carbohydrates, amino acids, terpenoids and vitamin C were also identified using GC–MS analysis since it allows detection of even the smallest amount of compounds present in the extracts ([Pobiega et al., 2019](#)).

## 2. Materials & methods

### 2.1. Sample collection

Propolis samples were collected from three different Indo-Malayan stingless bee species known as *Tetrigona apicalis*, *Tetrigona binghami*, and *Homotrigona fimbriata*. The samples were provided from Indo-Malayan Stingless Bee Repository at Malaysia Genome Institute, Selangor (N 2° 54' 16.8732" E 101° 46' 5.61") at the end of December 2020. Each sample was then stored in the dark at –20 °C prior analysis ([Mohamed et al., 2020](#)).

### 2.2. Propolis water extract preparation

The sample extraction technique was prepared according to [Salim et al. \(2018\)](#) with slight modifications. 40 g of crude sample was ground into powder form before mixing them with distilled water (1:5 *m/v*). The mixture was then heated at 70 °C for 5 min and left in the dark at room temperature overnight. After 24 h, the mixture was filtered using Whatman No. 1 filter paper and freeze-dried (Christ / RVC2-18/MZ2C) for 3 days to obtain dry extract in crystal-like form before being stored at –20 °C in the dark until further investigation.

### 2.3. GCMS analysis for biochemical constituents

Gas chromatography mass spectrometry was performed by using The Perkin Elmer Clarus 600 GCMS coupled to Turbo Matrix Headspace Sampler 40, equipped with GCMS column Elite 5MS (30 m × 250 mm × 0.25 μm). Helium was used as carrier gas at a flow rate of 1 mL/min and the injector temperature was 250 °C with a split mode (split ratio 100:1). The oven temperature was initially held at 80 °C for 4 min and increased to 250 °C at the rate of 20 °C/min and held for 15 min. The mass spectra were optimized by following parameters: Source temperature: 280 °C, Transfer temperature: 250 °C, Solvent delay time: 2 min, Scan range: 35–500 Da. Finally, the temperature was increased to 300 °C for 30 min. The compounds were identified by means of their reten-

tion time by comparison of their mass spectra with National Institute Standard and Technology (NIST) library data ([Pobiega et al., 2019](#)).

### 2.4. Quantification of total phenolic and flavonoid contents

The total phenolic content of each propolis extract was conducted using the Folin-Ciocalteu colorimetric method, as mentioned by [Zarate et al. \(2018\)](#) with minor modifications. 0.2 mL of each sample extract was placed into respective vials and 0.8 mL of 10% Folin-Ciocalteu's reagent was then added to make up the final volume of 1 mL in each vial. Next, 1 mL of 8% sodium carbonate solution was added to the mixture and mixed with 95% ethanol until final volume in each vial up to 3 mL after 5 min incubation. The solution was kept without presence of light for 50 min and the absorbance readings were measured at 725 nm using a UV–VIS Spectrophotometer (Beckman Coulter/ DU 730). Gallic acid (mg/mL GAE) was used as a standard solution to obtain the standard calibration curve.

The aluminium chloride (AlCl<sub>3</sub>) technique was performed as guided by [Fikri et al. \(2019\)](#) with little modifications to determine the flavonoid content present in each propolis extract. 0.2 mL of each standard and sample solution were placed into respective vials. Next, 0.5 mL aluminium chloride (10%) and 0.5 mL 1 M potassium acetate were completely homogenized with all mixtures and allowed to stand at ambient temperature for 30 min under dark condition. The absorbance reading for all mixtures were detected at a fixed wavelength of 510 nm through UV–VIS Spectrophotometer (Beckman Coulter/ DU 730). Rutin (mg/ml RE) was used as a standard reference to plot the standard calibration curve.

### 2.5. Quantification of alkaloid and carbohydrate contents

The quantification of alkaloid content was analyzed using 1, 10-phenanthroline technique as described by [Santo et al. \(2013\)](#) with slight modifications. 1 mL of 1, 10-phenanthroline (0.05 M) reagent was prepared in ethanol as a stock solution. Next, 100 μg of colchicine was diluted with 100 mL distilled water to be used as a standard reference to perform the calibration curve. 1 mL of each sample and standard solution was then mixed with 1 mL 0.025 M FeCl<sub>3</sub> in 0.5 M HCl and 1 mL of 1, 10-phenanthroline. All mixtures were shaken gently to homogenize completely and incubated in water bath (Bath WB Water Bath 18/30/45 Litre 100 °C ± 0, 1 °C) at 70 °C for 30 min. The absorbance measurements were detected using UV–VIS Spectrophotometer (Beckman Coulter/DU 730) at 510 nm against blank sample.

The analysis of carbohydrate content in sample extract was investigated as established by [Abdullah et al. \(2020\)](#) with minor modifications. This colorimetric method was conducted using 0.2 g of anthrone reagent with 100 mL of H<sub>2</sub>SO<sub>4</sub> as a stock solution. 100 mg of glucose was diluted with 100 mL of distilled water as a standard reference to plot the calibration curve. Next, 0.2 mL of sample extract was homogenized with 4 mL of anthrone reagent and all mixtures were incubated in boiling water for 10 min. Darker green appearance indicated higher content of carbohydrate in the tested sample. Finally, the absorbance was measured at 620 nm through UV–VIS Spectrophotometer (Beckman Coulter/ DU 730) against blank sample.

### 2.6. Quantification of terpenoid and lipid contents

Terpenoid content in sample extract was tested using linalool at different concentrations as standard reference to obtain the calibration curve. 1 mL of each sample extract was prepared in respective vials and mixed with 1.5 mL of chloroform. After gently mixing, 3 drops of sulphuric acid (95%) were slowly added into

**Table 1**

Concentration of different biochemical compounds found in stingless bee propolis water extracts (mg/mL).

No.	Compound	<i>T. apicalis</i>	<i>T. binghami</i>	<i>H. fimbriata</i>
1	Lipid	42.36 ± 0.05	15.98 ± 0.05	4.95 ± 0.01
2	Vitamin C	13.88 ± 0.12	4.40 ± 0.01	12.62 ± 0.05
3	Carbohydrate	9.01 ± 0.11	9.56 ± 0.14	4.67 ± 0.05
4	Terpenoid	0.66 ± 0.08	2.19 ± 0.05	1.15 ± 0.04
5	Alkaloid	1.73 ± 0.04	1.76 ± 0.04	1.82 ± 0.06
6	Flavonoid	34.50 ± 0.12	34.17 ± 0.15	34.53 ± 0.10
7	Phenolic	7.60 ± 0.26	10.11 ± 0.13	13.21 ± 0.11
8	Amino acid	0.62 ± 0.01	0.53 ± 0.04	0.70 ± 0.01

No significant difference between all samples since  $p > 0.05$ .

all mixtures. Finally, the absorbance readings of all samples and standard solutions were detected using UV–VIS Spectrophotometer (Beckman Coulter/DU 730) at a fixed wavelength of 538 nm against blank sample (Ramnath et al. 2015).

The assay of lipid content was carried out by preparing 0.75 g of vanillin reagent diluted with 125 mL of distilled water as a stock solution. Pure olive oil was prepared at different concentrations (0.2–1 mL) as a standard reference to perform the calibration curve. Next, 1 mL of each sample extract and standard solution were added with 1.5 mL H<sub>2</sub>SO<sub>4</sub> (95%) before incubated in a water bath (Bath WB Water Bath 18/30/45 Litre 100 °C ± 0, 1 °C) at 60 °C for 10 min. After cooling, 2.4 mL of vanillin reagent was added into each mixture and incubated at room temperature for 40 min. The absorbance was measured through UV–VIS Spectrophotometer (Beckman Coulter/DU 730) at a fixed wavelength of 490 nm (Abdullah et al. 2020).

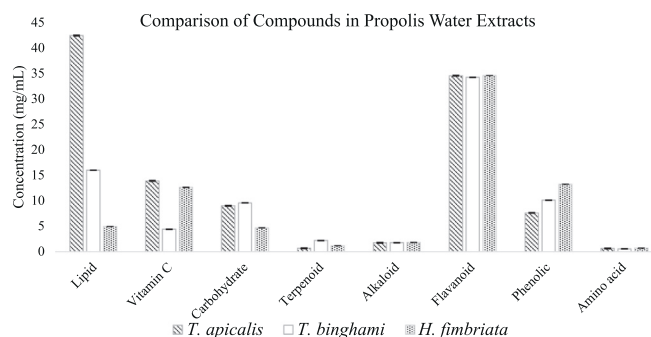
### 2.7. Quantification of amino acid and vitamin C contents

The total amino acid content was determined using the colorimetric method as established by Anjum et al. (2018) with little modifications. The stock solution was prepared using 2% ninhydrin in ethanol and leucine was used as a standard solution with different concentrations (0.2 mL–1 mL). 1 mL of each sample extract and prepared standard solution were mixed with 1 mL of ninhydrin. Next, the top of each vial containing reaction mixture was covered with aluminium foil and incubated in a water bath (Bath WB Water Bath 18/30/45 Litre 100 °C ± 0, 1 °C) at 60 °C for 15 min. Then, the mixtures were allowed to cool in ice water and 1 mL of 50% ethanol was added to all vials before detected through UV–VIS Spectrophotometer (Beckman Coulter/DU 730) at a fixed wavelength of 570 nm against blank sample. The negative reaction of ninhydrin test will be remained colourless at the end of reaction.

The quantification of vitamin C content was conducted as documented by Ramnath et al. (2015) method. 4% of oxalic acid diluted in ethanol was prepared as a stock solution. 1 mL of freshly prepared ascorbic acid and sample extract were placed in respective vials. Next, 2 mL of oxalic acid, 0.5 mL of H<sub>2</sub>SO<sub>4</sub>, and 2 mL of aluminium molybdate were added into all mixtures and shaken well. 3 mL of distilled water was added into each vial before incubating in a water bath (Bath WB Water Bath 18/30/45 Litre 100 °C ± 0, 1 °C) at 60 °C for 30 min. The absorbance readings were detected at 515 nm using UV–VIS Spectrophotometer (Beckman Coulter/DU 730).

### 3. Result

Table 1 summarizes the concentrations of total lipid, vitamin C, carbohydrate, terpenoid, alkaloid, flavonoid, phenolic and amino acid tested in propolis water extracts from three different Malaysian stingless bee species, namely *T. apicalis*, *T. binghami* and *H. fimbriata*. Analysis shows no significant difference between all



**Fig. 1.** Comparison of bioactive compounds in different propolis water extracts.

samples for the compounds studied. Fig. 1 also shows the comparison between the compounds obtained from different propolis extract samples. It can be seen from Fig. 1 that all three propolis samples contain highest concentration of flavonoids, and free amino acids the least.

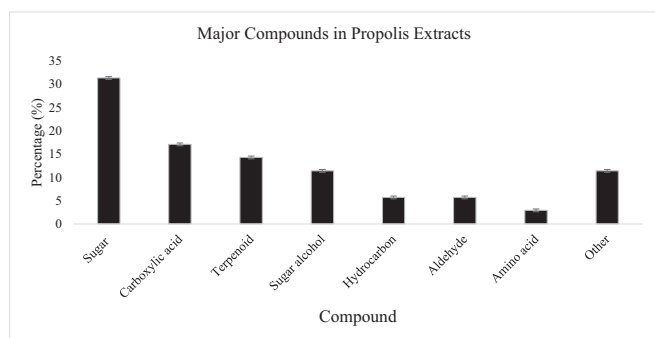
The concentration of total lipid detected in *T. apicalis* propolis water extract is the highest among all three samples with 42.36 mg/mL. This is then followed by *T. binghami* extract with 15.98 mg/mL and *H. fimbriata* extract with the lowest concentration, 4.95 mg/mL. Secondly, the concentration of vitamin C detected in *T. apicalis* propolis extract is the highest at 13.88 mg/mL, and is followed closely by *H. fimbriata* extract at 12.62 mg/mL. On the other hand, *T. binghami* extract has been shown to produce a very low amount of vitamin C at 4.40 mg/mL.

Next, *T. binghami* extract has recorded the highest amount of carbohydrate at 9.56 mg/mL while *T. apicalis* extract produced 9.01 mg/mL and *H. fimbriata* extract contain the lowest carbohydrate concentration of 4.67 mg/mL. Furthermore, it can also be seen that *T. binghami* extract produced the highest amount of terpenoids at 2.19 mg/mL, while *H. fimbriata* extract contains 1.15 mg/mL and *T. apicalis* extract contain the lowest terpenoid concentration of 0.66 mg/mL. Table 1 also showed that *H. fimbriata* extract contains the highest concentration of alkaloids at 1.82 mg/mL. This is followed closely by *T. binghami* and *T. apicalis* extracts with 1.76 and 1.73 mg/mL respectively.

Other than that, the total flavonoid contents found in *H. fimbriata* propolis extract is the highest at 34.53 mg/mL with *T. apicalis* extract came close at 34.50 mg/mL. The lowest total flavonoid content was observed in *T. binghami* extract at 34.17 mg/mL. *H. fimbriata* extract also produced the highest total phenolics at 13.21 mg/mL, while *T. binghami* extract recorded total phenolics of 10.11 mg/mL and *T. apicalis* extracts having the lowest amount of phenolics at 7.60 mg/mL. Finally, the concentration of free amino acids was found to be the highest in *H. fimbriata* extract at 0.70 mg/mL, followed by *T. apicalis* extract at 0.62 mg/mL and *T. binghami* extract at 0.53 mg/mL. According to Eroglu et al. (2016), amino acids found in propolis may come from saliva produced from bee metabolism

**Table 2**  
Major compounds detected in propolis water extracts.

Compound	<i>H. fimbriata</i>	<i>T. apicalis</i>	<i>T. binghami</i>
<b>Terpenoid</b>			
Germacrene D	+	–	–
Isolongifolol	+	–	–
Phorbol	+	–	–
$\alpha$ -Eudesmol	–	+	–
Isoaromadendrene epoxide	–	–	+
<b>Sugar</b>			
D-Fructose	+	+	+
d-Galactose	+	+	+
$\alpha$ -D-Glucopyranoside	+	–	–
$\alpha$ -D-Galactopyranoside	+	–	–
d-Ribose	–	+	–
d-Glucose	–	+	–
D-Glucose	–	+	+
$\alpha$ -D-Mannopyranoside	–	+	–
Glycoside	–	+	–
Hexopyranose	–	+	+
$\alpha$ -D-Glucofuranoside	–	–	+
<b>Carboxylic acid</b>			
$\alpha$ -Linoleic acid	+	–	–
Tricosadiynoic acid	+	–	–
Octadecatrienoic acid	+	–	–
Butanedioic acid	–	+	–
Propanoic acid	–	+	–
Cyclohexene	–	+	–
<b>Sugar alcohol</b>			
Arabinitol	+	+	+
Ribitol	+	+	+
Arabitol	–	+	+
D-Glucitol	–	–	+
<b>Hydrocarbon</b>			
Silane	–	–	+
Butane	–	–	+
<b>Aldehyde</b>			
Dioxolane-4-carboxaldehyde	–	–	+
Cyclohex-1-en-1-carboxaldehyde	–	–	+
<b>Amino acid</b>			
Pyroglutamic acid	–	+	–
<b>Others</b>			
Trimethylsilyl ether of glycerol	+	+	+
3,8-Dioxa-2,9-disiladecane	+	+	+
Silanol	–	+	–
2-Mono-isobutyryn	–	–	+



**Fig. 2.** Major chemical compounds detected in propolis water extracts.

as well as plants from which the resins were collected to build propolis.

Propolis is made up of various major chemical constituents and groups, depending on factors such as countries, botanical origins, bee species and many more (Awang et al. 2018). Table 2 listed the chemical groups and compounds detected in the propolis water extracts studied. A total of 35 major groups had been iden-

tified using GC–MS analysis. The Malaysian stingless bee propolis extracts were characterized with sugars (31.4%), carboxylic acids (17.1%), terpenoids (14.3%), sugar alcohols (11.4%), hydrocarbons (5.7%), aldehydes (5.7%), amino acids (2.9%) and other compounds (11.4%) as shown in Fig. 2.

#### 4. Discussion

According to Abdullah et al. (2020), lipid is usually the major compound found in propolis, regardless of bee species due to resins and waxes taken from trees. Moreover, it has also been noted that stingless bee propolis generally produce higher lipid content than honeybees due to their floral preferences, which makes the stingless bees hive to be more water resistant than honeybees (Devequi-Nunes et al. 2018). Next, vitamin analyses of stingless bee propolis have been scarcely explored and studied, especially in Malaysia and other tropical countries, and therefore need to be further investigated (Ahmad et al. 2019). Research on the chemical contents of honeybee propolis estimated that the propolis extract contained around 34–70  $\mu\text{g/g}$  of vitamin C concentration (Ramnath et al. 2015). Other than that, a study carried out by Usman et al. (2016) had also showed that honey derived from the *Trigona* sp. contain higher levels of vitamin C compared to vitamin A and E at 302.26  $\mu\text{g/g}$ .

According to Abdullah et al. (2019) propolis extract usually contains a low amount of carbohydrate making up of 0.17–0.48% of the whole chemical constituents and might change depending on the bee floral preferences, seasonality as well as availability around their hives. A study done by Lim et al. (2021) noted that stingless bee honeys from different origins contain around 68.33 to 72.25 g/100 g of total carbohydrate. Besides, it has also been reported that total carbohydrate of bee bread or pollen from stingless bees could range between 25 and 55% (Mohammad et al., 2019).

Terpenoids are the volatile components of the propolis and are the substances known to produce the odour or aroma given off by the propolis. It also plays a huge part in the biological activities of the propolis essential oil extract, such as antimicrobial and anti-inflammatory activities (Bankova et al. 2014). According to Šturm and Ulrih (2020), terpenoids were identified in the propolis for the first time in 2011, and 133 terpenes had been identified in propolis so far. Alkaloids were first discovered and isolated from propolis for the first time in 2015, and only 16 alkaloids from Brazil and Algeria had been identified to date (Šturm & Ulrih 2020). Hasan et al. (2014) had reported no alkaloids detected in different Indonesian *Trigona* sp. propolis extracts studied while Ramnath et al. (2015) found alkaloid concentrations in Indian *A. mellifera* propolis extract ranging from 62 to 98  $\mu\text{g/g}$ .

A study on Malaysian stingless bee propolis extracts, including *T. apicalis* extract showed a significant amount of total flavonoids, which are important in exhibiting potent antioxidant activity (Asem et al. 2019). Another study conducted on total flavonoids contents of Mexican *Melipona beecheii* and *A. mellifera* propolis extract yielded 7.68 and 17.23 mg/g, respectively (Ramón-Sierra et al. 2019). Variations observed in the amount of flavonoids found in the stingless bee propolis could be attributed to differences in plant preferences as well as vegetations pollinated by the bees (Rosli et al. 2016; Awang et al. 2018).

Moreover, polyphenols also play major roles in exhibiting bioactivities, including antioxidant activity displayed by the propolis extracts. This result obtained from this study also corroborated with those obtained by Awang et al. (2018) showing Malaysian *H. fimbriata* extract containing the highest phenolic contents, followed by *T. apicalis* and *T. binghami* extracts with 16.2, 13.9 and 5.7 mg/mL respectively. Besides, a study on several Indonesian

stingless bee propolis also recorded phenolics ranging between 10 and 28.65 mg/mL (Fikri et al. 2019). Polyphenols in propolis has been reported to enhance human health and biological activities such as antimicrobial, anticancer, anti-inflammatory effects and many more (Badiazaman et al. 2019).

Propolis is known to contain essential amino acids such as proline and arginine that are important for cell regeneration and could vary according to the vegetations and environments surrounding the bee hives (Mulyati et al. 2020). There had been several reports done on the amino acid contents in bee pollens, however, there is still a lack of information on free amino acids obtained in stingless bee propolis. According to de Oliveira et al. (2019), bee pollen collected from *Melipona* sp. contain leucine between 80 and 90 mg/g. On the other hand, da Silva et al. (2014) had identified about 0.63 mg/g of leucine in bee pollen due to differences in propolis collection site and bee species.

GC–MS analysis of the propolis extracts had revealed compounds such as sugar, carboxylic acid, terpenoid, sugar alcohol, hydrocarbon, aldehyde and amino acid. Sugar was the major compound identified in the propolis samples, making up of 31.4% of total compounds detected in the extracts. *T. apicalis* extracts contained the highest amount of sugars, followed by *T. binghami* and *H. fimbriata* extracts. Some of the reducing sugars found in the extracts tested were ribose, fructose, glucose and galactose. A study on propolis collected from different countries such as Spain, Romania and China showed the presence of fructose, glucose, galactose, stachyose and sucrose (Qian et al. 2008). Studies regarding sugars had been greatly studied on stingless bee honeys. A past research on Malaysian *H. itama* honey reported that it contained a high level of fructose and glucose due to bees' floral preferences (Cheng et al. 2019).

Secondly, carboxylic acids such as  $\alpha$ -linoleic, tricosadiynoic, octadecatrienoic, propanoic and butanedioic acids were also detected in propolis extracts studied. Table 2 showed carboxylic acids were identified in both *H. fimbriata* and *T. apicalis* extracts, but none in *T. binghami* extracts. A study on aqueous propolis extract from Czech Republic showed that it was mainly consisted of carboxylic acids and gave off effective antiviral activity against herpes simplex virus (Nolkemper et al. 2010). Besides, tricosadiynoic acid identified in *H. fimbriata* propolis extract had also been proven to be able to treat obesity and high-fat diet related diseases by reducing weight gain and insulin level in rats studied successfully (Liu et al. 2020; Zeng et al. 2017).

Next, terpenoids such as germacrene D, phorbol,  $\alpha$ -eudesmol and isolongifolol, was also an important group detected in the propolis samples since it played a significant role in the biological activities and aromatic properties exhibited by the propolis (Ramnath et al. 2015). *H. fimbriata* extract contained the highest amount of terpenoids, followed by *T. apicalis* and *T. binghami* extracts. A past study on Brazilian stingless and honeybee propolis revealed that germacrene D can only be found in stingless bee propolis, while  $\alpha$ -eudesmol and other components detected in propolis essential oil contained antibacterial properties (Bankova et al. 2014). Other than that, phorbol was also identified in Indian honeybee propolis extract for the first time in 2015 (Ramnath et al. 2015). However, there are limited studies regarding stingless bee propolis volatiles available compared to honeybees (Souza et al. 2018).

Furthermore, sugar alcohols such as arabitol, ribitol, arabinitol and D-glucitol were also identified in all the propolis samples. Most sugar alcohols were detected in *T. binghami*, followed by *T. apicalis* and *H. fimbriata* extracts. Sugar alcohols had also been reported in Brazilian stingless bees, *Tetragonisca angustula* and *Melipona* spp. propolis extracts (Bankova & Popova 2007). On the other hand, organic hydrocarbons such as silane and butane were only identified in *T. binghami* extracts. The application of silane in propolis as bio-friendly hydrophobic agent had been documented to enhance wood protection. Besides, butane was also

reported in a very low amount in Polish propolis extracts (Sawicka et al. 2012; Woźniak et al. 2018). Moreover, compounds such as carboxaldehydes and amino acids had also been determined in *T. binghami* and *T. apicalis* extracts respectively. Pyroglutamic acid had only been documented in Polish propolis extract recently and it had also been noted that other amino acids discovered in the propolis extracts were detected in very low amount (Kurek-Górecka et al. 2014).

Solvents used during propolis extraction process also play a major role in determining the nutritional values and chemical compositions exhibited by the propolis. Rocha et al. (2013) had noted that most studies regarding propolis had been done using ethanolic and other alcoholic extracts and very little is known on propolis water extracts and their compounds, including bioactivities. Besides, they had also reported that alcoholic extracts usually produce pungent taste and negative side effects in some cases when consumed, which could be highly disadvantageous in food and beauty industries. Furthermore, water extracts had also been proven to be more environmentally friendly, safe and biocompatible for utilization in the pharmaceutical and health sectors (Kubiliene et al. 2018). Other than that, a research done using propolis water extracts supplemented in diet also showed improvement in the antioxidant activity and immunity of the birds studied (Sahin & Ozturk 2018).

Studies on the biochemical constituents of stingless bee propolis are crucial as the therapeutic and medicinal properties of the propolis are directly related to their chemical compositions (Campos et al. 2015). Identifications of chemical compounds in stingless bee propolis are very much needed nowadays in order to ensure further investigations and applications of propolis are being carried out in various sectors and industries. Moreover, knowledge and data on the chemical compounds and compositions of the stingless bee propolis water extracts are still very limited and unclear. Therefore, it can be concluded from this study that Malaysian stingless bee propolis water extracts contain a significant amount of diverse chemical compounds that can be used as natural products to prevent diseases and improve the quality of life.

## 5. Conclusion

This study had been able to evaluate the concentrations of bioactive compounds such as total lipid, vitamin C, carbohydrate, terpenoid, alkaloid, flavonoid, phenolic and amino acid from Malaysian stingless bee propolis water extracts from *H. fimbriata*, *T. apicalis* and *T. binghami* successfully. Besides, chemical compounds from major groups such as sugar (31.4%), carboxylic acid (17.1%), terpenoid (14.3%), sugar alcohol (11.4%), hydrocarbon (5.7%), aldehyde (5.7%) amino acid (2.9%) and other constituents (11.4%) had also been analysed and identified by using GC–MS. There is also no significant difference detected between all samples since  $p \leq 0.05$ . It is hopeful that this study will be able to encourage further investigations and studies on the biochemical compounds and contents of stingless bee propolis in order to enhance the understanding and applications of propolis in the near future.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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