The Innovative Color of Emerald and Dark Violet Honeys from Stingless Bee: A Comparative of Chemical Profile, Physicochemical and Biological Properties

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Abstract

This research is aimed to report for the first discovery of emerald honey (green honey) from stingless bee in Narathiwat Province, Thailand. The objective of this research was to study the chemical composition of emerald honey and dark violet honey, as well as the physicochemical and biological properties of 4 species of the stingless bee from Narathiwat Province, namely *G. thoracica*, *H. itama*, *H. bakari* and *T. laeviceps*. The typical characteristic of the emerald honey is the color of the honey in the honey pots (cerumen pots) of the stingless bee raised in the *M. cajuputi* forest is green like the color of the emeralds. The intensity of the shade ranges from yellowish-green to dark green. The results analysis of LC-QTOF MS and GC MS found that the emerald honey contained of 195 important substances and 2 substances are particular interest comprised of Embelin and Lumichrome. And it has been previously reported to have properties to treat neurological conditions such as Alzheimer's disease, Parkinson's disease, and also many types of cancer. For the 16 stingless bee honey samples, total phenolic compound and DPPH radical scavenging activity at IC_{50} falling in the ranged of 45.20±2.80 to 165.27±0.23 mg GAE/100 g and 9.50±0.39 to 50.61±1.55 mg/mL, respectively. Glycemic index values were falling into the low to moderate range, while sugar profile showed low levels of glucose and maltose but the sucrose has a high percentage. The next research will be to isolate the valuable substances from the emerald stingless bee honey and the medical investigation purposes.

Keywords: Stingless bee honey, Emerald honey, Dark violet honey, LC-QTOF MS, LC/MS

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Introduction

Stingless bees are eusocial insects that do not have stings. They are also placed in the order of Hymenoptera, family Apidae, and subfamily Meliponinae, which are found in the tropical and subtropical regions of the world. Currently, more than 500 species of stingless bees are found in 32 genera around the world that distributed throughout the Neotropical, Afrotropical, and Indo-Australian areas (Eardley, 2004; Camargo & Pedro, 2007 & Michener, 2007). Stingless bee honey has different chemical and physical properties from general honey, such as taste, odor, color, texture and coagulation due to the process of chemical modification of flower nectar by enzymes from the salivary and cephalic glands in the abdomens of bees (Abd et al., 2017; Ávila et al., 2018).

Stingless bee honey has been used as an ingredient in traditional medicine for a long time from the past to the present. Many studies have reported the properties of stingless bee honey and found that it has various pharmacological properties such as antioxidant, antiaging, anti-inflammatory, anti-obesity, antidiabetic, anticancer, antiproliferative, antibacterial, antiviral, antimicrobial, improves memory and learning, reduces anxiety. Improve wound healing and improves fertility (Arshad et al., 2020; Biluca et al., 2020; Mustafa et al., 2019). Rich in phenolic compounds, triterpenes, amino acids, proteins, enzymes, carotenoids, organic acids, fatty acid, (Ávila et al., 2018; Chuttong et al., 2016). Phenolic compounds (phenolic acids and flavonoids) are the secondary metabolite obtained from plants.

The bees collected and transported these compounds from nectar, pollen, and propolis into the honeycomb. Phenolic compounds are the most important antioxidants in honey, which have therapeutic properties for human diseases in both traditional and modern medicine. Honey has been used to treat diseases related to oxidative stress, diabetes mellitus, hypertension, atherosclerosis, cancer, and Alzheimer's disease.

Material and Method

1. Identification of bioactive compounds in emerald and dark violet stingless bee honey

1.1 sample

Emerald honey of *Heterotrigona itama* from Muang District, Narathiwat Province (6°26'29.0"N 101°47'02.4"E), while dark violet honey of *Geneotrigona thoracica* from Thanto District, Yala Province (6°09'39.9"N 101°11'25.7"E) has been collected as shown in Figure 1. And collected nectar samples from *Melaleuca cajuputi* flowers in October 2023.

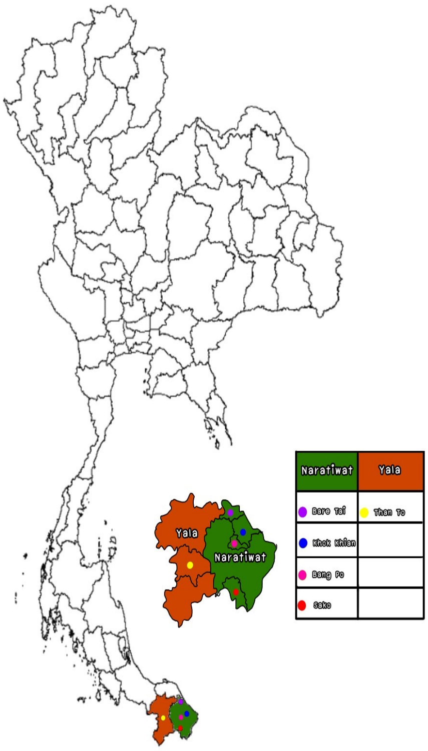

Figure 1. Location of Narathiwat and Yala districts, Thailand where the honey samples were collected from stingless bee beehives.

1.2 Extraction of bioactive compounds

Extraction of crude propolis honey using Thongsaiklaing et al. (2024) method by diluted propolis honey with water in the ratio of 1:1 (V/V), shaken to mix and then added ethyl acetate in the ratio of 1:1 (V/V), shook vigorously and then extracted by immersing in an Ultrasound cleaning bath (BANDELIN, SONOREX DIGITEC) using the frequency of 37 kHz at 40 ± 3 °C with 15 min 3 times. Then centrifuged at 6,000 rpm for 10 minutes, collecting and separating the fraction of ethyl acetate.

The remaining honey was extracted twice according to the same procedure. The ethyl acetate obtained from the three extractions was combined and evaporated to remove the ethyl acetate using a vacuum evaporator, and the extracted substance was stored at -20 °C.

1.3 Liquid chromatograph quadrupole time-of-flight mass spectrometer

Analysis of bioactive compounds using LC-QTOF MS according to the method of Thongsaiklaing et al. (2024) LC-QTOF MS, 1290 Infinity II LC-6545 Quadrupole-TOF (Agilent Technologies, USA) LC-6545 Quadrupole-TOF (Agilent Technologies, USA). The column used is UHPLC type Agilent: Zorbax Eclipse Plus C18 Rapid Resolution HD 100 mm length % acetic acid as mobile phase A and methanol as mobile phase B was employed: 0 min, 10% B; 35 min, 90% B; 40 min, 10% B; 50 min, 10% B. The data were then analyzed for negative and positive ionization mode using Mass Hunter WorkStation Software Qualitative Analysis Work flows V8, then identified and confirmed by comparison of their retention times and mass spectra with a Mass Hunter METLIN PCD and reference compounds.

1.4 Gas chromatograph mass spectrometer

Analysis of volatile composition of stingless bee honey by GC MS, GC Parameters Injection Volume 1 µL Inlet temperature 250 °C Injection mode Split Ratio 10 :1 Column HP-5ms Ultra Inert, 30 m x 250 µm x0.25 µm (Agilent Technologies) Oven Temperature Initial temp: 70 °C (hold time 2 min), rate 3 °C/min to 200 °C (hold time 20 min) Transfer Line Temperature 230 °C, Oven Temperature Initial temp: 70 °C (hold time 2 min), rate 3 °C/min to 200 °C (hold time 20 min) Transfer Line Temperature 230 °C Acquisition Type Scan Mass range m/z 35-500, Software control: MassHunter GC/MS Acquisition B.07.06.2704, and Data Analysis software: MassHunter Workstation Software Quantitative Analysis Version B.09.00 Unknown Analysis.

2. Analysis of physicochemical and biological properties

2.1 Sample

Four species of stingless bee honey were collected, namely *H. itama, H. bakari, G. thoracica* and *Tetragonula laeviceps*. Stingless bee honey from 3 districts were collected, namely Mueang district, 2 sub-districts, namely Khok khian (6°26’29.0”N 101°47’02.4”E), Bangpor subdistrict (6.342995, 101.823279), Sungai Padi district, Sako subdistrict (6.012368, 101.844945), and Bacho district, Barehtai subdistrict (6.517939, 101.712103) as shown in Figure 1.

2.2 Analysis of phenolic compounds and IC_{50} of antioxidant activity

The total phenolic compounds were determined according to the spectrophotometer methods (Total phenolic compound quantification by colorimetric assay) that was described by Bozin et al. (2008). The antioxidant activity was determined according to the methods of DPPH (2,2-diphenyl-1-picrylhydrazyl radical scavenging activity analysis) express the value of free radical reduction activity as IC_{50} values. That was described by van Amsterdam et al. (1992) with modification.

2.3 HPLC analysis of sugar profile

The sugar profile was determined by using performance liquid chromatography: HPLC (Column: VertiSepTM CMP, Germany). The experiment was repeated in each of 3 trials.

2.4 Physicochemical properties

The total solid content was measured using a refractometer (ATAGO, since 1940, Tokyo). The pH was measured by using the pH meter (LAQUAtwin-ph-33, Horiba Scientific, Japan). The color value was measured by using HunterLab ColourQuest XE colourimeter (ColourQuest XE spectrophotometer, Hunter Lab, USA). All experiments were repeated in three replications.

2.5 Analysis of estimated glycemic index (eGI)

The eGI was determined according to the method of Sopade and Gidley, (2009). The glucose concentration in the digesta was measured with an PGO enzyme assay (P7119, Sigma-Aldrich, USA). The eGI value was calculated according to the equation of Goni et al. (1997) as follow: eGI = 39.71+ 0.549HI (HI = Hydrolysis index).

Results and discussions

1. From the survey of stingless bee honey of *H. itama* and *G. thoracica* raised in the *M. cajuputi* forest area of Bacho swamp forest during September-November 2023.

Figure 2 represented the period *M. cajuputi* flowers blooming and found that the stingless bee honey obtained has a special characteristic of clearly visible in the honey collection pots (cerumen pots) which is green like the color of emera

The intensity of the shade ranges from yellowish green to dark green and the smell is similar to the smell of the *M. cajuputi*'s flowers. But when sucking the bee honey out of cerumen pots, it was found that the green color of the honey was yellowish-green and the taste of the bee honey was found to be sweet, sour, and slightly bitter. And the test result of synthetic dyes Allura Red, Amaranth, Brilliant Blue Carmoisine, Erythrosine, Fast Green, Indigo Carmine, Ponceau 4R, Quinoline Yellow, Tartrazine and Arsenic (As) in the emerald honey, it was found that there was no contamination of the synthetic dyes. The dark violet honey obtained from the bee strain *G. thoracica* as shown in Figure 3, has a pungent smell and a very sour taste. And the variation of the color of the stingless bee honey depending on its geographic origin. Conversely, the production and agricultural cultivation practice can also affect the color of the bee honey (Esa et al., 2022). The color of stingless bee honey generally ranges from pale amber to amber brown (Nordin et al., 2018), and this characteristic can be affected by factors such as exposure to light, heat, and storage time, as well as enzymatic reactions (de Sousa et al., 2016).

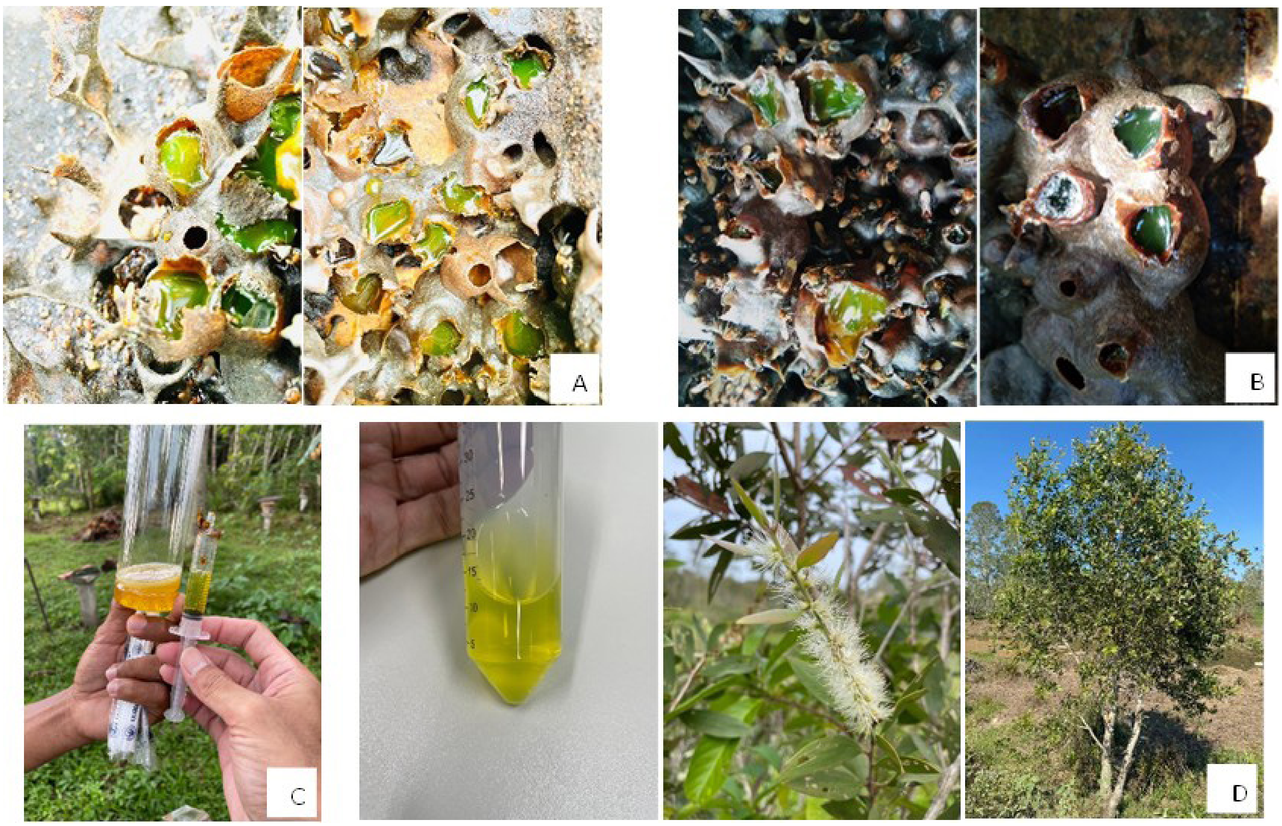

Figure 2. Emerald honey and nectar from *M. cajuputi*'s flowers in the Bacho swamp forest area (A) *H. itama* (B) *G. thoracica* (C), Emerald stingless bee honey in comparison with regular stingless bee honey (D) *M. cajuputi* flower nectar, *M. cajuputi* flower และ *M. cajuputi* tree

Figure 3 Dark violet honey

2. LC-QTOF-MS analysis

2.1 Chemical profiles comparison between emerald honey and *M. cajuputi* flower nectar

From the analysis of bioactive compounds in emerald honey and *M. cajuputi* flower nectar using LC-QTOF-MS and GC-MS as shown in Table 1. It was found that emerald honey contains 156 bioactive compounds while *M. cajuputi* flower nectar contains 89. When comparing the bioactive compounds, 24 of them were found to be directly related, namely: Galactonic acid, Sucrose, L-Phenylalanine, (Z)-2-Methyl-2-butene-1,4-diol 4-O-beta-D-Glucopyranoside, D-Vacciniin, 3-Hydroxycapric acid, (S)-(-)-2-Hydroxyisocaproic acid, L-Cladinose, Ala-Arg-Glu, 2-Methylhippuric acid, D-(+)-3-Phenyllactic acid, 2-Phenylethyl beta-D-glucopyranoside, Etrogol, N-Acetyl-L-phenylalanine, Mono-N-depropylprobenecid, 2,6-Dimethyl-6-O-beta-D-quinovopyranosyl-7-octadecenoic acid, Lumichrome, Embelin, Luteolin, PI(22:1(11Z)/18:3(9Z,12Z,15Z)), C16 Sphinganine, Xestoaminol C, 2-Hydroxyhexadecanoic acid, Phytosphingosine and Prosopinine. And the analysis of volatile compounds by GC-MS has been found that the emerald honey contains 39 important compounds, while in *M. cajuputi* flower nectar 8 were found and 3 were found to be consistent of Benzeneacetaldehyde, Benzeneethanol, 4H-Pyran-4-one and 2,3-dihydro-3,5-dihydroxy-6-methyl-). And 2 newly typical interested compounds have been found and presented in emerald honey composted of Embelin and Lumichrome.

Embelin is a class of benzoquinone compounds. Pharmacological properties of embelin extracted from *Embelia ribes* Burm seeds have been reported to include anti-inflammatory, antioxidant, analgesic, antitumor, wound healing, hepatoprotective, antibacterial activities (Danquah, 2022; Kamath et al., 2023). In addition, from recent research reports, this compound has neuroprotective properties. It has the potential to be used to treat neurological diseases, especially Alzheimer's disease (Bhuvanendran et al., 2019a; Bhuvanendran et al., 2019b; Bhuvanendran et al., 2022; Goal et al., 2024) and Parkinson's diseases (Jain, 2020; Koppal et al., 2021; Ramachandra et al., 2022).

Lumichrome is a derivative of vitamin B12 or riboflavin that has been shown to have anti-cancer properties against several cancers including lung cancer (Chantarawonga et al., 2019; Etua et al., 2021), colorectal cancer, liver carcinoma, breast adenocarcinoma (Etu et al., 2021; Robledo & Muhammad, 2021) and antioxidant. In addition, Liu et al. (2018) found that lumichrome has potential as a therapeutic agent for the treatment of osteoporosis. Ahmad et al. (2019) reported that Malaysian stingless bee honey from *H. itama* exhibited antiproliferation and early apoptosis activity in malignant glioma cell lines (U-87 MG cells), while Arung et al. (2021) reported that stingless bee honey from *Homotrigona fimbriata*, *H. itama*, *H. bakeri*, *Tetragonula* *sarawakensis*, *T. testaceitarsis*, *T. fuscobalteata* and *T. laeviceps* exhibited cytotoxic effect in MCF-7, HeLa and Caco-2 cancer cell lines.

2.2 Chemical profiles of dark violet stingless bee honey

The dark violet stingless bee honey from the LC-QTOF-MS test results showed that this type of honey contains 60 important compounds as shown in Table 2 which is contains the compounds that were previously reported and many new compounds. Most of the substances are phenolic flavonoid compounds and organic acids.

3. Analysis of volatile compounds profile in honeys between emerald honey and *M. cajuputi* flower nectar

From the analysis results of the volatile compound using GC MS found that in the emerald honey and in the nectar of *M. cajuputi* flower, 39 and 8 types of volatile compounds were found, respectively (Table 1). The comparison of the volatile compounds found that there were 3 types that matched, namely Benzeneacetaldehyde, Benzeneethanol and 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- volatile organic compounds in the stingless bee honey have a significant impact on the organoleptic properties of honey such as aroma, flavor, taste and texture. The type and composition of volatile organic compounds are influenced by entomological, geographical and botanical origin. Thus, these compounds have the potential to be chemical markers. Manickavasagam et al., (2024) and da Silva et al. (2017) had been analyzed the composition of the volatile compounds of 8 stingless bee honey from 3 regions in Brazil and found to contain 44 volatile compounds, consisting of monoterpenes, sesquiterpenes, alcohols, esters, ketones and aldehydes and also concluded that the volatile profiles of stingless bee honeys can be used to confirm the regional origin of honey. Manickavasagam et al. (2024) identified 62 volatile compounds from 30 *H. itama* honey samples from 3 different geographic origins, most of which belonged to the hydrocarbon and benzene derivatives classes. They concluded that the volatile organic compounds of stingless bee honeys originating from the same species but different geographic origins can be used as chemical markers.

Table 1 Chemical profile of emerald stingless bee honey from *H. itama* and *M. cajuputi* flower nectar

	LC MS/MS								GC MS			
No	Emerald honey				*Melaleuca cajuputi* flower nectar				Emerald honey		*M. cajuputi flower nectar*	
	Negative mode		Positive mode		Negative mode		Positive mode					
	Name	Formular	Name	Formular	Name	Formular	Name	Formular	Name	Formular	Name	Formular
1	Galactonic acid	$C_6H_{12}O_7$	Mebeverine metabolite (Veratric acid glucuronide)	$C_{15}H_{18}O_{10}$	Stearic acid	$C_{18}H_{36}O_2$	D-Fructofuranose 1,2':2,3'-dianhydride	$C_{12}H_{20}O_{10}$	1,2,3-Propanetriol	$C_3H_8O_3$	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	$C_6H_8O_4$
2	Pyroglutamic acid	$C_5H_7NO_3$	Sucrose	$C_{12}H_{22}O_{11}$	D-Glucuronic acid	$C_6H_{10}O_7$	Sucrose	$C_{12}H_{22}O_{11}$	4,5-Octanediol, 2,7-dimethyl-	$C_{10}H_{22}O_2$	2H-Pyran-2,6(3H)-dione	$C_5H_4O_3$
3	F-Honaucin A	$C_8H_9FO_4$	6-Acetyl-D-glucose	$C_8H_{14}O_7$	Citbismine C	$C_{37}H_{36}N_2O_{11}$	Dihydrocaffeic acid 3-O-glucuronide	$C_{15}H_{18}O_{10}$	4,5-Octanediol, 2,7-dimethyl-	$C_{10}H_{22}O_2$	Benzeneacetaldehyde	C_8H_8O
4	Deoxyuridine monophosphate (dUMP)	$C_9H_{13}N_2O_8P$	alpha,beta-Trehalose	$C_{12}H_{22}O_{11}$	D-Mannonate	$C_6H_{12}O_7$	Trigonelline	$C_7H_8NO_2$	Benzeneacetaldehyde	C_8H_8O	2-Furoic acid, anhydride with acetic acid	$C_7H_6O_4$
5	2,3-Dihydroxy-2-methylbutanoic acid	$C_5H_{10}O_4$	Flonicamid	$C_9H_6F_3N_3O$	Sucrose	$C_{12}H_{22}O_{11}$	Sedoheptulose	$C_7H_{14}O_7$	2,5-Hexanedione, 3,4-dihydroxy-3,4-dimethyl-	$C_8H_{14}O_4$	Benzeneethanol	$C_8H_{10}O$
6	(2-Methoxyethoxy)propanoic acid	$C_6H_{12}O_4$	Dambonitol	$C_8H_{16}O_6$	2,3-Dihydroxy-3-methylbutyric acid	$C_5H_{10}O_4$	Hexandraside E	$C3_2H_{38}O_{16}$	1,2-Heptanediol	$C_7H_{16}O_2$	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$
7	L-Phenylalanine	$C_9H_{11}NO_2$	Debromohymenialdisine	$C_{11}H_{11}N_5O_2$	Aminocaproic acid	$C_6H_{13}NO_2$	L-Leucine	$C_6H_{13}NO_2$	2,5-Hexanedione, 3,4-dihydroxy-3,4-dimethyl-	$C_8H_{14}O_4$	5-Hydroxymethylfurfural	$C_6H_6O_3$
8	Aspartyl-Asparagine	$C_8H_{13}N_3O_6$	Kynurenic acid	$C_{10}H_7NO_3$	Galactonic acid	$C_6H_{12}O_7$	Methyl 1-(propylsulfinyl) propyl disulfide	$C_7H_{16}OS_3$	2-Furanmethanol, 5-ethenyltetrahydro-.alpha.,.alpha.,5-trimethyl-, cis-	$C_{10}H_{18}O_2$	n-Hexadecanoic acid	$C_{16}H_{32}O_2$
7	Gly Tyr Asn	$C_{15}H_{20}N_4O_6$	(1S,2S,4R,8R)-p-Menthane-1,2,8,9-tetrol	$C_{10}H_{20}O_4$	1,6-Anhydro-N-acetyl-beta-muramate	$C_{11}H_{17}NO_7$	Pirimicarb	$C_{11}H_{18}N_4O_2$	2-Furancarboxylic acid, methyl ester	$C_6H_6O_3$	Octadec-9-enoic acid	$C1_8H_{34}O_2$
8	L-α-Hydroxyisovaleric acid	$C_5H_{10}O_3$	(Z)-2-Methyl-2-butene-1,4-diol 4-O-beta-D-Glucopyranoside	$C_{11}H_{20}O_7$	L-Phenylalanine	$C_9H_{11}NO_2$	1-(5-Ketohexyl)-3-methylxanthine	$C_{12}H_{16}N_4O_3$	trans-Linalool oxide (furanoid)	$C_{10}H_{18}O_2$	1-Heptadecanecarboxylic acid	$C_{18}H_{36}O_2$
9	1H-Imidazole-4-carboxamide, 5-[3-(hydroxymethyl)-3-methyl-1-triazenyl]-	$C_6H_{10}N_6O_2$	(1S,2S,4R,8R)-p-Menthane-1,2,8,9-tetrol	$C_{10}H_{20}O_4$			D-Vacciniin	$C_{13}H_{16}O_7$	acetin, mixture of 2 isomers	$C_5H_{10}O_4$		

Table 1 (Continued)

No	LC MS/MS								GC MS			
	Emerald honey				*Melaleuca cajuputi* flower nectar				Emerald honey		*M. cajuputi flower nectar*	
	Negative mode		Positive mode		Negative mode		Positive mode					
	Name	Formular	Name	Formular	Name	Formular	Name	Formular	Name	Formular	Name	Formular
10	Hydroxyphenyllactic acid	$C_{9}H_{10}O_{4}$	3-Hydroxycapric acid	$C_{10}H_{20}O_{3}$	Trp Glu Gln	$C_{21}H_{27}N_{5}O_{7}$	Idebenone Metabolite (Benzenebutanoic acid, 2,5-dihydroxy-3,4-dimethoxy-6-methyl-)	$C_{13}H_{18}O_{6}$	Benzeneethanol	C8H10O		
11	L-α-Hydroxyisovaleric acid	$C_{5}H_{10}O_{3}$	His Gly Ser	$C_{11}H_{17}N_{5}O_{5}$	N-Methylisoleucine	$C_{7}H_{15}NO_{2}$	Arg Ser Ser	$C_{12}H_{24}N_{6}O_{6}$	.alpha.-Hydroxyisocaproic acid	$C_{6}H_{12}O_{3}$		
12	2-[4-(3-Hydroxypropyl)-2-methoxyphenoxy]-1,3-propanediol	$C_{13}H_{20}O_{5}$	Nelarabine	$C_{11}H_{15}N_{5}O_{5}$	(S)-(-)-2-Hydroxyisocaproic acid	$C_{6}H_{12}O_{3}$	(Z)-2-Methyl-2-butene-1,4-diol 4-O-beta-D-Glucopyranoside	$C_{11}H_{20}O_{7}$	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_{6}H_{8}O_{4}$		
13	α-Cyano-3-Hydroxycinnamic Acid	$C_{10}H_{7}NO_{3}$	3-Methoxy-4-Hydroxyphenylglycol Sulfate	$C_{9}H_{12}O_{7}S$	L-Cladinose	$C_{8}H_{16}O_{4}$	2-O-Acetylarbutin	$C_{14}H_{18}O_{8}$	Benzoic acid	$C_{7}H_{6}O_{2}$		
14	2-O-p-Coumaroylhydroxycitric acid	$C_{15}H_{14}O_{10}$	PGDM	$C_{16}H_{24}O_{7}$	Ala Arg Glu	$C_{14}H_{26}N_{6}O_{6}$	Salidroside	$C_{14}H_{20}O_{7}$	4H-Pyran-4-one, 3,5-dihydroxy-2-methyl	$C_{6}H_{6}O_{4}$		
15	α-Cyano-3-Hydroxycinnamic Acid	$C_{10}H_{7}NO_{3}$	6-Hydroxy-4-decanolide	$C_{10}H_{18}O_{3}$	Ile-His-OH	$C_{17}H_{20}N_{4}O_{6}$	3-Hydroxycapric acid	$C_{10}H_{20}O_{3}$	5-Hydroxymethylfurfural	$C_{6}H_{6}O_{3}$		
16	3-Ethenyl-4-hydroxy-2,5-dimethylhex-5-en-2-yl acetate	$C_{12}H_{20}O_{3}$	Picrasin G	$C_{21}H_{28}O_{7}$	2-Methylhippuric acid	$C_{10}H_{11}NO_{3}$	Asp Asn Lys	$C_{14}H_{25}N_{5}O_{7}$	Ethanone, 2-hydroxy-1-phenyl-	$C_{8}H_{8}O_{2}$		
17	cis-3-Hexenyl pyruvate	$C_{9}H_{14}O_{3}$	Gibberellin A98	$C_{20}H_{26}O_{6}$	D-(+)-3-Phenyllactic acid	$C_{9}H_{10}O_{3}$	2-Phenylethyl beta-D-glucopyranoside	$C1_{4}H_{20}O_{6}$	Benzeneacetic acid	$C_{8}H_{8}O_{2}$		
18	Macrocarposide	$C_{21}H_{22}O_{11}$	7-Hydroxyheptaphylline	$C_{18}H_{17}NO_{3}$	3-(2,5-Dimethoxyphenyl)propanoic acid	$C_{11}H_{14}O_{4}$	Riboflavin (Vitamin B2)	$C_{17}H_{20}N_{4}O_{6}$	Epoxy-linalooloxide	$C_{10}H_{18}O_{3}$		
19	Anisole	$C_{7}H_{8}O$	Etrogol	$C_{13}H_{18}O_{2}$	S-Propyl-L-cysteine	$C_{6}H_{13}NO_{2}S$	Picraquassioside A	$C_{18}H_{22}O_{10}$	1,2-Ethanediol, 1-phenyl-	$C_{8}H_{10}O_{2}$		
20	Fraxetin	$C_{10}H_{8}O_{5}$	Lumichrome	$C_{12}H_{10}N_{4}O_{2}$	S-Propyl-L-cysteine	$C_{6}H_{13}NO_{2}S$	Balsalazide	$C_{17}H_{15}N_{3}O_{6}$	Phenol, 2,3,5-trimethyl-	$C_{9}H_{12}O$		
21	Fraxetin	$C_{10}H_{8}O_{5}$	Junipegenin B 7-O-glucoside	$C_{24}H_{26}O_{12}$	N-Acetyl-L-phenylalanine	$C_{11}H_{13}NO_{3}$	Gardoside	$C_{16}H_{22}O_{10}$	2,5-Dimethoxythiophenol	$C_{8}H_{10}O_{2}S$		
22	2E,6E,8E-Decatrienoic acid	$C_{10}H_{14}$O2	2,6-Dimethyl-6-O-beta-D-quinovopyranosyl-7-octadecenoic acid	$C_{16}H_{28}O_{7}$	Mono-N-depropylprobenecid	$C_{10}H_{13}NO_{4}S$	His Gly Ser	$C_{11}H_{17}N_{5}O_{5}$	3-hydroxy-4-phenyl-2-butanone	$C_{10}H_{12}O_{2}$		

Table 1 (Continued)

No	LC MS/MS								GC MS			
	Emerald honey				*Melaleuca cajuputi* flower nectar				Emerald honey		*M. cajuputi flower nectar*	
	Negative mode		Positive mode		Negative mode		Positive mode					
	Name	Formular	Name	Formular	Name	Formular	Name	Formular	Name	Formular	Name	Formular
23	butoxyacetic acid	$C_6H_{12}O_3$	Naftifine	$C_{21}H_{21}N$	Caprylic acid	$C_8H_{16}O_2$	2-Phenylethyl beta-D-glucopyranoside	$C_{14}H_{20}O_6$	.alpha.-Methyl-.alpha.-[4-methyl-3-pentenyl]oxiranemethanol	$C_{10}H_{18}O_2$		
24	L-Cladinose	$C_8H_{16}O_4$	4,5-Dihydrovomifoliol	$C_{13}H_{22}O_3$	3-Hydroxyisoheptanoic acid	$C_7H_{14}O_3$	3-O-(alpha-L-rhamnopyranosyl-(1-2)-alpha-L-rhamnopyranosyl)-3-hydroxydecanoic acid	$C_{22}H_{40}O_{11}$	trans-Linalool oxide (furanoid)	$C_{10}H_{18}O_2$		
25	Ala Arg Glu	$C_{14}H_{26}N_6O_6$	Visnadin	$C_{21}H_{24}O_7$	Lumichrome	$C_{12}H_{10}N_4O_2$	Lonicerin	$C_{21}H_{26}N_2O_4$	Benzeneethanol, .alpha.-(phenylmethyl)-	$C_{15}H_{16}O$		
26	(3E,8E)-suspensolide	$C_{10}H_{14}O_2$	5’-Deoxy-5’-fluoroadenosine	$C_{10}H_{12}FN_5O_3$	3-Hydroxymethyltriazolopthalazinone	$C_{11}H_9N_3O$	Pentyl heptanoate	$C_{12}H_{24}O_2$	Benzenepropanoic acid, .alpha.-hydroxy-, (S)-	$C_9H_{10}O_3$		
27	Tuberonic acid	$C_{12}H_{18}O_4$	(7’x,8’x)-4,7’-Epoxy-3,8’-bilign-7-ene-3,5’-dimethoxy-4’,9,9’-triol	$C_{20}H_{22}O_6$	Botrydial	$C_{17}H_{26}O_5$	dodecanamide	$C_{12}H_{25}NO$	2-Cyclohexen-1-one, 4-(3-hydroxy-1-butenyl)-3,5,5-trimethyl-	$C_{13}H_{20}O_2$		
28	2-Methylhippuric acid	$C_{10}H_{11}NO_3$	12alpha-Hydroxy-13,18-dehydroparain	$C_{21}H_{28}O_6$	Embelin	$C_{16}H_{32}O_2$	8-Methylnonan-2-one	$C_{10}H_{20}O$	3,6-DIMETHYL-4,5,6,7-TETRAHYDRO-1-BENZOFURAN	$C_{10}H_{14}O$		
29	D-(+)-3-Phenyllactic acid	$C_9H_{10}O_3$	Dihydroconiferin	$C_{16}H_{24}O_8$	Kirenol	$C_{20}H_{34}O_4$	3-(L-Menthoxy)-2-methylpropane-1,2-diol	$C_{14}H_{28}O_3$	6,7-DEHYDRO-7,8-DIHYDRO-3-OXO-.ALPHA.-IONOL	$C_{13}H_{20}O_2$		
30	Asp-Ile-OH	$C_{15}H_{18}N_2O_8$	6-Hydroxy-9,9-dimethyl-5-(3-methyl-1-oxobutyl)-1-propyl-3H,9H-[1,2]-dioxolo[3’,4’:4,5]furo[2,3-f][1]benzopyran-3-one	$C_{22}H_{26}O_7$			Trp Phe	$C_{20}H_{21}N_3O_3$	3-Buten-2-ol, 2-methyl-4-(1,3,3-trimethyl-7-oxabicyclo[4.1.0]hept-2-yl)-	$C_{14}H_{24}O_2$		
31	Methoxyhydroxymethylhydrocinnamic acid	$C_{11}H_{14}O_4$	Deguelin(-)	$C_{23}H_{22}O_6$			9,10-dihydroxy-hexadecanoic acid	$C_{16}H_{32}O_4$	2-Cyclohexen-1-one, 4-hydroxy-3,5,5-trimethyl-4-(3-oxo-1-butenyl)-	$C_{13}H_{18}O_3$		
32	N-Acetyl-L-phenylalanine	$C_{11}H_{13}NO_3$	Benzoyltropein	$C_{15}H_{19}NO_2$			Etrogol	$C_{13}H_{18}$O2	Hexadecanoic acid	C1$_6H_{32}O_2$		

Table 1 (Continued)

No	LC MS/MS								GC MS			
	Emerald honey				*Melaleuca cajuputi* flower nectar				Emerald honey		*M. cajuputi flower nectar*	
	Negative mode		Positive mode		Negative mode		Positive mode					
	Name	Formular	Name	Formular	Name	Formular	Name	Formular	Name	Formular	Name	Formular
33	γ-Glutamyl-β-cyanoalanine	$C_9H_{13}N_3O_5$	Bakkenolide B	$C_{22}H_{30}O_6$			Lumichrome	$C_{12}H_{10}N_4O_2$	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$		
34	Furfural diethyl acetal	$C_9H_{14}O_3$	Almotriptan	$C_{17}H_{25}N_3O_2S$			Luteolin	$C_{15}H_{10}O_6$	cis-Vaccenic acid	$C_{18}H_{34}O_2$		
35	L-Cladinose	$C_8H_{16}O_4$	PI(22:1(11Z)/18:3 (9Z,12Z, 15Z))	$C_{49}H_{87}O_{13}P$			2,6-Dimethyl-6-O-beta-D-quinovopyranosyl-7-octadecenoic acid	$C_{16}H_{28}O_7$	Octadecanoic acid	$C_{18}H_{36}O_2$		
36	Tetraneurin A	$C_{17}H_{22}O_6$	1,2,3,4-Tetrahydro-1-phenyl-4-(1-phenylethyl) naphthalene	$C_{24}H_{24}$			PI(O-16:0/16:0)	$C_{41}H_{81}O_{12}P$	Ethyl Oleate	$C_{20}H_{38}O_2$		
37	Mono-N-depropylprobenecid	$C_{10}H_{13}NO_4S$	Gingerenone B	$C_{22}H_{26}O_6$			PI(22:1(11Z)/18:3(9Z, 12Z,15Z))	$C_{49}H_{87}O_{13}P$	Abscisic acid	$C_{15}H_{20}O_4$		
38	7-Hydroxyheptaphylline	$C_{18}H_{17}NO_3$	Neobanone	$C_{21}H_{18}O_7$			2-Hydroxyhexadecanoic acid	$C_{16}H_{32}O_3$	1-Hexadecanol, acetate	$C_{18}H_{36}O_2$		
39	Rutagravine	$C_{19}H_{17}NO_5$	7-Hydroxydoxazosin	$C_{22}H_{23}N_5O_5$			Halaminol A	$C_{14}H_{29}NO$	Eicosen-1-ol, cis-9-	$C_{20}H_{40}O$		
40	Isovitexin	$C_{21}H_{20}O_{10}$	C16 Sphinganine	$C_{16}H_{35}NO_2$			Dodecan-3-one	$C_{12}H_{24}O$				
41	Apuleirin	$C_{20}H_{20}O_9$	2-[3-Ethyl-5-(4-methoxyphenyl)-1H-pyrazol-4-yl] phenol	$C_{18}H_{18}N_2O_2$			9,14-dihydroxy-octadecanoic acid	$C_{18}H_{36}O_4$				
42	(±)-Clausenamide	$C_{18}H_{19}NO_3$	3-Ethyltridecan-2-one	$C_{15}H_{30}O$			Decyl isobutyrate	$C_{14}H_{28}O_2$				
43	Cassythine	$C_{19}H_{19}NO_5$	Phytosphingosine	$C_{18}H_{39}NO_3$			Alprenolol	$C_{15}H_{23}NO_2$				
44	Butyl lactate	$C_7H_{14}O_3$	Xestoaminol C	$C_{14}H_{31}NO$			C16 Sphinganine	$C_{16}H_{35}O_2$				
45	D-Vacciniin	$C_{13}H_{16}O_7$	2-Hydroxyhexadecanoic acid	$C_{16}H_{32}O_3$			Gingerenone B	$C_{22}H_{26}O_6$				
46	Gibberellin A36	$C_{20}H_{26}O_6$	(3beta,19alpha)-3,19,23,24-Tetrahydroxy-12-oleanen-28-oic acid	$C_{30}H_{48}O_6$			C16 Sphinganine	$C_{16}H_{35}NO_2$				
47	Abscisic Acid (cis,trans)	$C_{15}H_{20}O_4$	Clausarinol	$C_{24}H_{30}O_6$			Phytosphingosine	$C_{18}H_{39}NO_3$				

Table 1 (Continued)

No	LC MS/MS								GC MS			
	Emerald honey				*Melaleuca cajuputi* flower nectar				Emerald honey		*M. cajuputi flower nectar*	
	Negative mode		Positive mode		Negative mode		Positive mode					
	Name	Formular	Name	Formular	Name	Formular	Name	Formular	Name	Formular	Name	Formular
48	Ixocarpalactone A	$C_{28}H_{40}O_8$	Prosopinine	$C_{16}H_{33}NO_3$			10,20-Dihydroxyeicosanoic acid	$C_{20}H_{40}O_4$				
49	(-)-trans-C75	$C_{14}H_{22}O_4$	Camelliagenin B	$C_{30}H_{48}O_5$			10,20-Dihydroxyeicosanoic acid	$C_{20}H_{40}O_4$				
50	Sequiterpene Lactone 326	$C_{15}H_{20}O_4$	D-erythro-Sphingosine C-15	$C_{15}H_{31}NO_2$			N-oleoyl threonine	$C_{22}H_{41}NO_4$				
51	Lumichrome	$C_{12}H_{10}N_4O_2$	(24E)-15alpha-Acetoxy-3alpha-hydroxy-23-oxo-7,9(11),24-lanostatrien-26-oic acid	$C_{32}H_{46}O_6$			Xestoaminol C	$C_{14}H_{31}NO$				
52	Junipegenin B 7-O-glucoside	$C_{24}H_{26}O_{12}$	Annosquamosin B	$C_{19}H_{32}O_3$			C17 Sphinganine	$C_{17}H_{37}NO_2$				
53	Genipinic acid	$C_{11}H_{14}O_6$	Montanol	$C_{21}H_{36}O_4$			3’,4’,5’-Trimethoxycinnamyl alcohol	$C_{12}H_{16}O_4$				
54	2-Methyl-1-phenyl-2-propanyl butyrate	$C_{14}H_{20}O_2$	PG(P-20:0/0:0)	$C_{26}H_{53}O_8P$			Panaquinquecol 7	$C_{17}H_{24}O_3$				
55	Hieracin	$C_{15}H_{10}O_7$	all-trans-heptaprenyl diphosphate	$C_{35}H_{60}O_7P_2$			17-hydroxy-heptadecanoic acid	$C_{17}H_{34}O_3$				
56	2-Phenylaminoadenosine	$C_{16}H_{18}N_6O_4$	Trp Val Trp	$C_{27}H_{31}N_5O_4$			Sphinganine	$C_{18}H_{39}NO2$				
57	(7R*,8R*)-3-Methoxy-3’,4,7,9,9’-pentahydroxy-8,4’-oxyneolignan 4-xyloside	$C_{24}H_{32}O_{11}$	D-Glucosyldihydrosphingosine	$C_{24}H_{49}NO_7$			Polyoxyethylene 40 monostearate	$C_{20}H_{40}O_3$				
58	3’,4’,5’-Trihydroxywogonin	$C_{16}H_{12}O_8$	13E-Docosenamide	$C_{22}H_{43}NO$			N-oleoyl GABA	$C_{22}H_{41}NO_3$				
59	Ibopamine	$C_{17}H_{25}NO_4$	26,26,26-trifluoro-25-hydroxy-27-norvitamin D3 / 26,26,26-trifluoro-25-hydroxy-27-norcholecalciferol	$C_{26}H_{39}F_3O_2$			Cyclandelate	$C_{17}H_{24}O_3$				

Table 1 (Continued)

No	LC MS/MS								GC MS			
	Emerald honey				*Melaleuca cajuputi* flower nectar				Emerald honey		*M. cajuputi flower nectar*	
	Negative mode		Positive mode		Negative mode		Positive mode					
	Name	Formular	Name	Formular	Name	Formular	Name	Formular	Name	Formular	Name	Formular
60	Matairesinol	$C_{20}H_{22}O_6$	Hexadecyl ferulate	$C_{26}H_{42}O_4$			Lauroyl diethanolamide	$C_{16}H_{33}NO_3$				
61	Cappariloside A	$C_{16}H_{18}N_2O_6$					Prosopinine	$C_{16}H_{33}NO_3$				
62	Cappariloside A	$C_{16}H_{18}N_2O_6$					Cyclandelate	$C_{17}H_{24}O_3$				
63	Fuberidazole	$C_{11}H_8N_2O$					5,7,4'-Trimethoxyflavan	$C_{18}H_{20}O_4$				
64	Apuleirin	$C_{20}H_{20}O_9$					alhpa-tocopherono-lactone	$C_{16}H_{22}O_4$				
65	N1,N5,N10-Tricoumaroyl spermidine	$C_{34}H_{37}N_3O_6$					16-Hydroxy-10-oxohexa-decanoic acid	$C_{16}H_{30}O_4$				
66	16a-Hydroxyprednisolone	$C_{21}H_{28}O_6$										
67	(±)-Naringenin	$C_{15}H_{12}O_5$										
68	Desacetylcolchicine	$C_{20}H_{23}NO_5$										
69	Luteolin	$C_{15}H_{10}O_6$										
70	Methylnorlichexanthone	$C_{15}H_{12}O_5$										
71	5,6-Dihydro-11-methoxyyangonin	$C_{16}H_{18}O_5$										
72	6,7-dihydroxy Bergamottin	$C_{21}H_{24}O_6$										
73	1-(4-Methoxyphenyl)-3-(4-morpholinyl)-1-propanone	$C_{14}H_{19}NO_3$										
74	3-Hydroxychavicol 1-glucoside	$C_{15}H_{20}O_7$										
75	Elephantopin	$C_{19}H_{20}O_7$										
76	2H-1-Benzopyran-2-one, 3-[1-(4-aminophenyl)-3-oxobutyl]-4-hydroxy-, (S)- (Acenocoumarol metabolite)	C19 H17 N O4										

Table 1 (Continued)

No	LC MS/MS								GC MS			
	Emerald honey				*Melaleuca cajuputi* flower nectar				Emerald honey		*M. cajuputi flower nectar*	
	Negative mode		Positive mode		Negative mode		Positive mode					
	Name	Formular	Name	Formular	Name	Formular	Name	Formular	Name	Formular	Name	Formular
77	4-Methylumbelliferyl heptanoate	$C_{17}H_{20}O_4$										
78	Tetranor-PGEM	$C_{16}H_{22}O_7$										
79	Embelin	$C_{17}H_{26}O_4$										
80	Broussoflavan A	$C_{25}H_{30}O_6$										
81	Estrone glucuronide	$C_{24}H_{30}O_8$										
82	Marasmene	$C_{15}H_{22}O_2$										
83	Bayogenin	$C_{30}H_{48}O_5$										
84	PG(19:0/0:0)	$C_{25}H_{28}O_5$										
85	2-Prenyl-6a-hydroxy-phaseollidin	$C_{25}H_{28}O_5$										
86	Tributyrin	$C_{15}H_{26}O_6$										
87	Vaccenic acid	$C_{18}H_{34}O_2$										
88	1-Phenyl-1,3-heptadec-anedione	$C_{23}H_{36}O_2$										
89	Maslinic Acid	$C_{30}H_{48}O_4$										
90	17-Octadecynoic Acid	$C_{18}H_{32}O_2$										
91	8a,11-Dihydroxy-D9-tet-rahydrocannabinol	$C_{21}H_{30}O_4$										
92	16a-Hydroxyprednisolone 16,17-acetonide	$C_{24}H_{32}O_6$										
93	2-Hydroxyhexadecanoic acid	$C_{16}H_{32}O_3$										
94	16(S)-Iloprost	$C_{22}H_{32}O_4$										
95	α-Mangostin	$C_{24}H_{26}O_6$										
96	16a-Hydroxyprednisolone 16,17-acetonide	$C_{24}H_{32}O_6$										
97	Ala Met Arg	$C_{14}H_{28}N_6O_4S$										

Table 2 Chemical profile of Dark violet stingless bee honey

Dark violet stingless bee honey									
No	Negative mode		Positive mode		No	Negative mode		Positive mode	
	Name	Formular	Name	Formular		Name	Formular	Name	Formular
1	D-Sorbitol	$C_6H_{14}O_6$	Temurin	$C_9H_{12}N_4O_3$	18	Sophoraflavanone G	$C_{25}H_{28}O_6$	C17 Sphinganine	$C_{17}H_{37}NO_2$
2	Galactonic acid	$C_6H_{12}O_7$	6-Acetyl-D-glucose	$C_8H_{14}O_7$	19	Phytomonic Acid	$C_{19}H_{36}O_2$	Clausarinol	$C_{24}H_{30}O_6$
3	Pyroglutamic acid	$C_5H_7NO_3$	(1alpha,2alpha,4betaH,6alpha,8R)-p-Menthane-2,6,8,9-tetrol	$C_{10}H_{20}O_4$	20	Lespedezaflavanone D	$C_{25}H_{28}O_6$	Prosopinine	$C_{16}H_{33}NO_3$
4	(R)-(+)-2-Pyrrolidone-5-carboxylic acid	$C_5H_7NO_3$	2,6-Dimethyl-1,8-octanedioic acid	$C_{10}H_{18}O_4$	21	10Z-nonadecenoic acid	$C_{19}H_{36}O_2$	Bayogenin	$C_{30}H_{48}O_5$
5	Succinic acid	$C_4H_6O_4$	(1S,2S,4R,8R)-p-Menthane-1,2,8,9-tetrol	$C_{10}H_{20}O_4$	22	beta-Mangostin	$C_{25}H_{28}O_6$	Diisobutyl phthalate	$C_{16}H_{22}O_4$
6	Dimethylallyl diphosphate (DMAPP)	$C_5H_{12}O_7P_2$	3-Hydroxycapric acid	$C_{10}H_{20}O_3$	23	4-Methylumbelliferyl heptanoate	$C_{17}H_{20}O_4$	16-Hydroxy-10-oxohexadecanoic acid	$C_{16}H_{30}O_4$
7	Hydroxyphenyllactic acid	$C_9H_{10}O_4$	3-Methoxy-4-Hydroxyphenylglycol Sulfate	$C_9H_{12}O_7S$	24	Kushenol A	$C_{25}H_{28}O_5$	D-erythro-Sphingosine C-15	$C_{15}H_{31}NO_2$
8	L-α-Hydroxyisovaleric acid	$C_5H_{10}O_3$	Dibutyl malate	$C_{12}H_{22}O_5$	25	Tributyrin	$C_{15}H_{26}O_6$	Ser Arg Tyr	$C_{18}H_{28}N_6O_6$
7	(S)-(-)-2-Hydroxyisocaproic acid	$C_6H_{12}O_3$	Perlolyrine	$C_{16}H_{12}N_2O_2$	26	6,8-Diprenylnaringenin	$C_{25}H_{28}O_5$	Annosquamosin B	$C_{19}H_{32}O_3$
8	trans-Cinnamic acid	$C_9H_8O_2$	(1R,2R,4S,6R)-2,6-Fenchanediol 2-O-b-D-glucoside	$C_{16}H_{28}O_7$	27	Stearic acid	$C_{18}H_{36}O_2$	Montanol	$C_{21}H_{36}O_4$
10	Asp-Ile-OH	$C_{15}H_{18}N_2O_8$	PI(22:1(11Z)/18:3(9Z,12Z,15Z))	$C_{49}H_{87}O_{13}P$	28	Maslinic Acid	$C_{30}H_{48}O_4$	PG(18:3(9Z,12Z,15Z)/0:0)	$C_{24}H_{43}O_9P$
11	Luteolin	$C_{15}H_{10}O_6$	GlcNAcbeta1-4Manbeta1-4Glcbeta-Cer(d18:1/24:1(15Z))	$C_{62}H_{114}N_2O_{18}$	29	α-Mangostin	$C_{24}H_{26}O_6$		
12	10-hydroxy capric acid	$C_{10}H_{20}O_3$	Gingerenone B	$C_{22}H_{26}O_6$	30	Macrourone C	$C_{30}H_{36}O_6$		
13	Embelin	$C_{17}H_{26}O_4$	C16 Sphinganine	$C_{16}H_{35}NO_2$	31	Isopalmitic acid	$C_{16}H_{32}O_2$		
14	Exiguaflavanone M	$C_{25}H_{30}O_7$	Phytosphingosine	$C_{18}H_{39}NO_3$	32	(3R, 6'Z)-3,4-Dihydro-8-hydroxy-3-(6-pentadecenyl)-1H-2-benzopyran-1-one	$C_{24}H_{36}O_3$		
15	Lonchocarpol B	$C_{25}H_{30}O_7$	C16 Sphinganine	$C_{16}H_{35}NO_2$		(+)-Isomyristic acid	$C_{14}H_{28}O_2$		
16	2',4',6',3,4-Pentahydroxy-3',5-diprenyldihydrochalcone	$C_{25}H_{30}O_6$	Xestoaminol C	$C_{14}H_{31}NO$					
17	Abyssinin III	$C_{25}H_{28}O_6$	2-Hydroxyhexadecanoic acid	$C_{16}H_{32}O_3$					

3. Total phenolic compounds and antioxidant activity

From the analysis of total phenolic compounds in stingless bee honey from 4 species in 4 farms in Table 3, it was found that the stingless bee honey from *T. laeviceps* in Khok khian (TLKK) had the highest value at 165.27±0.23 mg GAE/100 g and had a DPPH radical scavenging activity at IC_{50} of 9.50±0.39 mg/mL. The stingless bee honey from *H. itama* in Barehtai (HIBR) had the lowest total phenolic compounds at 37.67±1.01 mg GAE/100 g and a DPPH radical scavenging activity at IC_{50} of 50.61±1.55 mg/mL.

DPPH radical scavenging activity was used to examine the total hydrogen or electron donating activity of a single antioxidant. Shamsudin et al. (2022) investigated the total phenolic content of stingless bee honey from three *H. itama* strains in three fruit farms in Malaysia and found that the content ranged from 58.39 to 74.72 mg GAE/100 g with DPPH radical scavenging activity IC_{50} values ranging from 11.27 to 21.41 mg/mL. It can be observed that the total phenolic content of stingless bee honey depended on the botanical characteristics of each area. In *G. thoracica* honey, Ahmad et al. (2019) reported that the total phenolic compounds were 5.47±0.26 mg GAE/100g with DPPH radical scavenging activity at IC_{50} of 57.60±1.20%, while in *T. laeviceps*, Agussalim et al. (2022) found the total phenolic content and DPPH radical scavenging activity to be between 0.65-2.30 mg GAE/100g and 61.43-90.28%, respectively.

4. Sugar profile

From the analysis of four sugars, namely Fructose, Glucose, Sucrose and Maltose, in four strains of stingless bee honey and four ecosystems as showed in Table 3 represented that sucrose was the most abundant, with the values in the range of 12.37±0.06 - 46.95±1.27 percent, followed by glucose 7.46±0.13 - 29.72±0.17 percent, fructose 5.54±0.07 - 18.42±0.02 percent and maltose 0.25±0.00 - 1.79±0.08 percent.

It can be seen that the amounts of monosaccharides and disaccharides sugars that are the components of stingless bee honey will be in a wide range, except for maltose, which is much higher than the international standard for honey from *Apis mellifera*, which the limitation of sucrose level less than 5 g/100 g, while the fructose and glucose content are falling into the standard and not exceed more than 60 g/100g (Codex Alimentarius, 2001).

Suntiparapop et al. (2012) reported that *T. leaviceps* honey from Chantaburi and Trat provinces contained fructose of 28.84 ± 0.28 and 25.31 ± 0.04, glucose of 21.44 ± 0.19 and 20.15 ± 0.02, and sucrose of 19.45 ± 0.12 and 18.85 ± 0.40, respectively. Also, Chuttong et al. (2016) has been analyzed the carbohydrate profile in 28 samples of *T. leaviceps* honey from 11 species of stingless bees in Chiang Mai province. with mean values of total sugar (51 ± 21 g/100 g), fructose (17 ± 9.7 g/100 g), glucose (14 ± 8.6 g/100 g), maltose (41 ± 15 g/100 g) and sucrose (1.2 ± 2.7 g/100 g).

5. Analysis of estimated glycemic index (eGI)

Glycemic index (GI) is a value that indicates how quickly carbohydrate foods are absorbed into the body after eating and converted to sugar when compared to glucose. GI is divided into 3 levels composed of low glycemic or low glycemic with a value of ≤55, medium glycemic or moderate glycemic with a value between 56-69 and high glycemic or high glycemic with a value of ≥70. Therefore, the glycemic index of food has a significant impact on the health of consumers. And it is useful for those who have to control their sugar levels, especially with high blood sugar and diabetes patient. From the results of the study, it was found that 13 samples of stingless bee honey as demonstrated in Table 3, had a GI value between 50.34±0.02 to 61.26±0.67, which could be divided into 2 levels of low glycemic level, found in 5 samples in 3 areas (Khok Khian, Barehtai and Sako), and medium glycemic level, found in 8 samples in 3 areas. While honey bee had a GI value of 63.98±0.06. Krishnasree and Ukkuru (2017) found that raw honey bee had a higher GI value of 61 than stingless bee honey, which was 54. From the previous study, the highest GI value of raw honey bee had been in the range of 69.1-109.0 due to the chemical composition of honey bee is not constant and varies according to the botanical and geographical origin (Atayoglu et al., 2016).

Table 3 Total phenolic compounds, sugar profile and glycemic index of stingless bee honey

Sample	Total phenolic compounds (mg GAE/100 g)	IC_{50} (mg/mL)	Sugar profile (% w/v)				eGI	eGI level
			Fructose	Glucose	Sucrose	Maltose		
Control (Honey bee)	48.53±1.36^{j}	36.26±0.62^{c}	17.89±0.31^{b}	29.53±0.08^{a}	0.65±0.01^{h}	6.08±0.07^{a}	63.98±0.0^{6}a	Medium
GTKK	86.73±3.06^{e}	17.79±0.65^{h}	15.90±0.37^{d}	7.46±0.13^{i}	24.25±0.02^{d}	0.25±0.0^{0}k	50.82±0.76^{h}	Low
GTBR	78.33±0.61^{f}	44.90±0.56^{b}	16.39±0.12^{c}	19.66±0.12^{e}	18.68±0.14^{e}	1.35±0.02^{d}	57.44±0.34^{f}	Medium
GTBP	121.00±0.53^{c}	19.22±0.66^{g}	5.11±0.01^{i}	7.48±0.23^{i}	46.95±1.27^{a}	0.68±0.02^{g}	61.26±0.67^{b}	Medium
HIKK	101.00±3.12^{d}	12.13±0.37^{i}	17.97±0.04^{b}	8.25±0.20^{h}	23.76±0.04^{d}	1.10±0.01^{e}	52.40±0.06^{g}	Low
HIBR	37.67±1.01^{l}	50.61±1.55^{a}	18.36±0.01^{a}	15.48±0.19^{g}	24.07±0.01^{d}	0.51±0.00^{hi}	52.12±0.07^{g}	Low
HIBP	64.07±1.72^{h}	27.70±1.55^{e}	18.17±0.02^{ab}	26.46±0.13^{c}	12.37±0.06^{g}	0.45±0.01^{j}	59.07±0.14^{de}	Medium
HISK	74.20±2.65^{g}	23.99±0.30^{f}	14.12±0.02^{f}	6.95±0.17^{j}	23.86±0.01^{d}	1.72±0.03^{c}	50.34±0.0^{2}h	Low
HBKK	52.67±1.01^{i}	26.66±0.79^{e}	18.14±0.03^{ab}	17.14±0.31^{f}	23.78±0.02^{d}	0.55±0.00^{h}	52.81±0.07^{g}	Low
HBBR	45.20±2.80^{k}	37.41±0.67^{c}	18.16±0.07^{ab}	28.12±0.31^{b}	13.61±0.17^{f}	0.64±0.01^{g}	58.88±0.10^{e}	Medium
HBBP	118.40±2.09^{c}	11.30±0.55^{i}	18.42±0.0^{2}a	29.72±0.1^{7}a	13.11±0.02^{fg}	0.47±0.01^{ij}	59.70±0.06^{cd}	Medium
TLKK	165.27±0.23^{a}	9.50±0.^{3}9j	7.25±0.27^{g}	6.03±0.20^{k}	41.86±1.32^{c}	1.79±0.08^{b}	60.23±0.39^{c}	Medium
TLBR	99.93±0.76^{d}	33.40±0.68^{d}	5.54±0.0^{7}h	5.36±0.09^{l}	45.72±1.71^{b}	1.38±0.02^{d}	56.77±0.32^{f}	Medium
TLBP	160.53±0.42^{b}	11.80±0.65^{i}	15.31±0.05^{e}	20.31±0.19^{d}	13.01±0.10^{fg}	0.97±0.00^{f}	59.43±0.66^{de}	Medium

Note: Different letter indicates significant different ($p<0.05$) between honey samples.

Abbreviations: GTKK; *G. thoracica* Khok khian, GTBR; *G. thoracica* Barehtai, GTBP; *G. thoracica* Bangpor, HIKK; *H. itama* Khok khian, HIBR; *H. itama* Barehtai, HIBP; *H. itama* Bangpor, HISK; *H. itama* Sako, HBKK; *H. bakari* Khok khian, HBBJ; *H. bakari* Barehtai, HBBP; *H. bakari* Bangpor, TLKK; *T. laeviceps* Khok khian, TLBR; *T. laeviceps* Barehtai, TLBP; *T. laeviceps* Bangpor.

6. Physicochemical properties of bee the honey

The color of the bee honey varies greatly depending on its geographic origin. In addition, the production methods and agriculture practice affect the color of honey (de Almeida-Muradian et al., 2013). The color of honey ranges from light amber to amber brown and darker colored honey has a higher ash value than lighter colored honey (Rao et al., 2016).

From the 13 stingless bee honey samples in Table 4, it was found that the total amount of solids compared to the controlled stingless bee honey samples. The samples of stingless bee honey had a lower amount of total solids than the controlled stingless bee honey and had the values between 63.67-73.10 °Brix. The samples of Stingless bee honey also had a higher acidity value than the controlled stingless bee honey, which had values between 2.94-3.62. And from the analysis of the color value of the sample, it was found that the controlled stingless bee honey samples had luminance values L*, a* and b* equal to 73.40, 7.00 and 46.37 and had a clear and not cloudy golden yellow appearance. And it was found that most of the samples of stingless bee honey had a lower brightness value than the controlled sample and had a relatively high b* value due to the cloudy appearance and had a more yellow color than the controlled sample as shown in figure 4 and Table 4. The color characteristics of honey can be affected by factors such as exposure to light, heat and storage time, including enzymatic reactions (de Sousa et al., 2016). Darker honey has a higher total amount of phenolics. This indicates that it may have high levels of biological activity (Majid et al., 2019).

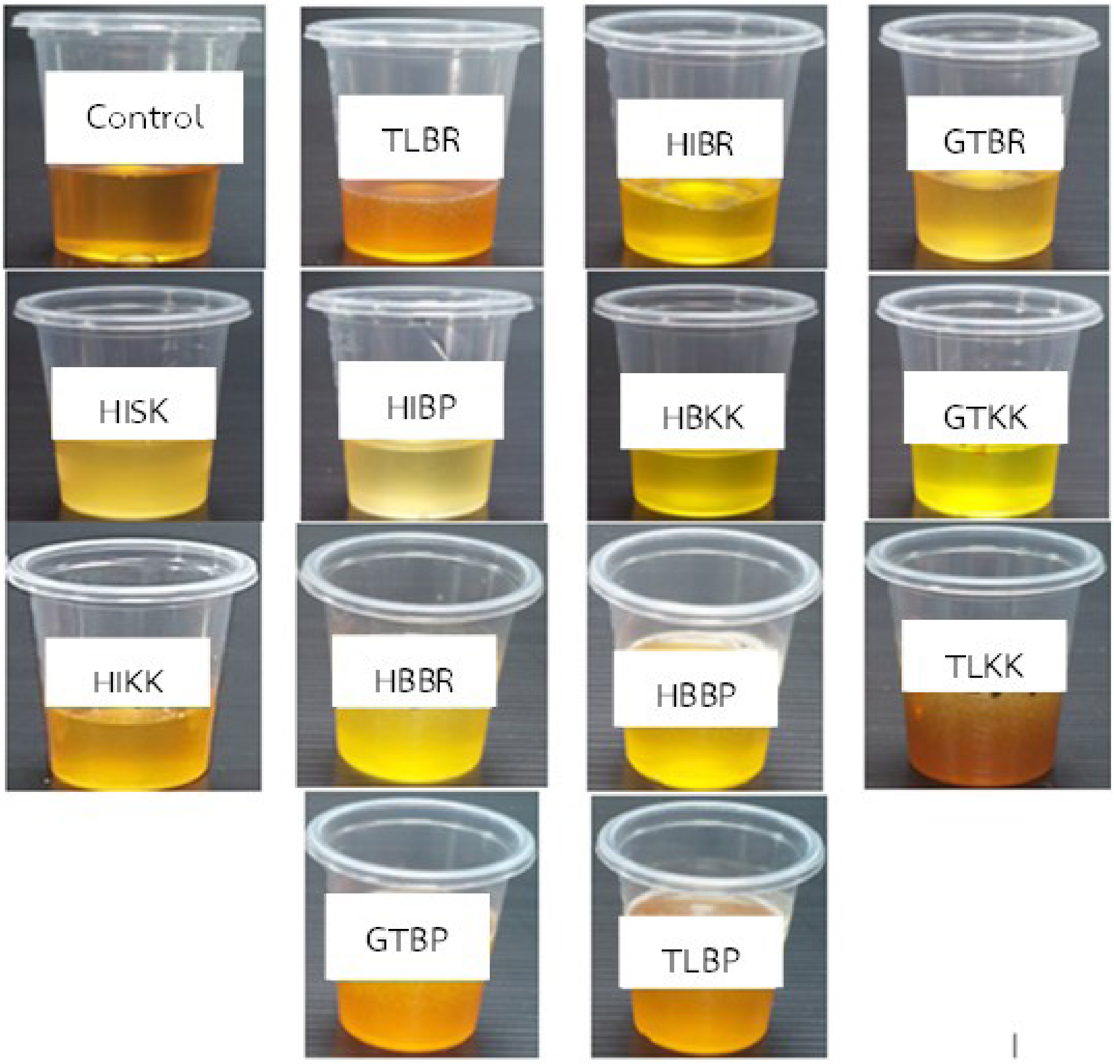

Figure 4 Appearance of stingless bee honey

Table 4. Total solids (Brix) and pH of stingless bee honey.

Sample	Total solid (Brix)	pH	Color index		
			L*	a*	b*
Control	79.80 ± 0.10^{a}	4.04 ± 0.19^{a}	73.40 ± 1.59^{a}	7.00 ± 1.45^{e}	46.37 ± 1.66^{bc}
GTKK	68.67 ± 0.50^{fg}	2.98 ± 0.05^{bc}	70.16 ± 1.97^{ab}	-2.79 ± 0.13^{k}	36.64 ± 1.27^{e}
GTBR	69.17 ± 0.42^{ef}	2.98 ± 0.07^{bc}	46.97 ± 2.35^{h}	3.72 ± 0.56^{h}	27.43 ± 1.39^{fg}
GTBP	67.93 ± 0.42	3.14 ± 0.08^{b}	58.56 ± 7.22^{de}	7.55 ± 1.91^{de}	38.23 ± 2.60^{e}
HIKK	68.63 ± 0.40^{fg}	2.96 ± 0.06^{bc}	57.49 ± 3.94^{de}	8.82 ± 1.39^{d}	49.17 ± 1.99^{b}
HIBR	70.43 ± 0.61^{d}	3.15 ± 0.03^{b}	54.10 ± 2.29^{fg}	8.11 ± 0.62^{de}	48.68 ± 1.86^{b}
HIBP	68.17 ± 0.12^{g}	2.94 ± 0.06^{bc}	66.49 ± 3.75^{bc}	1.88 ± 0.06^{i}	21.16 ± 2.68^{h}
HISK	63.67 ± 0.50^{h}	3.00 ± 0.04^{bc}	61.10 ± 1.59^{de}	3.60 ± 0.19^{h}	26.05 ± 0.56^{g}
HBKK	69.13 ± 0.57^{ef}	3.13 ± 0.04^{b}	62.24 ± 4.79^{cd}	0.26 ± 1.16^{j}	47.90 ± 1.65^{bc}
HBBR	69.70 ± 0.40^{de}	3.36 ± 0.03^{ab}	54.81 ± 1.24^{fg}	5.10 ± 0.58^{gh}	38.61 ± 2.15^{e}
HBBP	70.27 ± 0.32^{d}	3.15 ± 0.04^{b}	58.32 ± 1.95^{de}	5.94 ± 0.36^{fg}	30.74 ± 0.55^{f}
TLKK	71.50 ± 0.52^{c}	3.62 ± 0.04^{ab}	41.69 ± 4.58^{i}	10.55 ± 0.89^{c}	26.07 ± 3.23^{g}
TLBR	73.10 ± 0.30^{b}	3.07 ± 0.05^{b}	51.87 ± 6.69^{g}	16.52 ± 2.47^{a}	43.11 ± 4.01^{d}
TLBP	71.30 ± 0.69^{c}	3.38 ± 0.10^{ab}	56.57 ± 2.40^{efg}	12.05 ± 1.17^{b}	53.44 ± 3.57^{a}

Note: Different letter indicates significant different ($p<0.05$) between honey samples.

Abbreviations: GTKK; *G. thoracica* Khok khian, GTBR; *G. thoracica* Barehtai, GTBP; *G. thoracica* Bangpor, HIKK; *H. itama* Khok khian, HIBR; *H. itama* Barehtai, HIBP; *H. itama* Bangpor, HISK; *H. itama* Sako, HBKK; *H. bakari* Khok khian, HBBJ; *H. bakari* Barehtai, HBBP; *H. bakari* Bangpor, TLKK; *T. laeviceps* Khok khian, TLBR; *T. laeviceps* Barehtai, TLBP; *T. laeviceps* Bangpor.

Conclusions

The comparison of chemical composition of the emerald stingless bee honey and *M. cajuputi* nectar using LC-QTOF-MS, found that 24 compounds were found to be consistent, with 2 prominent compounds comprised of Lumichrome and Embelin. Total phenolic compounds in *T. laeviceps* honey were at high levels, especially in Khok khian and Bangpor sub-districts. For the eGI (estimated glycemic index) level in stingless bee honey were found in the range of low to moderate, which is suitable for controlling starch and sugar. This study found that the ecology and botanical characteristics of plants influenced the phytochemical composition and antioxidant activity of the stingless bee honey. In addition, such data can be used to support the consumption of stingless bee honey as a health food and also provide some evidence on the emerald stingless bee honey showing its potential to prevent health problems and to promote and increase the value and worth of the stingless bee honey products.

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