Original paper

GC-MS ANALYSIS OF CHEMICAL CONSTITUENTS IN ETHANOLIC BEE POLLEN EXTRACTS FROM THREE SPECIES OF MALAYSIAN STINGLESS BEE

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Abstract

The pollen of stingless bees is derived from flower pollen mixed with bee digestive enzymes and preserved with honey and nectar. In this study, the volatile compounds present in ethanolic bee pollen extracts (BPEs) from three species of the Malaysian stingless bee were analyzed using gas chromatography-mass spectrometry (GC-MS). Hydrocarbons, sugars and its derivatives, fatty acids, amino acids, alcohol, uridine, aldehyde and an unknown carbamate were detected. Mannitol, the main sugar compounds, represented 54.34% in *Trigona thoracica*, 39.11% in *Trigona apicalis* and 33.05% in *Trigona itama*. Propanoic acid and hexadecanoic acid were the main hydrocarbons present in the extract of *Trigona apicalis* (4.04%) and *Trigona thoracica* pollen (1.28%) respectively. The polyunsaturated fatty acids linoleic acid and α-linolenic acid were found in small amounts in all BPEs (0.07-1.11%). The chemical compounds found in BPEs had biological activities, thus bee pollen may be useful in traditional medicine and as a health supplement.

Keywords: gas chromatography-mass spectrometry (GC-MS), Malaysian stingless bee pollen, trigona, volatile compounds

INTRODUCTION

Bee pollen (or bee bread) from stingless bees is derived from flower pollen mixed with bee digestive enzymes and preserved with honey and nectar (Nagai et al., 2005; Silva et al., 2006). It has medicinal qualities and is used commonly as a treatment complement in patients with cancer, hyperlipidaemia, depression, and nutritional deficiency and as an immune booster (Komosinska-Vassev et al., 2015).

We recently reported that ethanolic bee pollen extracts (BPE) from common domesticated Malaysian stingless bee species (*Trigona (Tetrigona) apicalis, Trigona (Heterotrigona) itama*, and *Trigona (Geniotrigona) thoracica*) have strong antioxidant activity and high polyphenol contents (Harif Fadzilah et al., 2017). Bee pollen had been previously reported to contain at least 250 substances, including sugars, lipids (triglycerides, phospholipids), carbohydrates, proteins, amino acids, vitamins, minerals, carotenoids, flavonoids, and macro- and micronutrients (DeGrandi-Hoffman, Eckholm, & Huang, 2013; Graikou et al., 2011; Komosinska-Vassev et al., 2015).

Analyses of BPE from stingless bees by gas chromatography-mass spectrometry (GC-MS) are sparse, and to date, there is a lack of GC-MS data for ethanolic BPE from *T. thoracica, T. itama*, and *T. apicalis*. The goal of this study was to identify the volatile components of BPE from Malaysian stingless bees to further understand the potential of this resource for future medicinal applications.

MATERIAL AND METHODS

Chemicals and reagents

Ethanol (Acros Organics, New Jersey, USA), methanol HPLC grade (Acros Organics, New Jersey, USA), Bis-(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS) (Supelco, Pennsylvania,

USA), pyridine (Sigma, Saint Louis, USA) were purchased for use in this study.

Collection of bee pollen

Bee pollen samples from *T. thoracica, T. itama*, and *T. apicalis* colonies were obtained from Syamille Agrofarm, Kuala Kangsar, Perak, Malaysia. The species of stingless bee were identified and confirmed by the Entomology Section, Malaysian Agriculture and Research Development Institute, Serdang, Malaysia. Collected samples were kept at 4°C in a refrigerator for GC-MS analysis.

Preparation of ethanolic BPE

Bee pollen samples from each species were suspended in ethanol at a concentration of 1:10 (w:v) or 10 g in 100 mL. The samples were mixed and sonicated in an ultrasound bath at 41°C for 90 min. The samples were then centrifuged at 5,000 rpm for 5 min. The supernatant was filtered into a round bottom flask and dried in an Eyela OSB210 rotary evaporator (Eyela, Tokyo, Japan). The extract was freeze-dried for four days in a Martin Christ Alpha freeze dryer (Martin Christ, Germany). Freeze-dried extracts were kept refrigerated at 4°C for further use.

Derivatization of BPE for GC-MS analysis

Bee pollen extracts were derivatized according to a previously published method (Graikou et al., 2011). Briefly, 5 mg of the BPE was mixed with 75 µL of BSTFA and 50 µL of pyridine before being heated at 80°C for 20 min. The derivatized extract was analyzed using GC-MS.

GC-MS conditions

The analyses were performed using an Agilent 7890A gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) equipped with an Agilent mass selective detector (MSD) 5975C. Chromatographic separations were performed on a HP-5 MS fused-silica capillary column (30 m × 0.25 mm I.D., 0.25 µm film thickness) with helium as the carrier gas at 1.0 mL min^{-1} in a constant flow rate mode. The injector was operated at 200°C, and the oven temperature was programmed from 100 to 300°C at a rate of 5°C/min (Graikou et al., 2011). Two microliters of the sample were injected in a split mode (1:20). The transfer line and the ion source temperatures were fixed at 280°C. The MSD was operated under the electron impact ionization mode. Scan mode was adopted to determine the retention time and the mass fragment fingerprint of the sample. Chromatographic data were processed using MSD Chemstation software (Agilent). Identification of the compounds was based on a comparison of mass spectra with those from the NIST 05 Mass Spectral (MS) Library. Only compounds with data matching quality > 80% are reported in this study. The abundance of the volatile compounds in the sample was calculated as a relative percentage of total peak area.

RESULTS

Compounds found in the BPE of each stingless bee species consisted of many chemical groups, with variations seen among bee species. Four major compounds with quality > 80% were detected in each species (Fig. 1, Tab. 1). All three species shared the same major compounds, glycerol and D-mannitol, and both *T. itama* and *T. thoracica* shared the compound xylitol. In addition, propanoic acid, D-fructose, D-glucopyranose and ribitol were the major compounds detected in different species.

Other compounds detected with quality > 80% were illustrated in Tab. 2-5. Analysis of the volatile compounds after derivation with BSTFA showed that compounds in the BPE from three species of Malaysian stingless bee consisted mainly of sugar and its derivatives (46.90-65.05%), hydrocarbon (0.44-5.73%), fatty acids (0.58-1.58%), amino acids (0.26-0.60%), alcohol (2.22-7.92%), aldehyde (0.18-1.39%), uridine (0.06-0.34%) and an unknown carbamate (0.21%).

Sugar and its derivatives constituted the majority of compounds detected in all species - 49.84% of total peak area in *T. apicalis*, 46.90% in *T. itama*, and 65.05% in *T. thoracica*. Mannitol had the biggest peak area in all species (33.05-54.34%), followed by fructose (7.17%), xylitol

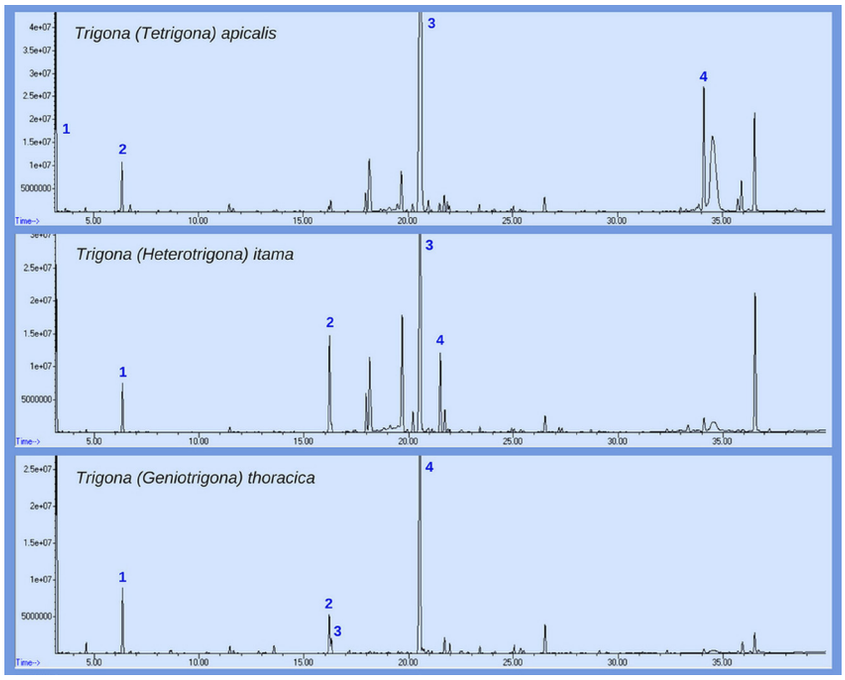

Fig. 1. Total ion chromatogram of *T. apicalis, T. itama*, and *T. thoracica* ethanolic extract. Identifications of numbered peaks are given in Tab. 1.

Table 1.
Major compounds identified from GC-MS of *T. apicalis*, *T. itama*, and *T. thoracica* ethanolic extracts. Peak numbers correspond to those given in the chromatogram shown in Figure 1. Identification of the compounds was based on the NIST 05 MS Library, and the data matching quality was > 80%

Peak No.	RT (min)	Major compounds identified in T. apicalis ethanolic extract	Area (%)	Quality (%)
1.	3.19	Propanoic acid, 2-[(trimethylsilyl)oxy]-, trimethylsilyl ester	4.04	91
2.	6.35	Trimethylsilyl ether of glycerol	2.22	91
3.	20.61	D-Mannitol, 1,2,3,4,5,6-hexakis-O-(trimethylsilyl)-	39.11	93
4.	34.14	D-Fructose, 1,3,4,5,6-pentakis-O-(trimethylsilyl)-	7.17	90
Peak No.	**RT (min)**	**Major compounds identified in *T. itama* ethanolic extract**	**Area (%)**	**Quality (%)**
1.	6.34	Trimethylsilyl ether of glycerol	2.98	91
2.	16.22	Xylitol, 1,2,3,4,5-pentakis-O-(trimethylsilyl)-	6.97	91
3.	20.56	D-Mannitol, 1,2,3,4,5,6-hexakis-O-(trimethylsilyl)-	33.05	87
4.	21.52	.beta.-D-Glucopyranose, 1,2,3,4,6-pentakis-O-(trimethylsilyl)-	5.14	95
Peak No.	**RT (min)**	**Major compounds identified in *T. thoracica* ethanolic extract**	**Area (%)**	**Quality (%)**
1.	6.34	Trimethylsilyl ether of glycerol	7.92	90
2.	16.21	Xylitol, 1,2,3,4,5-pentakis-O-(trimethylsilyl)-	4.97	93
3.	16.30	Ribitol, 1,2,3,4,5-pentakis-O-(trimethylsilyl)-	1.78	90
4.	20.54	D-Mannitol, 1,2,3,4,5,6-hexakis-O-(trimethylsilyl)-	54.34	87

Table 2.

Sugar compounds identified in BPE of Malaysian stingless bee by GC-MS

No	Sugar Compounds	RT (min)	Area (%)		
			T. apicalis	*T. itama*	*T. thoracica*
1	Arabinose, 2,3,4,5-tetrakis-O- [MW: 438.21]	14.53	0.08	0.09	ND
2	Xylitol, 1,2,3,4,5-pentakis-O- [MW: 512.27]	16.21	ND	6.97	4.97
3	Ribitol, 1,2,3,4,5-pentakis-O- [MW: 512.27]	16.30	0.81	ND	1.78
4	D-Ribonic acid, 2-C-methyl-2,3 [MW: 378.17]	17.12	0.07	ND	ND
5	Sorbopyranose, 1,2,3,4,5-pentakis-O- [MW: 540.26]	18.19	ND	ND	0.40
6	Galactonic acid, 2,3,5,6-tetrakis-O-(trimethylsilyl)-, lactone [MW: 466.21]	19.48	ND	ND	0.25
7	.beta.-D-Xylopyranose, 1,2,3,4-tetrakis-O-(trimethylsilyl) [MW: 438.21]	19.69	ND	ND	0.28
8	D-Galactose, 2,3,4,5,6-pentakis-O- [MW: 540.26]	19.96	0.11	0.22	0.26
9	Talose, 2,3,4,5,6-pentakis-O- [MW: 540.26]	19.94	ND	ND	0.19
10	D-Mannitol, 1,2,3,4,5,6-hexakis-O- [MW: 614.32]	20.55	39.11	33.05	54.34
11	Ether of glucitol [MW: 614.32]	20.65	ND	ND	0.57
12	Galactitol, 1,2,3,4,5,6-hexakis-O- [MW: 614.32]	20.73	ND	ND	0.52
13	Inositol, 1,2,3,4,5,6-hexakis-O, allo [MW: 612.30]	20.94	0.65	0.48	0.57
14	.beta.-D-Glucopyranose, 1,2,3,4,6-pentakis-O-(trimethylsilyl) [MW: 540.26]	21.51	0.41	5.14	ND
15	Myo-Inositol, 1,2,3,4,5,6-hexakis-O- [MW: 612.30]	23.40	0.37	0.40	0.92
16	Galactopyranose, 1,2,3,4,6-pentakis-O-(trimethylsilyl)-, .beta.-d [MW: 540.26]	24.43	0.05	ND	ND
17	D-Glucose, 2,3,4,5,6-pentakis-O [MW: 540.26]	27.18	ND	0.32	ND
18	.alpha.-D-Glucopyranoside, 1,3,4,6-tetrakis-O-(trimethylsilyl) [MW: 918.43]	32.71	0.04	ND	ND
19	Maltose, octakis [MW: 918.43]	33.03	0.20	ND	ND
20	D-Glucose, 4-O-[2,3,4,6-tetrakis-O-(trimethylsilyl)-.beta.-D-galactopyranosyl]- [MW: 918.43]	33.28	0.18	ND	ND
21	D-Altrose, 2,3,4,5,6-pentakis-O-[MW: 540.26]	33.91	0.59	ND	ND
22	D-Fructose, 1,3,4,5,6-pentakis-O [MW: 540.26]	34.14	7.17	ND	ND
23	D-Turanose, heptakis [MW: 846.39]	33.60	ND	0.23	ND
	Total Area (%)		49.84	46.90	65.05

(4.97-6.97%), glucopyranose (0.41-5.14%), ribitol (0.81-1.78%), myo-inositol (0.37-0.92%) and inositol (0.48-0.65%) (Tab. 2).

Table 3 showed eight hydrocarbon compounds with quality > 80% detected in all species and constituted about 5.73% of the total peak area in *T. apicalis*, 0.44% in *T. itama* and 1.89% in *T. thoracica*. Propanoic acid was the most abundant hydrocarbon detected in *T. apicalis* (4.04%), whereas hexadecanoic acid was the major compound presented in *T. thoracica* (1.28%).

Seven fatty acid compounds were detected, representing about 1.04% of total peak area in *T. apicalis*, 0.58% in *T. itama*, and 1.58% of total peak area in *T. thoracica*. α-linolenic acid (ALA) had the highest peak area (0.30-1.11%) followed by 9,12-octadecadienoic acid/linoleic acid isomer (0.16-0.28%) (Tab. 4).

Table 5 reported other compounds detected in the BPE which were amino acids, alcohol, aldehyde, uridine and an unknown carbamate. Only a small percentage of volatile amino

Table 3.

Hydrocarbon compounds identified in BPE of Malaysian stingless bee by GC-MS

No.	Hydrocarbon Compounds: Ester-carboxylic acid, Alkane, Alkene	RT (min)	Area (%)		
			T. apicalis	*T. itama*	*T. thoracica*
1.	Propanoic acid, 2-[(trimethyl silyl) oxy]-, trimethyl silyl ester [MW: 234.11] *Propionic acid	3.19	4.04	ND	ND
2.	Pentanoic acid, 4-methyl-2-[(trimethylsilyl) oxy]-, trimethyl silyl ester [MW: 276.16] *Valeric acid	5.58	ND	ND	0.13
3.	Benzenepropanoic acid, .alpha.-[(trimethylsilyl)oxy]-, trimethyl silyl ester [MW: 310.14] *Hydrocinnamic acid	12.84	ND	ND	0.29
4.	Mannonic acid, 2,3,4,6-tetrakis-O-(trimethyl silyl)-, lactone [MW: 466.21]	19.50	0.89	ND	ND
5.	D-Gluconic acid, 2,3,4,5,6-pentakis-O-(trimethylsilyl)-, trimethylsilyl ester [MW: 628.30] *Dextronic acid	21.88	0.48	0.24	ND
6.	Hexadecanoic acid, trimethyl silyl ester [MW: 328.28] *Palmitic acid	21.98	0.29	0.20	1.28
7.	Eicosen-1-ol, cis-9 [MW: 296.31]	27.73	ND	ND	0.19
8.	Heptacosane [MW: 380.44]	32.53	0.03	ND	ND
	Total Area (%)		5.73	0.44	1.89

Table 4.

Fatty acid compounds identified in BPE of Malaysian stingless bee by GC-MS

No.	Fatty Acid Compounds	RT (min)	Area (%)		
			T. apicalis	*T. itama*	*T. thoracica*
1.	Butanedioic acid, bis(trimethyl silyl) ester [MW: 262.11] *Succinic acid	6.98	0.05	ND	ND
2.	Butanoic acid, 4-[bis(trimethylsilyl) amino]-,trimethylsilyl ester [MW: 319.18] *Butyric acid	11.64	0.18	ND	ND
3.	Linoleic acid ethyl ester [MW: 308.27]	24.01	0.07	ND	ND
4.	9,12,15-Octadecatrienoic acid, ethyl ester MW: 306.26]	24.12	0.16	ND	0.27
5.	9,12-Octadecadienoic acid (Z,Z)-, trimethyl silyl ester [MW: 352.28] *Linoleic acid (Z,Z)-isomer	24.92	0.16	0.28	0.20
6.	.alpha.-linolenic acid, trimethyl silyl ester [MW: 350.27]	25.03	0.32	0.30	1.11
7.	Dodecanoic acid, tetradecyl ester [MW: 396.40] *Lauric acid	33.52	0.10	ND	ND
	Total Area (%)		1.04	0.58	1.58

Table 5.
Other compounds (amino acids, alcohol, uridine, aldehyde, and unknown carbamate) identified in BPE of Malaysian stingless bee by GC-MS

No.	Amino acid	RT (min)	Area (%)		
			T. apicalis	*T. itama*	*T. thoracica*
1.	L-Alanine, N-(trimethylsilyl)-, trimethylsilyl ester [MW: 233.13]	3.64	0.13	ND	ND
2.	L-Valine, N-(trimethylsilyl)-, trimethylsilyl ester [MW: 261.16]	5.28	0.04	ND	ND
3.	L-Proline, 1-(trimethylsilyl)-, trimethylsilyl ester [MW: 259.14]	6.71	ND	ND	0.42
4.	Trimethylsiloxy (trimethylsilyl) proline [MW: 275.14]	10.34	ND	ND	0.18
5.	L-Asparagine, N,N2-bis (trimethylsilyl)-, trimethylsilyl ester [MW: 348.17]	14.85	0.09	ND	ND
6.	Total Area (%)		0.26	ND	0.60
No.	**Alcohol**	**RT (min)**	***T. apicalis***	***T. itama***	***T. thoracica***
1.	Trimethylsilyl ether of glycerol [MW: 308.17]	6.34	2.22	2.98	7.92
No.	**Aldehyde**	**RT (min)**	***T. apicalis***	***T. itama***	***T. thoracica***
1.	3-Bromo-5-ethoxy-4-hydroxy benzaldehyde [MW: 243.97]	34.53	ND	0.18	1.39
No.	**Uridine**	**RT (min)**	***T. apicalis***	***T. itama***	***T. thoracica***
1.	Uridine, 2',3',5'-tris-O-(trimethylsilyl) [MW: 460.19]	29.12	0.06	0.09	0.34
No.	**Unknown Carbamate**	**RT (min)**	***T. apicalis***	***T. itama***	***T. thoracica***
1.	[4-Bromo-2-(hydrazono-phenyl-methyl)-phenyl]-carbamic acid, ethyl ester [MW: 361.04]	33.03	ND	0.21	ND

acids were detected in BPE of *T. apicalis* and *T. thoracica*. Amino acids in *T. apicalis* were composed of a few such compounds as L-alanine (0.13%), L-valine (0.04%) and L-asparagine (0.09%). L-proline and trimethylsiloxy proline were represented as 0.42% and 0.18% of total peak area in *T. thoracica.*

Glycerol was found abundantly in all BPE, with the highest peak area in *T. thoracica* (7.92%), followed by *T. itama* (2.98%) and *T. apicalis* (2.22%). Hydroxybenzaldehyde was found in *T. itama* (0.18%) and *T. thoracica* (1.39%) but not in the *T. apicalis* BPE. Uridine and carbamate were also detected in small portion of the BPE.

DISCUSSION

Variations of different chemical groups found in BPE showed that each bee species had different foraging activities that depended on geographical location and its preferences for flower types. Stingless bees are known to collect pollen from numerous types of flowers, and it was reported that the bee pollen collected from one species can represent > 30 flower species (Poolprasert,

2014).

Our bee pollen samples were collected from Syamille Agrofarm, a 12-acre farm that contains diverse flora, including *Cocos nullifera, Antigonan leptopus, Cuphea hyssofolia, Averrhoea bilimbi, Citrus microcarpa*, *Durio zibethinus*, and *Syzygium* spp., with a surrounding dipterocarp forest. The multitude of flower species in this agrofarm may be the source of a diverse group of volatile compounds, some of which might be useful for medicinal applications for humans.

The compounds detected in this study were mainly comprised of sugar and its derivatives, hydrocarbon, fatty acids, and other such compounds as amino acid, alcohol, aldehyde, uridine and carbamate. A previous GC-MS study of the methanolic extract of bee pollen from the honeybee also showed that it was rich in sugar, fatty acids, fatty acid esters and phenolic compounds (Graikou et al., 2011).

Mannitol had the biggest peak area in *T. apicalis*, *T. itama*, and *T. thoracica*. Mannitol had been reported to be the most common sugar compound found in the methanolic BPE of the honeybee (Graikou et al., 2011). A high mannitol content (34.9% of dry weight) was also found in bee pollen from the Brazilian stingless bee *Melipona subnitida* (Silva et al., 2006). The activity of enzymes in stingless bee pollen has been suggested to convert sugar compounds to mannitol regardless of the nectar sources (Silva et al., 2014). Mannitol is generally used in food products for diabetics because of its low-calorie sugar characteristic.

Other such common sugars as galactose, myo-inositol and inositol were present in small amounts (0.11-0.92% of total peak area) in BPE from all three species. Galactose, a monosaccharide, is crucial for human metabolism and plays a role in energy delivery and galactosylation of complex molecules (modification of protein and cell signalling function) (Coelho, Berry, & Rubio-Gozalbo, 2015).

Inositol and its isomer, myoinositol (commonly occurs in nature), is a sugar alcohol. Inositol is important in physiological regulation. Deregulated inositol metabolism occurs in cancer (Vucenik & Shamsuddin, 2003). Inositol in the form of inositol hexakisphosphate was shown to inhibit growth and invasiveness of several types of cancers. Myoinositol synergized with inositol hexakisphosphate was found to exert a strong chemopreventive effect (Unfer et al., 2017).

Eight hydrocarbon compounds were detected in BPE, where propanoic acid was the most abundant hydrocarbon presented in *T. apicalis* and hexadecanoic acid was the major compound presented in *T. thoracica.*

Propanoic acid has been shown to enhance the colonic barrier function, exert immunosuppressive action, have anti-lipid activity and improve insulin sensitivity. These effects are beneficial in the prevention of obesity and diabetes type 2 (Al-Lahham et al., 2010; Xia et al., 2017).

Aparna et al. (2012) reported that hexadecanoic acid could inhibit phospholipase A2, which is a precursor of inflammation (Aparna et al., 2012). In addition, the hexadecanoic acid in the form of monoacyl glycerol-palmitic acid was shown to have antioxidant, antidiabetic and anticholesterol activities (Feltes et al., 2013).

All three stingless bee species were also composed of fatty acid compounds. α-linolenic acid (ALA) had the highest peak area followed by 9,12-octadecadienoic acid/linoleic acid isomer. Both of them were also commonly found in honey bee pollen (Graikou et al., 2011).

ALA is an omega-3 fatty acid, which is important for bee colony hygiene due to its bactericidal and antifungal properties (Manning, 2001). High consumption of ALA in humans was able to prevent cardiovascular diseases and strokes (Connor, 1999; Hadjighassem et al., 2015). In addition, ALA supplementation in the diet of diabetic patients was shown to improve metabolic profiles and insulin sensitivity (Gomes et al., 2015).

9,12-octadecadienoic acid/linoleic acid isomer was also present in small amounts in all three BPEs (Tab. 2(c)). Linoleic acid is a polyunsaturated omega-6 fatty acid and involved in many physiological functions, including inflammation and wound healing. Rodrigues et al. (2016) reported that linoleic acid enhanced wound closure in diabetic rats through the regulation of the inflammatory phase and angiogenesis.

The amino acid compounds - alanine, valine and asparagine were detected in *T. apicalis* and

only one - proline and its isomer, trimethylsiloxy proline was detected in *T. thoracica* species. A high-performance liquid chromatography analysis revealed that the bee pollen from *M. subnitida* contained seventeen amino acids, including all of the essential amino acids except for tryptophan; proline and serine were the predominant amino acids (Silva et al., 2014).

Glycerol was found abundantly in all BPE, with the highest peak area in *T. thoracica*. Glycerol, a humectant, can reduce water activity when added to the diet. In humans, glycerol also provides energy via lipolysis metabolism under energy-demanding conditions (Ahmadian et al., 2007).

Hydroxybenzaldehyde was found in *T. itama* and *T. thoracica* BPE but not in the *T. apicalis* BPE. In the rat model of epilepsy, hydroxybenzaldehyde in the form of 4-hydroxybenzaldehyde exhibited strong positive modulation of the GABAergic receptor, which may contribute to antiepileptic and anticonvulsive activity (Ha et al., 2000). Koong et al. (2016) showed that 4-hydroxybenzaldehyde had vasculoprotective activity by inhibiting inflammatory markers and signalling molecules. They also reported that it could prevent angiogenesis formation in a carotid artery balloon injury of the Sprague Dawley rat model of induced thrombus generation through increased blood circulation after the formation of the thrombus and attenuated neointima formation (Kong et al., 2016).

The volatile compounds present in bee pollen may derive from such various sources as plant nectar, compounds transformed or generated by bees, bee-pollen processing, storage and contamination through the environment or microbes (Igor Jerković, 2010). Although the variability of bee pollen compounds depends on floral and geographical origin (Kaškonienė, Venskutonis, & Čeksterytė, 2008), the isolation and detection techniques of volatiles may also influence the results (Nurul Syazana et al., 2013). These factors may yield different volatile compounds than those seen in our study and others. One limitation of our study was that the GC-MS peaks were identified through direct comparison of the mass spectra of volatile compounds obtained with those reported in the NIST 05 MS Library, and no internal standards were included in the analyses.

As a conclusion, the volatile compounds found in Malaysian BPE from stingless bee species were diverse and consisted of sugar compounds, hydrocarbons, fatty acids, amino acids, alcohol, aldehyde, uridine and an unknown carbamate. Sugar and its derivatives were a major component of the ethanolic BPE, along with hydrocarbons and fatty acids. The chemical compounds found in BPE have biological activities, so bee pollen may be useful in traditional medicine and as a health supplement.

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