Molecular Identification of Three Stingless Bees and Chemical Profiles of Their Honeys

Thanaset Thongsaiklaing[1,4*], Kitti Satjawattana[2], Wasan Palasai[3],
Jirika Nutalai[2] and Sulaiman Cheabu[1]

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Abstract

Stingless bee is honey is widely consumed because it has high pharmacological properties. In addition, stingless bee honey also has color. It has a unique smell and taste as well. This depends on the ecosystem and species in the area where bees live. Thailand is a tropical country with a high abundance and diversity of plant life. But, studies on the composition of compounds substance in stingless bee honey are relatively few. Therefore, this research focused on the detection of the effective substances of the stingless bee honey in Narathiwat Province. From the study on the effective compound substances in 3 species of stingless bee honey composed of *H. itama*, *H. bakari* and *T. laeviceps*, using the liquid chromatograph quadrupole time-of-flight mass spectrometer (LC-QTOF MS) technique, the effective substances were examined negatively and positive ion modes found that the stingless bee honey contained of flavonoids, phenolic compounds, terpenes, di-amino acids and tripeptides and also other types of organic acids. The most effective substances found in *T. laeviceps*, H.itama, and *H. bakari* stingless bee honey were 191, 79, and 70 types, respectively. All of the substances found were both new substances that had never been reported before and substances that had been previously reported. For the amount of honey, it was found that the *H. bakari* species gave the highest amount and *T. laeviceps* honey has the highest percentage of sweetness. Storing honey at the room temperature increased acidity in all varieties. This research showed that the ecosystem and stingless bee species affect the chemical properties of honey including fermentation and the increasing of pH of the honey.

Keyword: Stingless bee honey, Stingless bee, LC-QTOF MS, Chemical profile, CO1

[1] Faculty of Agriculture, Princess of Naradhiwas University, 96000,

[2] Thailand Program Management Unit on Area Based Development (PMU A), Pathumwan, Bangkok, 10330, Thailand

[3] Faculty of Engineering, Princess of Naradhiwas University, 96000, Thailand

[4] Biotechnology and molecular biology laboratory (BMBL), Faculty of Agriculture, Princess of Naradhiwas University, 96000, Thailand

[*]Corresponding Author Email: thanasetfdr@gmail.com

Introduction

Stingless bee is a Eusocial insect that does not have a stinger and lives in the tropics and found in southern and central America, Africa, and southwest Asia. and Australia which is classified in the order of Hymenoptera, family Apidae and subfamily Meliponinae. Only 6 genera around the world. Currently, more than 600 species of hymenoptera are found in worldwide (Michener, 2000: Avila et al., 2018). Recently, nucleotide sequences have been used for classification along with morphological characteristics. This will provide more detailed and accurate information. It can also be used to classify complex characteristics that are morphologically difficult to distinguish. The *cytochrome c oxidase 1* (*CO1*) gene is mostly used for classification (Ndungu et al., 2017; Francoso et al., 2019). Besides, being useful in pollination, stingless bees also have other products such as royal jelly, propolis, flower pollen and honey. Honey is a product that has been widely consumed since ancient times. It is a natural product that is rich in various biologically active substances and has unique properties. Most of the compounded substances in stingless bee honey are in the group of phenolic compounds, flavonoids and terpenes include phenolic acids, Mandelic acid, Cinnamic acid, Sinapic acid, Abscisic acid (trans,trans-Abscisic acid, cis,trans-Abscisic acid), Coniferic acid, Trans -ferulic acid, p-Coumaric acid, Caffeic acid, Chlorogenic acid, Rosmarinic acid, Ellagic acid (Phenolic compound), Carnosol (Phenolic diterpene) Flavonoid compounds include Hispidulin, Aromadendrin, Myricetin, Chrysin, Eriodictyol, Catechol, Luteolin, Naringenin, Quercetin, Apigenin, Quercetin, Catechin, Taxifolin, Kaempferol, Isoquercitrin, Hesperetin, flavanon-glycoside, Galangin, Pinocembrin, Hesperitin, Isoquercetrin Other phenolic compounds include Vanillin, Umbelliferone, Syringaldehyde, 3,4-Dihydroxybenzoic acid, Sinapaldehyde, 4-Hydroxybenzoic acid or called p-hydroxybenzoic acid (PHBA) (Pasupuleti et al., 2017; Al-Hatamleh et al. al., 2020) contains the sugars maltose, glucose, fructose and trehalulose (Fletcher et al., 2020). There are also proteins and enzymes involved in the detoxification process, such as superoxide dismutase (SOD), catalase (CAT) and reduce glutathione (GSH) (Rao et al., 2016). However, the composition of the effective substances in stingless bee honey is different, depending on the ecosystem in which stingless bee lives, differences in plant types, climate and geography also affects the physical and chemical properties of honey. This makes the properties of stingless bee honey in each area have the unique properties. In addition, the area has abundant of biodiversity, this makes honey have a greater variety of biologically active substances with unique properties. From many studies that tested the properties of honey that have been published in scientific journals around the world, it was found that honey has anti-microbial properties, antioxidants, anti-inflammatory, anti-tumor, anti-cancer, and reduces the fat in blood vessel. And has properties in heart disease protection, eye treatment, digestive diseases, abnormal nervous system, reproductive system protection and used to heal wounds treatment, etc. The aims of this research was to analyze the chemical composition of 3 types of stingless bee honey by using molecular techniques to confirm the type of honey from the stingless bee using the nucleotide sequence of the *CO1* gene and examine the amount of the sweetness and pH of stingless bee honey in Narathiwat Province.

Materials and Methods

1. Collecting samples of honey and 5 bees each from stingless bee farms of 3 species: *Heterotrigona itama*, *Heterotrigona bakari* and *Tetragonula laeviceps*, and 1 kg each of honey species.

2. Study of preliminary morphological characteristics of stingless bee

Samples of stingless bees were anesthetized by freezing in a freezer -20 o C for 5 minutes and then cut into different sections to study the front wings (forewing), antennae (antennae), teeth (mandible) and the third pair of legs (hind legs) using a stereo camera Olympus brand and camera (Moticcam) size 2.20 PM and record the images.

3. Polymerase chain reaction (PCR) using the *CO1* gene of stingless bee

3.1 DNA extraction

The whole stingless bee has been grinded thoroughly in digestion buffer, volume 350 microliters, added proteinase K (concentration 20mg/ml) 20 microliters, grind together, incubate at 55ºC for 24 hours, then added 6M $NaCl_2$, volume 300 microliters, mix well and then centrifuge at a speed of 14,000 rpm for 10 minutes. Then aspirate 1.5 ml of the clear portion into a tube, added 100% ethanol twice the amount of the clear portion and turn the tube over and refrigerate at -20°C overnight, then centrifuge at the speed of 14,000 rpm for 15 minutes. Then pour out all the solution and collect the DNA precipitate at the bottom of the tube and wash the resulting DNA precipitate with 500 microliters of 70% ethanol. And then, centrifuged at 8,500 rpm for 5 minutes (repeat twice). The solution was discarded and the DNA precipitate was dried. Dissolved the precipitate with 50 microliters of TE buffer. The resulting DNA was tested for concentration and purity by using a Nanodrop (Microvolume Spectrophotometer/Nanodrop: Thermo Scientific) and 1% agarose gel, images were captured under UV light (Gel Doc, BioRad), and the extracted DNA was stored at -20°C.

3.2 Testing the optimum conditions of the genetic amplification reaction for the *CO1* gene in stingless bees.

3.2.1 Testing the efficiency of the primers and optimum conditions of the gene amplification reaction.

The primers used were designed using the nucleotide sequences of the *CO1* gene from three species of stingless bees, namely *H. Itama*, *Geniotrigona thoracica* and *Lepidotrigona terminata*.

(1) Selection of appropriate primer pairs for genetic material amplification reactions. Using a total of 5 primers, consisting of 2 forward primers and 3 reward primers, the reaction consists of 10X Taq DNA polymerase buffer, quantity 1.25 microliters, 50 $MgCl_2$ quantity 0.25 microliters, dNTPs mix volume 0.25 µl, 10 pmol forward primer (SBCO1_F1; 5' TTCCAAATTCAGGAACTGG 3' ; SBCO1_F2; 5' ATGAACTGTGTATCCTCCTC 3') volume 0.25 µl, 10 pmol reward primer (SBCO1_R1_2; 5' CCTCCAATTGTAAATATTAA 3' ; SBCO1_R3 ; 5' GCAATAATTGAAAATACAGC 3' ; SBCO1_R4; 5' AGCATAATTCCCGTTAGTCC 3') volume 0.25 microliters, DNA Template (concentration 50 ng/microliter) 0.25 microliters, DNA polymerase amount 0.125 microliters and adjust the amount with distilled water to reach 12.5 microliters. Then the reaction was put into a gradient amplification reaction machine (Biorad) for 41 cycles under the following conditions: Preheat 95ºC for 3 minutes, Denature for 95ºC 30 seconds, Annealing according to the gradient program as follows: H : 50 ; G : 50.7; F : 51.9; E : 53.8; D : 56.1; C : 58; B : 59.2 and A : 60ºC for 30 seconds respectively, Extension 68ºC for 60 seconds and Final extension for 68ºC 10 minutes, then the obtained PCR product was checked for DNA size using a 1% agarose gel and recorded under UV light (Gel Doc, BioRad).

(2) Magnesium chloride suitability test by using the primer pairs SBCO1_F1 and SBCO1_R3, the genetic amplification reaction was the same as in (1), except 50 $MgCl_2$ was used in volumes of 0, 0.25, 0.75, and 1 µL, respectively. The temperature in the Annealing step was used according to the primer pair test results in the topic (1)

3.3 Increasing the amount of the genetic material of the CO1 gene

Gene amplification reactions were used as in (1), except the primers 1, SBCO1_F1 and SBCO1_R3, were used to amplify in samples of *H. itama* and *H. bakari*. Primers 2, SBCO1_F2 and SBCO1_R3, were used to amplify in samples of *T. Laeviceps*. DNA Template by Annealing steps 51ºC (TB2 and BP1) and 48ºC (*T. Laeviceps*). Then the PCR product was purified using FavorPrepTM GEL/PCR Purifcation Mini Kit, Favorgen Biotech

Corp. and subjected to sequencing. Kleotide at ATGC Company Limited, Thailand Science Park Khlong Luang District Pathum Thani Province.

3.4 Phylogenetic analysis

Three samples of 601 base pair nucleotide sequences were analyzed for evolutionary genetic relationships using MEGA X software (Molecular Evolutionary Genetics Analysis) Neighborjoining of 1,000 bootstrap method by using the *CO1* genes of *T. pegdeni*, *H. itama* and *Geneotrigona thoraciga* as a baseline.

4. Crude extraction of bioactive substances from stingless bee honey and identification of effective substances in stingless bee honey

4.1 Extraction of crude bioactive compounds from honey.

Take 1 kilogram of each honey sample and dilute it with water at a ratio of 1:1 (V/V). Then shake vigorously and then add ethyl acetate at a ratio of 1:1 (V/V) with shake vigorously again. Then, continue extraction by soaking in an Ultrasound cleaning bath (BANDELIN, SONOREX DIGITEC) using a frequency of 37 kHz at 40 ± 3 degrees Celsius for 15 minutes 3 times. Afterward, centrifuged at 6,000 rpm for 10 minutes, keeping the separated ethyl acetate portion. The remaining honey was extracted twice according to the same procedure. Then, the ethyl acetate obtained from the 3 extractions was combined and evaporated to remove the ethyl acetate using a vacuum evaporator and the extract was stored at -20°C.

4.2 Liquid-chromatograph and Mass spectroscopy

Liquid chromatograph quadrupole time-of-flight mass spectrometer (LC-QTOF MS), 1290 Infinity II 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 Workflows V8, then identified and confirmed by comparison of their retention times and mass spectra with a Mass Hunter METLIN PCD and reference compounds.

5. Quantity analysis of the sweetness and acidity of stingless bee honey

Samples of honey from 9 honeycomb of each species were used with a vacuum cleaner and then weighed. Measure sweetness using a refractometer and measure acidity using a pH meter. Statistical data analysis was performed using R version 3.6.1 program.

Results and discussions

1. Study of the morphological characteristics of stingless bee

The *H. Itama* and *H. bakari* species have the same black external organs except for the color of the wings. The *H. Bakari* species is dark black and the size of the body is larger (pictures 1-3) and (Table 1) while *T. Laeviceps* has light brown external organs. The body size is smaller. The wings are light brown. The number of hamuli depends on the size of the body. Larger species have more hamuli.

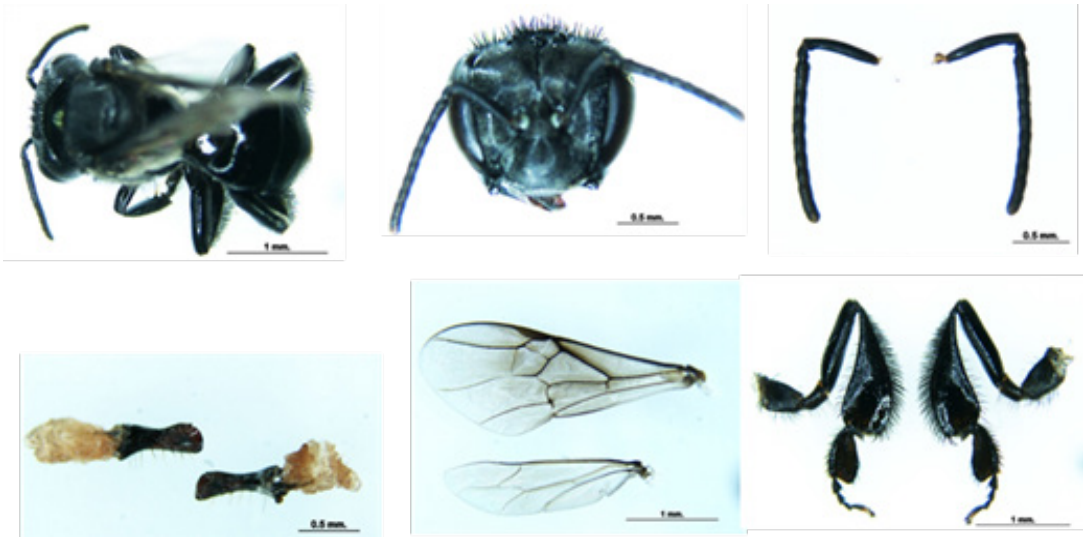

Figure 1. Morphology of the bee *H. Itama*. A) body B) head C) teeth D) antennae E) wings F) 3rd pair of legs

Figure 2. Morphology of the bee specimen *H. bakari*.
A) body B) head C) teeth D) antennae E) wings F) 3rd pair of legs

Figure 3. Morphology of the bee specimen *T. Laeviceps*
A) Body B) Head C) Teeth D) Antennae E) Wings F) 3rd pair of legs

Table 1. External morphology of stingless bees

Name	Body		Head		Teeth		Antennae		Wings		3rd pair of legs		Hamuli	
	Length (mm)	Color	Length (mm)	Color	Length (mm)	Color	Length (mm)	Color	Length (mm)	Color	Length (mm)	Color	Length (mm)	Color
H. Itama	4.5	Black	2	Black	1	Black	3	Black	5	Black	4.7	Black	7/7	Black
H. bakari	5.8	Black	2.7	Black	1.2	Black	2.6	Black	8	Light brown	7.2	Black	7/7	Black
T. Laeviceps	3.5	Dark brown	1.8	Dark brown	0.6	Light brown	2	Dark brown	3	Light brown	4	Dark brown	5/5	Black

Table 2. Concentration and quality of extracted stingless bee DNA

Sample	Replication	DNA concentration (Microgram/Microliter)	OD 260/280	OD 230/280
H. iltama	1	415.6	1.87	1.55
	2	736.0	1.88	1.40
	3	586.4	1.89	1.58
	4	316.9	1.93	1.67
H. bakari	1	343.7	1.75	0.92
	2	548.1	1.58	0.97
	3	258.9	1.79	0.97
	4	343.7	1.75	0.92
T. laeviceps	1	125.9	1.64	0.75
	2	453.9	1.80	1.27
	3	403.9	1.94	1.33
	4	237.3	1.77	0.93

2. Increasing the amount of genetic material of the *CO1* gene in stingless bees

2.1 Quality and purity of DNA

From the DNA extraction from three species of stingless bees, it was found that the DNA concentration in the samples was extracted from 125.9 to 736.0 micrograms/microliter. The quality of the extracted DNA had an absorbance value of 260/280 between 0.80 and 2.11 and an absorbance value of 260/230 between 0.42 and 2.10 (Table 2). This range of light is used as an index of DNA purity. The range of light at 260 and 280 nanometers, with a ratio of approximately 1.8, is considered pure DNA. But, if the ratio is lower than or equal to 1.6, it indicates protein and phenol contamination. The 260 and 230 nm photoperiods are the second index used to indicate the purity of DNA. The accepted value for purity is between 2.0 and 2.2. If the ratio is lower than this, it means that the DNA has been contaminated with EDTA, fat, carbohydrates, and salt (Lucena-Aguilar et al., 2016). Most of the extracted stingless bee DNA was in high concentration and of good quality that could be used as a model DNA for the reaction to increase the amount of genetic material of the CO1 gene.

2.2 Testing on the appropriate annealing temperature and magnesium chloride concentration for genetic amplification reactions.

The results from the experiment with all 5 primers found that there were 2 sets of appropriated primer pairs for amplifying the CO1 gene, namely primer set 1 consisted of forward SBCO1_F1 and reverse SBCO1_R3 and primer set 2 consisted of forward SBCO1_F2 and reverse SBCO1_R3 with PCR product sizes of 820 and 798 bp, respectively. Primer set 1 had an optimum temperature of 50 and 50.7 o C. Primer set 2 had an optimum temperature of 48 o C (A) as shown on (Table 3). Each stingless bee sample had the specific to a different set of primer as represented on Figure 4.

2.3 Magnesium chloride concentration

The optimum magnesium chloride concentration in the amplification reaction was found to be 0.5 mM when the DNA concentration was approximately 100 µg/µL (Figure 5).

2.4 Increasing the amount of genetic material of the *CO1* gene

From using 2 pairs of primers consisting of SBCO1_F1/SBCO1_R3 and SBCO1_F1/ SBCO1_R4 with annealing temperatures (Tm°) of 51 and 48 degrees Celsius, respectively. In the process of increasing the amount of genetic material of the *CO1* gene in all 3 samples of bees, it was found that the PCR product obtained was of good quality. No non-specific band contamination was found (Figure 6), which was suitable for further purification and sending for nucleotide sequencing.

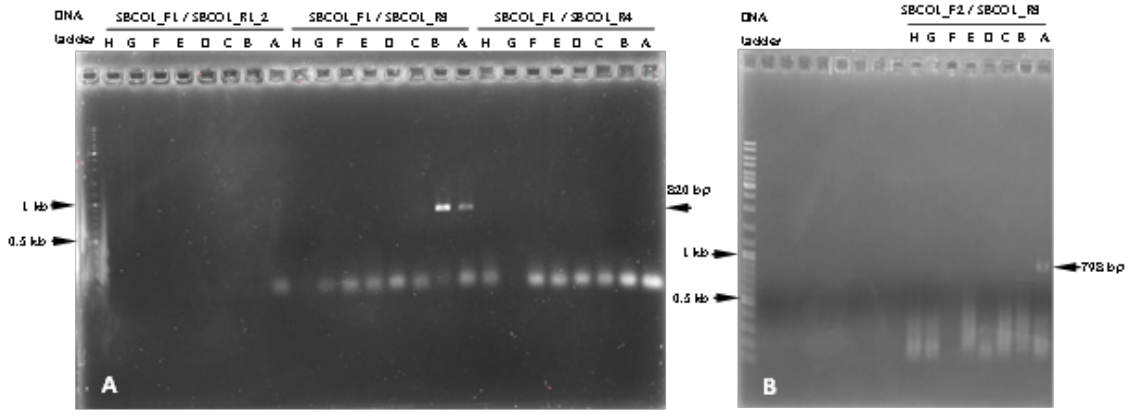

Figure 4. Optimal temperature of the DNA primer used to amplify the *CO1* gene in stingless bees on a 1% agarose gel with ethidium bromide under UV light. A) Suitable temperature of DNA primer forward SBCO1_F1 reverse SBCO1_R1_2 forward SBCO1_F1 reverse SBCO1_R3 and forward SBCO1_F1 reverse SBCO1_R4 B) Suitable temperature of DNA primer forward SBCO1_F2 reverse SBCO1_R3

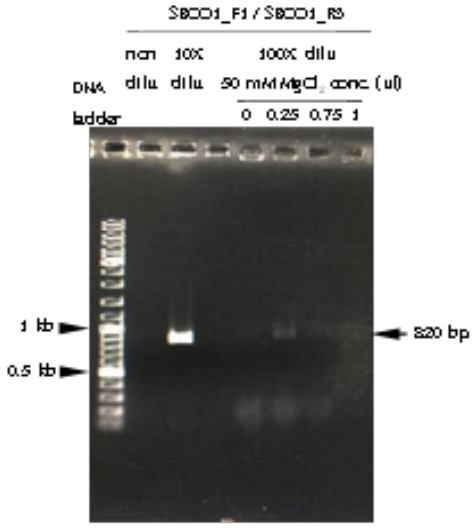

Figure 5. Concentrations of DNA template and magnesium chloride suitable for the CO1 gene amplification reaction on a 1% agarose gel with ethidium bromide under UV light.

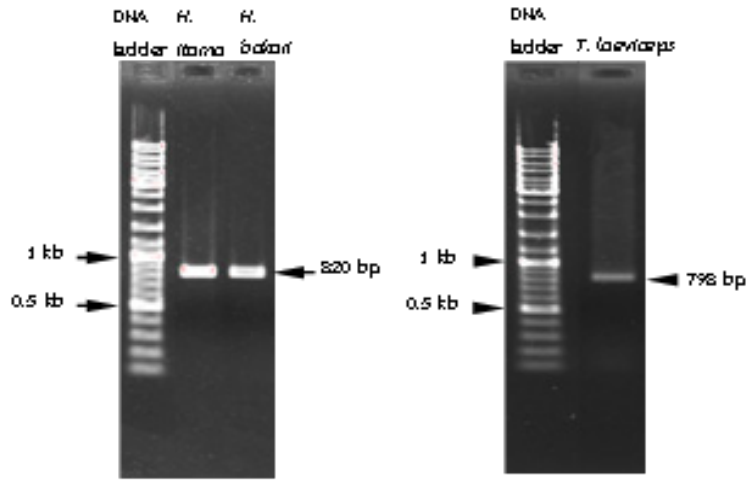

Figure 6. PCR products of 820 bp and 798 bp of the *CO1* gene from *H. itama, H. bakari*, and *T. laeviceps* stingless bee samples on 1% agarose gels with ethidium bromide under UV light

Table 3. Blast results using partial nucleotide sequences of the stingless bee *CO1* gene from the BOLD and NCBI databases.

Sample	BOLD Systems Database			NCBI Database		
	Closest species	Similarity (%)	Accession	Closest species	Similarity (%)	Accession
H. itama	*Heterotrigona itama*	97.87	KX113629	*Heterotrigona itama*	97.66	KX113629.1
	Lepidotrigona sp. aff terminata	87.16	Early-Release	*Melipona bicolor*	86.52	AF466146.2
	Lepidotrigona sp. aff terminata	87.16	Early-Release	*Melipona bicolor bicolor voucher MP83*	86.52	EU163158.1
	Lepidotrigona cf. terminata	87.16	Private	*Melipona bicolor*	86.12	AF370439.1
	Lepidotrigona terminata	86.24	Early-Release	*Melipona rufiventris rufiventris voucher MP77*	84.99	EU163151.1
H. bakari	No match	-	-	*Heterotrigona itama*	87.13	KX113629.1
	No match	-	-	*Melipona crinita voucher MP92*	85.77	EU163164.1
	No match	-	-	*Melipona panamica voucher MP1*	85.64	EU163096.1
	No match	-	-	*Melipona solari voucher MP86*	85.51	EU163160.1
	No match	-	-	*Melipona sp. MP93*	85.53	EU163165.1
T. laeviceps	*Tetragonula cf. laeviceps*	99.12	Private	*Tetragonula pagdeni*	86.72	NC_066054.1
	Tetragonula cf. laeviceps	98.82	Private	*Tetragonula iridipennis voucher DOGR Voucher*	85.39	NC_081039.1
	Tetragonula SE Asia 03	92.33	Private	*etragonula iridipennis voucher DOGR Voucher*	85.39	OQ103112.1
	Tetragonula cf. laeviceps ssp 7	87.32	Early-Release	*Cephalotrigona sp. MP106*	85.17	EU163102.1
	Tetragonula cf. minor ssp 1	86.69	Early-Release	*Cephalotrigona sp. MP89*	85.04	EU163161.1

2.5 Nucleotide sequence analysis using databases Bioinformatics and evolutionary relationships (Phylogenetic tree)

By comparing the similarity of the first 5 nucleotide sequences from the database (Table 3), it was found that the *H. itama* sample had the highest percentage of similarity to *H. itama* with the Bold systems and NCBI databases, equal to 97.87. and 97.66 percent, respectively, while *T. laeviceps* was 99.12 percent similar to *T. cf. laeviceps* and 87.13 percent to *Tetragonula pagdeni*, respectively.

By establishing a phylogenetic relationship using the nucleotide sequence of the *CO1* gene using the 1,000 bootstrap method, Kimura 2-parameter model, it was found that the strains *H. itama* and *H. bakari* were in the same group. while the smaller *T. laeviceps* species has been separated into another group (Figure 7). Francoso et al. (2019) used the nucleotide sequence of the *COX1* gene to identify the Carbonaria stingless bee species, which is a species complex or cryptic species that cannot be definitely separated using morphological characteristics. It was found that the stingless bee species *Tetragonula carbonaria* and *T. hockingsi* were different and different from other species. Hurtado-Burillo et al. (2013) used DNA barcode techniques to classify stingless bee species Scaptotrigona that has been moved from another source. It was found that the species *S. mexicana* and *S. hellwegeri* were closely related. In addition, *S. mexicana* is a complex species.

From many studies, it has been found that the application of morphological characteristics in combination with DNA barcode techniques allows for the separation and identification of stingless bee species in more detail. Ndungu et al. 2017 identified the stingless bee species and complex species within the same species (cryptic variation within species) in Kenya using morphological characteristics and the CO1 gene as a DNA barcode, it was found that the stingless bee can be divided into 3 groups: Group 1 *Meliponula bocandei*, Group 2 *M. lendliana* and *Plebeina hildebrandti*, Group 3 *Dactylurina schmidti*, *M. ferruginea* black and *M. ferruginea* reddish brown, respectively. The *CO1* gene is a DNA barcode used to separate *M. ferruginea* black and *M. ferruginea* reddish brown into different types. Sayusti et al. (2021) used nest structure characteristics along with morphology and DNA barcode techniques to identify the species *Tetragonula sapiens*, *T. clypearis*, *T. fuscobalteata*, *Lepidotrigona terminata* and *Wallacetrigona incisa* in Indonesia. It was found that the above method can be used to separate stingless bees in the genus Tetragonula as well.

3. Chemical profile of stingless bee honey

From the analysis of the effective compounded substances in the three species of stingless bee honey (Table 4), it was found that the compounded substances in the honey of the *T. laeviceps* specie were the most numerous, followed by *H. itama* and *H. bakari*. There were 191, 79, and 70 types, respectively. The substances found included both substances that had been previously reported and substances that had not previously been reported to be found in stingless bee honey. Biluca et al. (2017) extracted phenolic compounds from 9 species of stingless bee honey from Brazil using the LC–ESI–MS/MS (Liquid chromatography-electrospray ionization-tandem mass spectrometry) technique. It was found that there were compounded substance

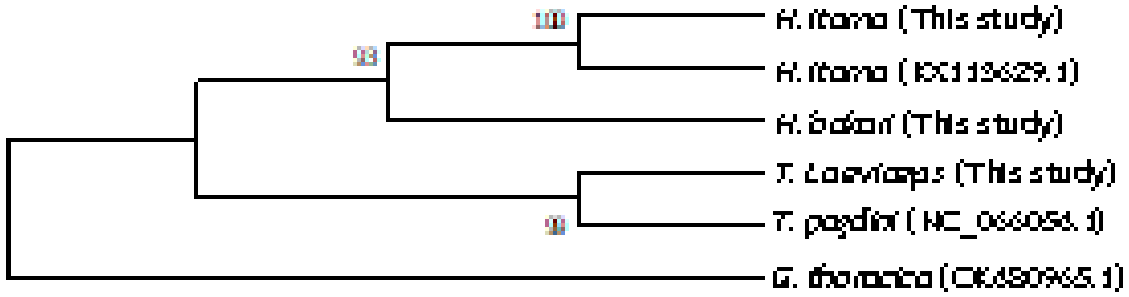

Figure 6. Evolutionary relationship of the stingless bee

in all 9 species of honey. It contains 26 phenolic compounds, 12 phenolic acids, 9 flavonoids, 3 phenolic aldehydes, 1 coumarin and 1 diterpene. Nisar et al. (2019) used a reversed-phase technique HPLC analysis of the compounds in stingless bee honey found that it consisted of gallic acid, rutin, ascorbic acid, quercetin, and kaempferol in *Apis dorsata* honey. Chew et al. (2018) used LC MS/MS to separate bioactive compounds from *A. dorsata* honey, which found organic acids including gluconic acid, succinic acid, hydroxybenzoic acid, hydroxydecanoic acid, abscisic acid, hydroxyoctanic acid, and phenic acid. Nolic acid includes caffeic acid and salicylic acid, while flavonoids include luteolin, hesperetin, kaempferol, apigenin, 3,7,4-trihydroxyflavone, naringenin, chrysin, fisetin, vitexin, isoorientin, and xanthohumol. Avila et al. (2018a, b) analyzed phenolic compounds of 32 samples of farm-raised scaptotrigona honey from 4 species of Scaptotrigona: *M. bicolor*, *M. quadrifasciata*, *M. marginate* and *S. bipuncata*. using HPLC-PDA (photodiode array detector) and analyzing the structure of the substance using Q-TOF–MS, it was found to contain phenolic compounds. The bioactive ingredients include p-coumaric acid, quercetin and hesperetin for anti-bacterial and antioxidant properties. In addition, it was found that stingless bee honey had 45 percent higher antioxidants and biological activities than *Apis mellifera*. Biluca et al. (2017) found that stingless bee honey is rich in potassium, calcium, sodium, and magnesium as components. From comparing the chemicals that are the main components, it was found that the honey extracted by the experimental method was more effective in extracting more substances than other methods.

4. Quantity analysis of the sweetness and acidity of stingless bee honey

From the analysis of the amount of honey from the *H. bakari* and *H. itama* species, it was found that the month of harvest had a significant effect on the amount of honey. Also, the amount of honey from the stingless bee was higher in May than in July. And different bee species have different effects on the amount of honey. The *H. bakari* specie gave more honey than *H. itama*, in both May and July (Figure 7). As for the percentage of sweetness, it was found that the different months of harvest resulted in significantly different percentages of sweetness in the *H. itama* species, but no differences were found in the *H. bakari* species. Also, in May, the percentage of sweetness was significantly different sweetness had higher values than July (Figure 8). Stingless bee honey from the *T. laeviceps* species, which is a small species. Mostly, they collect only once a year. The average nectar for the whole year is approximately 1.03 kilograms. As for the acidity, it was found that both species of stingless bee honey had an increased acidity value (Figure 9). General honey is different from stingless bee honey that has high moisture and low sweetness. After the stingless bee honey is stored in a cerumen pot, microorganisms, mainly bacteria in the genus bacillus and yeast, convert some of the sugar into alcohol through anaerobic fermentation and converted to acetic acid. Sugars are also converted to lactic acid and water through lactic fermentation and other types of acidification (Vit et al., 2013; Souza et al., 2021).

Table 4. Chemical profile of stingless bee honey 3 species

No	*T. laeviceps*				*H. itama*				*H. bakari*			
	Negative mode		Positive mode		Negative mode		Positive mode		Negative mode		Positive mode	
	Name	Formu-lar	Name	Formu-lar	Name	Formu-lar	Name	Formu-lar	Name	Formu-lar	Name	Formular
1	D-Sorbitol	C6 H14 O6	Pyroglutamic acid	C5 H7 N O3	D-Sorbitol	C6 H14 O6	Sucrose	C12 H22 O11	D-Sorbitol	C6 H14 O6	Poppy acid	C7 H4 O7
2	Galactonic acid	C6 H12 O7	Adenine	C5 H5 N5	Hyaluronic acid	C16 H27 N O12	Acetyl-maltose	C14 H24 O12	Galactonic acid	C6 H12 O7	N1-(5-Phospho-a-D-ribo-syl)-5,6-dimethylbenzimidaz-ole	C14 H19 N2 O7 P
3	Pyroglutamic acid	C5 H7 N O3	L-3-Amino-2-(oxalylami-no)propanoic acid	C5 H8 N2 O5	Pyroglutamic acid	C5 H7 N O3	Debromohymenialdisine	C11 H11 N5 O2	Mecarbinzid	C13 H16 N4 O3 S	Sucrose	C12H22 O11
4	Succinic acid	C4 H6 O4	9-Aminoacridine	C13 H10 N2	Deoxyuridine monophosphate (dUMP)	C9 H13 N2 O8 P	Debromohymenialdisine	C11 H11 N5 O2	Hyaluronic acid	C16 H27 N O12	Theophylline	C7 H8 N4 O2
5	1,2,3-Trihydroxybenzene	C6 H6 O3	Reduced pyocyanine	C13 H12 N2 O	2-Deoxy-D-Ri-bose	C5 H10 O4	(1alpha,2alpha,4betaH,6al-pha,8R)-p-Men-thane-2,6,8,9-tetrol	C10 H20 O4	2,5-Dimeth-yl-3-(meth-ylthio)furan	C7 H10 O S	4-(Hydroxymethyl)benzenedi-azonium(1+)	C7 H7 N2 O
6	2-hydroxy-butanoic acid	C4 H8 O3	Dihydroeuparin	C13 H14 O3	Succinic acid	C4 H6 O4	(1alpha,2alpha,4betaH,6al-pha,8R)-p-Men-thane-2,6,8,9-tetrol	C10 H20 O4	Deoxyuridine monophos-phate (dUMP)	C9 H13 N2 O8 P	6-Acetyl-D-glucose	C8 H14 O7
7	Methyl hydrogen fumarate	C5 H6 O4	Eupatoriochromene	C13 H14 O3	L-Phenylalanine	C9 H11 N O2	2,6-Dimethyl-1,8-octanedi-oic acid	C10 H18 O4	Succinic acid	C4 H6 O4	1-O-Galloylglycerol	C10 H12 O7
8	4-Aminocatechol	C6 H7 N O2	(R)-Bitalin A	C13 H14 O3	L-Phenylalanine	C9 H11 N O2	(1S,2S,4R,8R)-p-Menthane-1,2,8,9-tetrol	C10 H20 O4	Isopentenyl py-rophosphate	C5 H12 O7 P2	alpha,beta-Trehalose	C12 H22 O11
7	Hydroquinone	C6 H6 O2	2,6-Dimethyl-1,8-octane-dioic acid	C10 H18 O4	Pyrocatechol	C6 H6 O2	Methionyl-Histidine	C11 H18 N4 O3 S	1,2,3-Trihy-droxybenzene	C6 H6 O3	Clitoriacetal	C19 H18 O9
8	DL-Phenylalanine	C9 H11 N O2	(1S,2S,4R,8R)-p-Men-thane-1,2,8,9-tetrol	C10 H20 O4	L-α-Hydroxyiso-valeric acid	C5 H10 O3	3-Hydroxycapric acid	C10 H20 O3	Pyrocatechol	C6 H6 O2	Tryptophyl-Lysine	C17 H24 N4 O3
9	2-Acetylfuran	C6 H6 O2	Xestoaminol C	C14 H31 N O	TEPP	C8 H20 O7 P2	3-Methoxy-4-Hydroxy-phenylglycol Sulfate	C9 H12 O7 S	L-Phenylalanine	C9 H11 N O2	Gibberellin A98	C20 H26 O6

Table 4. (Continued 1)

No	*T. laeviceps*				*H. itama*				*H. bakari*			
	Negative mode		Positive mode		Negative mode		Positive mode		Negative mode		Positive mode	
	Name	Formu-lar	Name	Formu-lar	Name	Formu-lar	Name	Formu-lar	Name	Formu-lar	Name	Formular
10	L-α-Hydroxyisovaleric acid	C5 H10 O3	Dambonitol	C8 H16 O6	D-(+)-3-Phenyl-lactic acid	C9 H10 O3	3-Methoxy-4-Hydroxy-phenylglycol Sulfate	C9 H12 O7 S	3,4-Dihydroxy-benzoic acid	C7 H6 O4	Lys Trp	C17 H24 N4 O3
11	Hydroxyphenyllactic acid	C9 H10 O4	6-Acetyl-D-glucose	C8 H14 O7	Asp-Ile-OH	C15 H18 N2 O8	Imiquimod	C14 H16 N4	L-α-Hydroxyiso-valeric acid	C5 H10 O3	α-Toxicarol	C23 H22 O7
12	1-(4-Methoxyphenyl)-2-ni-troethylene	C9 H9 N O3	3,7-Dimethyl-2E,6E-deca-dien-1,10-dioic acid	C12 H18 O4			(3S,5R,6R,7E)-3,5,6-Trihy-droxy-7-megastigmen-9-one	C13 H22 O4	1H-Imidaz-ole-4-carbox-amide, 5-[3-(hy-droxymeth-yl)-3-meth-yl-1-triazenyl]-	C6 H10 N6 O2	Robustic Acid	C22 H20 O6
13	Vanilpyruvic acid	C10 H10 O5	Dipropyl hexanedioate	C12 H22 O4	trans-Cinnamic acid	C9 H8 O2	10-hydroxy-2E-decenoic acid	C10 H18 O3	Nithiazide	C6 H8 N4 O3 S	Vellokaempferol 3,5-dimethyl ether	C22 H20 O6
14	2-hydroxy pelargonic acid	C9 H18 O3	Limonen-6-ol-pivalate	C15 H24 O2	Indolelactic acid	C11 H11 N O3			4-formyl Indole	C9 H7 N O	Decarboxy-Norlobaric Acid	C23 H26 O6
15	Fraxetin	C10 H8 O5	Humulene diepoxide A	C15 H24 O2	(3S,4S)-3-hy-droxytetradec-ane-1,3,4-tricar-boxylic acid	C17 H30 O7	(4S,6R)-p-Mentha-1,8-di-ene-6,7-diol 7-glucoside	C16 H26 O7	Fraxetin	C10 H8 O5	Deguelin(-)	C23 H22 O6
16	(S)-(-)-2-Hydroxyisocaproic acid	C6 H12 O3	Perlolyrine	C16 H12 N2 O2	Abscisic Acid (cis,trans)	C15 H20 O4	Linalool 3,7-oxide be-ta-primeveroside	C21 H36 O11	(S)-(-)-2-Hy-droxyisocaproic acid	C6 H12 O3	Bisindolylmaleimide I	C25 H24 N4 O2
17	Zanthobisquinolone	C21 H18 N2 O4	Polyethylene, oxidized	C12 H20 O5	Matairesinol	C20 H22 O6	(-)-trans-C75	C14 H22 O4	Asarinin (-)	C20 H18 O6	PI(22:1(11Z)/18:3 (9Z,12Z,15Z))	C49 H87 O13 P
18	Acetyl-DL-Leucine	C8 H15 N O3	Penciclovir	C10 H15 N5 O3	Quercetin	C15 H10 O7	(1S,2S,4S,5S)-2,4-Thujane-diol 4-O-beta-D-Glucopy-ranoside	C16 H28 O7	Indolelactic acid	C11 H11 N O3	6,8-Dihydroxy-1,7-diprenylxan-thone-2-carboxylic acid	C24 H24 O6
19	gamma-L-Glutamyl-L-me-thionine sulfoxide	C10 H18 N2 O6 S	(±)-Naringenin	C15 H12 O5	3',4',5'-Trihy-droxywogonin	C16 H12 O8	Gibberellin A67	C19 H24 O6	Sequiterpene Lactone 326	C15 H20 O4	(4E,8E,10E-d18:3)sphingosine	C18 H33 N O2

Table 4. (Continued 2)

No	*T. laeviceps*				*H. itama*				*H. bakari*			
	Negative mode		Positive mode		Negative mode		Positive mode		Negative mode		Positive mode	
	Name	Formu-lar	Name	Formu-lar	Name	Formu-lar	Name	Formu-lar	Name	Formu-lar	Name	Formular
20	D-(+)-3-Phenyllactic acid	C9 H10 O3	C16 Sphinganine	C16 H35 N O2	Luteolin	C15 H10 O6	2,6-Dimethyl-6-O-beta-D-quinovopyranosyl-7-octadecenoic acid	C16 H28 O7	Abscisic Acid (cis,trans)	C15 H20 O4	C16 Sphinganine	C16 H35 N O2
21	Asp-Ile-OH	C15 H18 N2 O8	D-erythro-Sphingosine C-15	C15 H31 N O2	Dihydroroseoside	C19 H32 O8	Unshuoside A	C16 H28 O7	Matairesinol	C20 H22 O6	Phytosphingosine	C18 H39 N O3
22	N-Acetyl-L-phenylalanine	C11 H13 N O3	Saphenic acid methyl ester	C16 H14 N2 O3	Embelin	C17 H26 O4	Florilenalin	C15 H20 O4	Quercetin	C15 H10 O7	Clausarinol	C24 H30 O6
23	Erioflorin acetate	C21 H26 O7	Florilenalin	C15 H20 O4	Prosopinine	C16 H33 N O3	Hydroxymyricanone	C21 H24 O6	(±)-Naringenin	C15 H12 O5	Prosopinine	C16 H33 N O3
24	(7'R*,8'S*)-Methyl 4,7'-epoxy-3,8'-bilign-7-ene-4',9'-dihydroxy-3',5-dimethoxy-9-oate	C21 H22 O7	Prosopinine	C16 H33 N O3	JWH 073 2'-naphthyl-N-(1-methylpropyl) isomer	C23 H21 N O	6-Epi-7-isocucurbic acid glucoside	C18 H30 O8	Jasmonic acid	C12 H18 O3	15-oxo-hexadecanoic acid	C16 H30 O3
25	Duartin, Dimethyl Ether	C20 H24 O6	3',4',5,7-Tetrahydroxyisoflavanone	C15 H12 O6	Phytomonic Acid	C19 H36 O2	PI(22:1(11Z)/18:3(9Z,12Z,15Z))	C49 H87 O13 P	6,7-dihydroxy Bergamottin	C21 H24 O6	3,5-Dimethoxy-8,8-dimethyl-2-phenyl-4H,8H-benzo[1,2-b:3,4-b']dipyran-4-one	C22 H20 O5
26	Isoeugenitol	C11 H10 O4	2-Hydroxyhexadecanoic acid	C16 H32 O3	JWH 073 2'-naphthyl isomer	C23 H21 N O	GlcNAcbeta1-4Manbeta1-4Glcbeta-Cer(d18:1/24:1(15Z))	C62 H114 N2 O18	Kaempferol	C15 H10 O6	Verimol C	C18 H20 O4
27	Rubone	C20 H22 O7	5-methyl-tetradecanedioic acid	C15 H28 O4	Tributyrin	C15 H26 O6	1-aminopyrene	C16 H11 N	Isorhamnetin	C16 H12 O7	Epicatechin-(2beta->5,4beta->6)-ent-epicatechin	C30 H24 O12
28	Deoxyelephantopin	C19 H20 O6	3,4-Dimethyl-5-pentyl-2-furanheptanoic acid	C18 H30 O3	1-Phenyl-1,3-heptadecanedione	C23 H36 O2	Gingerenone B	C22 H26 O6	LY364947	C17 H12 N4	Monoacetoxyscirpenol	C17 H24 O6
29	Pinostilbenoside	C21 H24 O8	Diisobutyl phthalate	C16 H22 O4	Stearic acid	C18 H36 O2	C16 Sphinganine	C16 H35 N O2	Pinocembrin	C15 H12 O4	Deoxycorticosterone	C21 H30 O3

Table 4. (Continued 3)

No	*T. laeviceps*				*H. itama*				*H. bakari*			
	Negative mode		Positive mode		Negative mode		Positive mode		Negative mode		Positive mode	
	Name	Formu-lar	Name	Formu-lar	Name	Formu-lar	Name	Formu-lar	Name	Formu-lar	Name	Formular
30	Koaburanin	C21 H24 O8	Linoleamide	C18 H33 N O	1α,25-dihy-droxy-25,25-di-phenyl-26,27-di-norvitamin D3 / 1α,25-dihy-droxy-25,25-di-phenyl-26,27-di-norcholecalcif-erol	C37 H48 O3	Phytosphingosine	C18 H39 N O3	Embelin	C17 H26 O4	Androst-4-ene-3alpha,17be-ta-diol diacetate	C23 H34 O4
31	Gibberellin A51-catabolite	C19 H22 O5	16-Hydroxy-10-oxohexa-decanoic acid	C16 H30 O4	Stearic acid	C18 H36 O2	Xestoaminol C	C14 H31 N O	Hypercalin B	C33 H42 O5	Gingerglycolipid A	C33 H56 O14
32	Lariciresinol	C20 H24 O6	Phytosphingosine	C18 H39 N O3	1α,25-dihy-droxy-25,25-di-phenyl-26,27-di-norvitamin D3 / 1α,25-dihy-droxy-25,25-di-phenyl-26,27-di-norcholecalcif-erol	C37 H48 O3	2-Hydroxyhexadecanoic acid	C16 H32 O3	Arg Asn Arg	C16 H32 N10 O5		
33	(2S)-5,6,7,8,4'-Pentame-thoxyflavanone	C20 H22 O7	Valacyclovir	C13 H20 N6 O4	Isopalmitic acid	C16 H32 O2	Clausarinol	C24 H30 O6	Tributyrin	C15 H26 O6		
34	Sequiterpene Lactone 326	C15 H20 O4	Annosquamosin B	C19 H32 O3	Kolanone	C33 H42 O4	Prosopinine	C16 H33 N O3	1α,25-dihy-droxy-25,25-di-phenyl-26,27-di-norvitamin D3 / 1α,25-dihy-droxy-25,25-di-phenyl-26,27-di-norcholecal-ciferol	C37 H48 O3		

Table 4. (Continued 4)

No	*T. laeviceps*				*H. itama*				*H. bakari*			
	Negative mode		Positive mode		Negative mode		Positive mode		Negative mode		Positive mode	
	Name	Formu-lar	Name	Formu-lar	Name	Formu-lar	Name	Formu-lar	Name	Formu-lar	Name	Formular
35	Triptonide	C20 H22 O6	8-HpODE	C18 H32 O4			JWH 073 2'-naphth-yl-N-(1-methylpropyl) iso-mer	C23 H21 N O	all-trans-hepta-prenyl diphos-phate	C35 H60 O7 P2		
36	Eriodictyol	C15 H12 O6	1-Hydroxy-3,5-dime-thoxy-2-prenylxanthone	C20 H20 O5			Pro Met Leu	C16 H29 N3 O4 S	1-Phenyl-1,3-heptadec-anedione	C23 H36 O2		
37	Deoxysappanone B Trimethyl Ether	C19 H20 O5	9,10,18-TriHOME(12Z)	C18 H34 O5			N-Desethylquinagolide glucuronide	C24 H37 N3 O9 S	Linoleic acid	C18 H32 O2		
38	Hieracin	C15 H10 O7	Lansiumarin C	C21 H22 O5			Diisobutyl phthalate	C16 H22 O4	Kolanone	C33 H42 O4		
39	Dihydrosamidin	C21 H24 O7	2,6-Dimethyl-6-O-be-ta-D-quinovopyrano-syl-7-octadecenoic acid	C16 H28 O7			Amdinocillin	C15 H23 N3 O3 S	Phytomonic Acid	C19 H36 O2		
40	N1,N5,N10-Tricaffeoyl spermidine	C34 H37 N3 O9	(1S,2R,4R)-p-Menth-8-ene-2,10-diol 2-glucoside	C16 H28 O7			16-Hydroxy-10-oxohexa-decanoic acid	C16 H30 O4				
41	Matairesinol	C20 H22 O6	Deoxysappanone B 7,3'-Dimethyl Ether Ac-etate	C20 H20 O6			D-erythro-Sphingosine C-15	C15 H31 N O2				
42	1-(4-Hydroxy-3,5-dime-thoxyphenyl)-2-[2-me-thoxy-4-(1-propenyl)phe-noxy]-1-propanol	C21 H26 O6	Batyl Alcohol	C21 H44 O3			Acetyl tributyl citrate	C20 H34 O8				
43	8-C-beta-D-Glucopyrano-syldiosmetin	C22 H22 O11	Kanzonol P	C22 H24 O5			Annosquamosin B	C19 H32 O3				
44	Quercetin	C15 H10 O7	(+)-Fargesin	C21 H22 O6			Montanol	C21 H36 O4				
45	Bluensomycin	C21 H39 N5 O14	(7'x,8'x)-4,7'-Epoxy-3,8'-bilign-7-ene-3,5'-dime-thoxy-4',9,9'-triol	C20 H22 O6			PG(18:3(9Z,12Z,15Z)/0:0)	C24 H43 O9 P				

Table 4. (Continued 5)

No	*T. laeviceps*				*H. itama*				*H. bakari*			
	Negative mode		Positive mode		Negative mode		Positive mode		Negative mode		Positive mode	
	Name	Formu-lar	Name	Formu-lar	Name	Formu-lar	Name	Formu-lar	Name	Formu-lar	Name	Formular
46	(±)-Naringenin	C15 H12 O5	Lariciresinol	C20 H24 O6								
47	Lanceoletin	C16 H14 O6	Kanzonol O	C22 H22 O6								
48	N1,N5,N10-Tricoumaroyl spermidine	C34 H37 N3 O6	7-Hydroxy-1,7-bis(4-hydroxy-3-methoxyphenyl)-1-heptene-3,5-dione	C21 H22 O7								
49	Luteolin	C15 H10 O6	Hydroxymyricanone	C21 H24 O6								
50	Pedalitin	C16 H12 O7	Deguelin(-)	C23 H22 O6								
51	6’’’-Deamino-6’’’-dehydro-6’’’-oxoneomycin C	C23 H43 N5 O14	8-Hydroxypinoresinol	C20 H22 O7								
52	Elephantin	C20 H22 O7										
53	Isoorientin 2’’-O-(E)-ferulate	C31 H28 O14	Cyclonormammein	C21 H26 O6								
54	6,7-dihydroxy Bergamottin	C21 H24 O6	2-(4-Allyl-2,6-dimethoxyphenoxy)-1-(4-hydroxy-3-methoxy-phenyl)-1-propanol	C21 H26 O6								
55	(2S)-5,6,7,3’,4’-Pentamethoxyflavanone	C20 H22 O7	alpha-Peroxyachifolide	C20 H24 O7								
56	17β-hydroxy Wortmannin	C23 H26 O8	Gibberellin A102	C20 H26 O7								
57	Kaempferol	C15 H10 O6	Lepidiumterpenyl ester	C23 H42 O4								
58	Apigenin	C15 H10 O5	3,5-Di-O-methyl-8-prenylafzelechin-4beta-ol	C22 H26 O6								

Table 4. (Continued 6)

No	*T. laeviceps*				*H. itama*				*H. bakari*			
	Negative mode		Positive mode		Negative mode		Positive mode		Negative mode		Positive mode	
	Name	Formu-lar	Name	Formu-lar	Name	Formu-lar	Name	Formu-lar	Name	Formu-lar	Name	Formular
59	Isorhamnetin	C16 H12 O7	MG(20:0/0:0/0:0)	C23 H46 O4								
60	Diosmetin	C16 H12 O6	Edulisin III	C21 H24 O7								
61	7,3',4'-Trihydroxy-3,8-dimethoxyflavone	C17 H14 O7	Visnadin	C21 H24 O7								
62	9,12,13-trihydroxy-10,15-octadecadienoic acid	C18 H32 O5	Garcimangosone C	C23 H24 O7								
63	Deoxysappanone B 7,3'-Dimethyl Ether Acetate	C20 H20 O6	Rosmic acid	C21 H26 O7								
64	2,3-dinor Thromboxane B1	C18 H32 O6	Clusin	C22 H26 O7								
65	11,12,13-trihydroxy-9-octadecenoic acid	C18 H34 O5	Antiarone K	C22 H26 O7								
66	2alpha,3alpha-(Difluoromethylene)-5alpha-androstan-17beta-ol acetate	C22 H32 F2 O2	Sphenostylin D	C22 H26 O7								
67	Embelin	C17 H26 O4	(+)-Tephropurpurin	C24 H24 O7								
68	19(R)-hydroxy-PGF1α	C20 H36 O6	Acetyl tributyl citrate	C20 H34 O8								
69	4beta-Hydroxyobovatachromene	C21 H22 O6	Cinnzeylanine	C22 H34 O8								
70	Simvastatin acid	C25 H40 O6	Armillaric acid	C23 H28 O7								
71	Bayogenin	C30 H48 O5	Lupinisoflavone H	C25 H26 O7								

Table 4. (Continued 7)

No	*T. laeviceps*				*H. itama*				*H. bakari*			
	Negative mode		Positive mode		Negative mode		Positive mode		Negative mode		Positive mode	
	Name	Formu-lar	Name	Formu-lar	Name	Formu-lar	Name	Formu-lar	Name	Formu-lar	Name	Formular
72	Abyssinin III	C25 H28 O6	Lupinisoflavone I	C25 H26 O7								
73	(±)12,13-DiHOME	C18 H34 O4	(+)-Syringaresinol	C22 H26 O8								
74	5'-Demethoxydeoxypodo-phyllotoxin	C21 H20 O6	(R)-Byakangelicinn 2'-(3-methylbutanoate)	C22 H26 O8								
75	Quillaic acid	C30 H46 O5	8-Acetoxy-4'-methoxy-pinoresinol	C23 H26 O8								
76	7,8-(2,2-Dimethylpyra-no)-3,4'-dihydroxy-5-me-thoxyflavan	C21 H22 O5	Melledonal A	C23 H28 O8								
77	Vaccenic acid	C18 H34 O2	7,8,3',4',5'-Pentame-thoxy-6'',6''-dimeth-ylpyrano[2'',3'':5,6] flavone	C25 H26 O8								
78	Euchrenone a6	C30 H34 O6	5,2',4',5'-Tetra-hydroxy-3-(3-hy-droxy-3-methylbu-tyl)-6'',6''-dimethylpyra-no[2'',3'':7,8]flavone	C25 H26 O8								
79	(24E)-15alpha-Acetoxy-3alpha-hydroxy-23-oxo-7,9(11),24-lanostatrien-26-oic acid	C32 H46 O6	(-)-Salvisyriacolide	C25 H40 O6								
80	Alisol C	C30 H46 O5	Diferuloylputrescine	C24 H28 N2 O6								
81	12R-hydroxy-9Z-octadece-noic acid	C18 H34 O3	Quasiprotopanaxatriol	C30 H50 O3								

Table 4. (Continued 8)

No	*T. laevicep*s				*H. itama*				*H. bakari*			
	Negative mode		Positive mode		Negative mode		Positive mode		Negative mode		Positive mode	
	Name	Formu-lar	Name	Formu-lar	Name	Formu-lar	Name	Formu-lar	Name	Formu-lar	Name	Formular
82	Tributyrin	C15 H26 O6	Panaxadiol	C30 H52 O3								
83	gamma-Mangostin	C23 H24 O6	23-Hydroxy-3-oxocy-cloart-24-en-26-oic acid	C30 H46 O4								
84	6Z,11Z-octadecadienoic acid	C18 H32 O2	Quillaic acid	C30 H46 O5								
85	Mangostinone	C23 H24 O5	Melilotigenin	C30 H46 O5								
86	17-Octadecynoic Acid	C18 H32 O2	Actinidic acid	C30 H46 O5								
87	Kolanone	C33 H42 O4	21beta-Hydroxyheder-agenin	C30 H48 O5								
88	Cholesterol glucuronide	C33 H54 O7	Ganoderiol I	C31 H50 O5								
89	16beta-16-Hydroxy-3-oxo-1,12-oleanadien-28-oic acid	C30 H44 O4	Lappaol C	C30 H34 O10								
90	Betulonic acid	C30 H46 O3	N1,N5,N10-Tricoumaroyl spermidine	C34 H37 N3 O6								
91	dimethoxy Curcumin	C23 H24 O6	Baccatin III	C31 H38 O11								
92	Maslinic Acid	C30 H48 O4	N1,N5,N10-Triferuloyl spermidine	C37 H43 N3 O9								
93	α-Mangostin	C24 H26 O6	Neoarctin A	C42 H46 O12								

Table 4. (Continued 9)

No	*T. laeviceps*				*H. itama*				*H. bakari*			
	Negative mode		Positive mode		Negative mode		Positive mode		Negative mode		Positive mode	
	Name	Formu-lar	Name	Formu-lar	Name	Formu-lar	Name	Formu-lar	Name	Formu-lar	Name	Formular
94	2-Hexyldecanoic acid	C16 H32 O2										
95	Kirenol	C20H34 O4										
96	Betulinic Acid	C30 H48 O3										
97	9R-hydroxy-12E-octadece-noic acid	C18 H34 O3										
98	Pinolenic Acid	C18 H30 O2										

3.4 Quantity analysis of the sweetness and acidity of stingless bee honey

From the analysis of the amount of honey from the *H. bakari* and *H. itama* species, it was found that the month of harvest had a significant effect on the amount of honey. Also, the amount of honey from the stingless bee was higher in May than in July. And different bee species have different effects on the amount of honey. The *H. bakari* specie gave more honey than *H. itama*, in both May and July (Fig. 7). As for the percentage of sweetness, it was found that the different months of harvest resulted in significantly different percentages of sweetness in the *H. itama* species, but no differences were found in the *H. bakari* specie. Also, in May, the percentage of sweetness was significantly different sweetness had higher values than July (Fig. 8). Stingless bee honey from the *T. laeviceps* species, which is a small species. Mostly, they collect only once a year. The average nectar for the whole year is approximately 1.03 kilograms. As for the acidity, it was found that both species of stingless bee honey had an increased acidity value (Fig. 9). General honey is different from stingless bee honey that has high moisture and low sweetness. After the stingless bee honey is stored in a cerumen pot, microorganisms, mainly bacteria in the genus bacillus and yeast, convert some of the sugar into alcohol through anaerobic fermentation and converted to acetic acid. Sugars are also converted to lactic acid and water through lactic fermentation and other types of acidification (Vit *et al*., 2013; Souza *et al*., 2021).

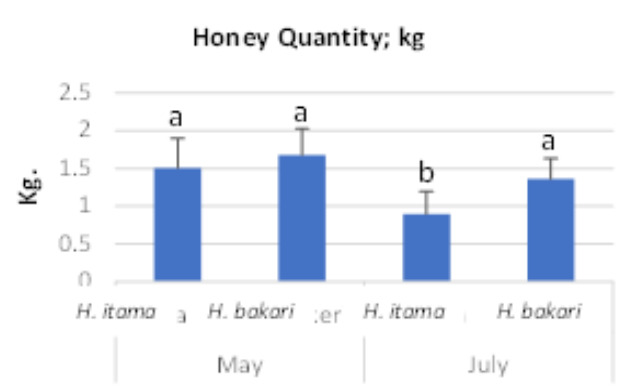

Figure 7. Quantity of stingless bee honey (kilograms) from *H. itama* and *H. bakari* species

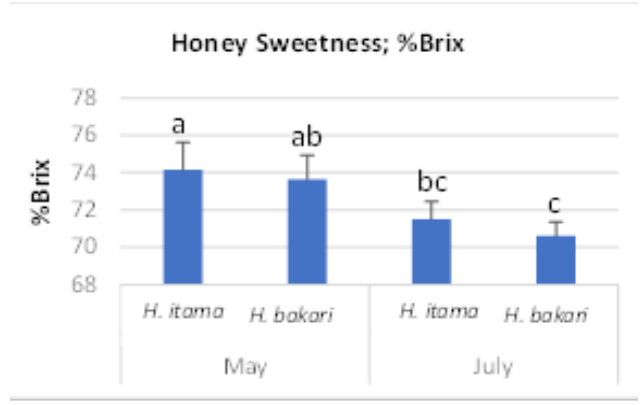

Figure 8. Percentage of sweetness (Brix) of *H. itama* and *H. bakari* honey

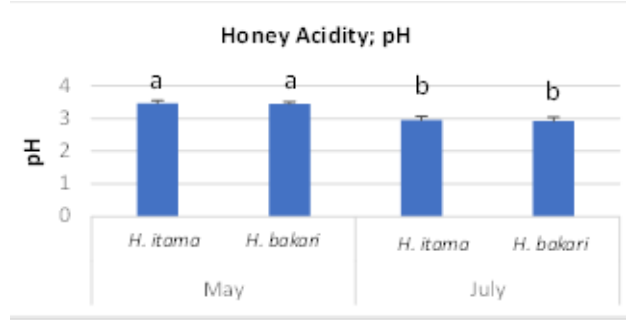

Figure 9. pH of *H. itama* and *H. bakari* honey

Characteristics Relationship of various factors that affect the stingless bee honey

The typical characteristics of stingless bee honey not only its sweet and sour taste but its important substances are more diverse than general honey also. This depends upon on the two influences: the foraging behavior of each species of stingless bee and the typical of food plants within its range, which has a great influence on determining the composition of important substances in the honey. In addition, the type of honey bee, the type of food plant and the season are also related to sweetness and acidity. It was found that when collecting the stingless honey during the rainy season, the sweetness decreased and the acidity increased. When curing the honey at a temperature of 35 degrees Celsius for 15, 30, and 45 days, it was found that the acidity increased significantly. The information from this research can be used as a guideline for the quality improvement of the stingless bee honey in commercialize purpose.

Conclusions

Effective compounded substances in all 3 species of stingless bee honey are in the flavonoid, phenolic compound, and terpene groups and different depend on the species of stingless bee. The diversity and abundance of the ecosystem in which stingless bees live. Combining biomolecular techniques with stingless bee morphology has more potential accurately classify especially the complex type of stingless bee.

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