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Original Research Article

Physicochemical Properties and Biochemical Profiling of Local Commercial Forest and Tualang Honeys

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ABSTRACT

Local varieties of honey from Sabah, such as Forest honey (FH) and Tualang honey (TH), are renowned for their manifold health benefits and diverse compounds, which are influenced by floral sources and geographical locations. Despite their prevalence, the physicochemicals, microbial properties, and metabolite compounds of these honey variants remain unexplored. The study aims to analyze and present the physicochemical and biochemical compositions of two local honeys purchased from the local market in Kundasang, Sabah, Malaysia. Both honey samples exhibited comparable characteristics in terms of moisture, fat, sugar content, and pH values, falling within the standard range for honey. However, FH demonstrated higher ash and protein levels than TH, with FH's ash and protein contents at 0.23±0.04% and 0.72±0.01%, respectively, contrasting with TH's 0.11±0.01% and 0.34±0.01%. Hydroxymethylfurfural (HMF) levels differed significantly between the two samples, registering 1.78±0.12% for FH and 2.86±0.04% for TH, both of which are within acceptable consumption ranges. Total plate count findings indicated a significant difference, with 1.02±0.03 log CFU/g for FH and 0.41±0.002 log CFU/g for TH, yet within recommended safe limits. Liquid Chromatography-Quadrupole Time of Flight-Tandem Mass Spectrometry (LC-QTOF-MS) revealed TH yielded 20 compounds, surpassing FH's 16 compounds, including previously unreported compounds. Given the diverse compounds' versatility, this study advocates for their application in healthcare and as biomarkers for honey identity.

Keywords: Forest honey, Tualang honey, Metabolite profiling, Liquid chromatography-quadrupole time of flight-tandem mass spectrometry.

Introduction

Honey, which naturally possesses sweetness, exhibits a sophisticated chemical composition and a range of health-promoting attributes.[1] Honey typically consists of around 180 compounds, including sugars, water, proteins, vitamins, free amino acids, enzymes, organic acids, and phenolic compounds.[2] Bees produce honey using invertase enzymes from their hypopharyngeal glands to break down the sugars present in plant nectar, specifically the disaccharide sucrose, into the monosaccharides glucose (dextrose) and fructose (levulose).[3] Honey is a natural sweetener, offering essential nutrients and acting as a significant source of energy due to its high sugar content.[4] Numerous studies have uncovered various benefits of honey, including, antioxidant effects, antimicrobial properties and nematocidal properties, anti-cancer activity, and anti-inflammatory effects, in addition to wound healing properties.[5,6]

The advantageous qualities of honey can only be experienced by consuming high-quality natural honey.[7] The composition of honey is influenced by numerous factors, including the specific botanical and floral sources, the method of processing, and surrounding environmental conditions.[2]

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Geographical location is an especially determinant factor of the quality and physicochemical properties of honey. Accordingly, honey originating from different areas is typically found to have different properties, which are also often associated with unique traditional applications.

The properties of honey are modulated by many factors, including geographical origin. The same tree planted in two different areas might grow and produce different metabolites, which further regulate its nectar composition, which is then used by the bees for honey production. Brzosko and Mirski [8] supported this assumption through a finding that geographical region plays a significant role in the sugar concentration and composition of an orchid's nectar. Furthermore, Satriadi *et al.* (2023) [9] observed variations in the proximate analysis results of Kelulut honey from Indonesia and Malaysia, with these differences attributed to distinct geographical locations.

Tualang, Acacia, Forest, and Kelulut are among the most prevalent honey types found in Malaysia, including the Sabah region.[10] Tualang honey, produced by *Apis dorsata*, derives its name from the Tualang tree, also known as *Koompassia excelsa*, one of the tallest tropical rainforest trees in Southeast Asia, where bees build their hives.[11] Acacia honey, a monofloral variety, is obtained from *Apis mellifera*, a cultivated bee that collects extrafloral nectar from the *Acacia mangium* tree, a member of the *Fabaceae pea* family, commonly referred to as the forest mangrove, and which typically reaches heights of up to 30 meters.[12] Finally, Kelulut honey is created by *Trigona* spp., a stingless bee that forages nectar from a variety of polyfloral sources.[13] In brief, the various honey varieties display specific attributes tied to their origins and the bee species involved in the production, with these disparities being shaped by the plant environments where these bees flourish.

These honeys are not only widely available in various local markets in Sabah, Malaysia, but are also believed to exhibit many health benefits

according to traditional knowledge. Nevertheless, the quality of honey might be found to be different, as it was directly collected from the wild trees with no standardized production and handling procedures.[14] In light of extant literature on various local honey varieties in Malaysia, including Sabah, it is noteworthy that this investigation has identified incongruities in their physicochemical properties. This disparity may be attributed to the diverse sources or temporal parameters from which the honey specimens under examination were collected, differing from those utilized in antecedent studies. Nevertheless, to date, no reports have comprehensively described the physicochemical and microbiological properties of local honey from Sabah, coupled with metabolite compound contents. This study, therefore, aims to provide the physicochemical and biochemical compounds of two local honeys, TH, and FH from street vendors in Sabah, Malaysia. This study describes, for the first time, compounds in both honeys that promise future applications.

Materials and Methods

Honey collection

TH and FH were purchased from a local vendor in Kundasang, Sabah, Malaysia, in May 2023. The vendors obtained TH and FH from the honey collectors approximately one week after harvesting. All samples were stored at 4℃ until further analysis.

Physicochemical analysis

Moisture

Moisture content was determined according to Kumar *et al.* (2010).[15] Briefly, five grams of each sample were carefully weighed and placed in a hot air oven, which was maintained at a temperature of 105˚C ± 2˚C. Each sample was left to dry for a minimum of two hours and allowed to cool in a desiccator. The lowest weight observed was taken. The moisture content was then calculated based on the following formula:

Moisture content (%) = Loss in weight after drying / Initial weight of samples x 100

Ash content

Ash content was determined based on AOAC Official Method 990.11.[16] Each desiccated sample was first incinerated using a blue flame from a burner until smoke ceased to emanate. Subsequently, the porcelain or crucible dish was placed in a muffle furnace held at a temperature of 500˚C ± 5˚C for a duration of 1 to 2 hours. Afterward, the sample was allowed to cool in a desiccator, and its weight was then measured. The ash content was then calculated based on the following formula:

Ash content (%) = Weight of ash (g)/ weight of sample (g) x 100

Fat content

Fat content was determined using the Soxhlet extraction method according to AOAC 2003.06 and following Aneni *et al*. (2023)[17] A moisture-free sample of approximately 1g was wrapped in filter paper, placed within a fat-free thimble, and subsequently inserted into the extraction tube. The receiving beaker, having been weighed, cleaned, and dried, was charged with petroleum ether and secured within the apparatus. The water and heater were activated to initiate the extraction process. Following 4-6 cycles of siphoning, the ether was allowed to evaporate, and the beaker was disconnected before the final siphoning. The extracted content was then transferred to a pristine glass dish and underwent an ether wash. The ether was then evaporated using a water bath. Subsequently, the dish was positioned in an oven at 105℃ for a duration of 2 hours and subsequently cooled within a desiccator. The percentage of fat content was calculated according to the following equation:

Fat content (%) = Weight of fat obtained (g) / Weight of sample (g) × 100

Protein content

The protein content was quantified using the Kjeldahl method as described in AOAC Official Method 973.48.[16] The Kjeldahl procedure consists of three sequential steps: digestion, distillation, and titration. During the digestion phase, the organic nitrogen present in honey is transformed into ammonium sulfate in the presence of a mixed catalyst, typically at around 370 ℃. In the subsequent distillation step, the digested sample is rendered alkaline with sodium hydroxide, causing the nitrogen to be distilled as ammonia nitrogen. This ammonia nitrogen is captured in a boric acid solution and subsequently quantified through titration using 0.1 N hydrochloric acid. The protein content is then determined by multiplying the nitrogen content by a universal conversion factor of 6.25.[18]

Sugar content

Sugar content was determined based on Khalil *et al*. (2012)[19] Twenty-five percent (w/v) of honey solution was suspended in distilled water. A refractor metric method was used to determine the total sugar content for each honey sample. An ambient temperature is required in measuring the refractive indices of honey samples by using an Atago handheld refractometer (ATAGO Co., Ltd, Japan). Meanwhile, the percentage of sucrose content was calculated per g/mL of honey. Ultra-pure water was employed to calibrate the refractometer to a zero reading.

pH value

The measurement was conducted using a digital Cyberscan pH meter (Eutech Instrument, Singapore) at 28±2℃, with 10-fold diluted sample used in the measurement. Calibration of the pH meter was conducted prior to the measurement using standard buffer solutions (pH 4.0 and 7.0).

Hydroxymethylfurfural (HMF) content

HMF content was measured according to Lim *et al.* (2022).[14] Five grams of honey were weighed and dissolved in approximately 25 mL of distilled water. Subsequently, 0.5 mL of Carrez solution I was introduced and thoroughly mixed. Following that, 0.5 mL of Carrez solution II was added, and mixed in, and the volume was adjusted to 50 mL using water. A drop of ethanol was included to eliminate foam formation. The resulting mixture was then filtered using filter paper, discarding the initial 10 mL of filtrate.

Further, five milliliters of this filtered mixture was separately transferred into two test tubes. To the first test tube, identified as the sample solution, an additional 5 mL of water was added. In the second test tube, labeled as the reference solution, 5 mL of 0.2% sodium bisulphite solution was introduced.

The absorbance of the sample and reference solutions were measured using a fluorescence spectrophotometer (excitation=284; emission=336 nm), using a quartz cell, which was performed within an hour after sample preparation. Further dilution with water or sodium bisulphite was conducted for the sample or reference solution, respectively, whenever the reading exceeded 0.6.

Total plate count (TPC)

TPC was determined based on Khadra *et al.* (2018)[20] Briefly, 10 grams of honey were suspended in 90 ml of 0.1% phosphate buffer solution. A series of dilutions were then carried out and 0.1 ml was spread on Plate Count Agar (PCA) (OXOID). The culture was incubated for 72 h at 37 °C.

Sample Preparations for Phenolic Analysis

Sample preparation was performed according to Seraglio *et al*. (2016).[21] Each sample was dissolved in 0.5 mL of deionized water, vortexed (IKA®, USA) for 40 seconds, and shaken in a Thermo shaker (Eppendorf, Germany) for 4 minutes. A 100 µL aliquot of extract and 900 µL of mobile phase (98:2; A:B) were then transferred to a 1.5-mL polypropylene microtube, centrifuged (Eppendorf, Germany) at 14,000 rpm for 20 minutes, filtered through 0.22 µM membrane filters (Membrane Solution, WA, USA) and transferred to a vial before injection.

Chromatographic separation and mass spectrometric analysis
The analysis was also performed according to Seraglio *et al.* (2016).[21] Samples were analyzed using LC-QTOF-MS, BRUKER IMPACT II (Bruker Daltonics GmbH Fahrensheitstr, Germany) operating in electron spray ionization (ESI) in positive ionization mode that scans between 50-1500 m/z, dry gas as 10 L/min, dry heater at 250 °C and nebulizer at 2.0 bar. A reversed-phase C18 column (3µm 2.1 x 150 mm) (Thermo Scientific, USA) with a flow rate adopted was 300 µL min^{-1}, and a volume of 5 µL was injected for all sample solutions. The mobile phase was composed of solvent A (water with 0.1% formic acid) and solvent B (acetonitrile with 0.1% formic acid). The mobile phase gradient was programmed as follows: 98% A (v/v) from 0 to 4.0min, 98–80% A (v/v) from 4.0–7.0 min, 80–10% A (v/v) from 7.0–14.0 min, 10% A (v/v) from 14.0– 15.0 min, 10–98% A (v/v) from 15.0–17.0 min. The total run time was 20 minutes, and the column equilibration time between each run was 4 min. Bruker Compass DataAnalysis 4.3 was used for data acquisition and processing. Accurate mass and MS/MS spectral data were compared to the HR_msms_nist and plant metabolites databases.

Statistical analysis
Data are presented as mean ± standard deviation from 3 independent replications. The differences among the means were analyzed using Analysis of Variance (ANOVA) with a post-hoc test of Tukey. P values of less than 0.05 and 0.01 are considered significant and very significant differences, respectively.

Results and Discussion

Physicochemical properties
The findings (Table 1) indicated that FH exhibits comparably higher moisture content (10.79 ±0.03%) compared to TH (10.69±0.42%) (P>0.05). These values are considerably acceptable according to the Codex standard for Honey [22] where the moisture content of honey is acceptably below 20%. The moisture parameter is an important factor in assessing the maturity, density, viscosity, state, stability, and overall quality and processing characteristics of honey.[23] Nevertheless, some reports have also indicated that there were discrepancies in moisture content among different honeys. For example, Dan *et al.* (2018) [7] reported that other types of honey from West Malaysia have a higher content of moisture such as rubber (29.3 ± 0.00%), Kelulut (27.4 ± 0.03 %), Tualang (22.5 ± 0.09%) and Acacia (20.2 ± 0.09%). The differences are acceptable because the honey used in this study differed from that studied by Dan *et al*. (2018)[7], which was obtained from different locations, trees, or types of bees. Notably, Chen [24] indicated that a high content of moisture is relatively unfavorable, as high water content directly correlates with a higher likelihood of fermentation and spoilage. Honey that has a high moisture content is more prone to spoilage compared to honey with a low moisture content, while the latter tends to have a longer shelf-life life.[25, 26]
Further, Table 1 showed that FH contained significantly higher ash content as compared to TH (P < 0.05). The ash content directly indicates the number of inorganic residues, particularly minerals and vitamins, remaining after the process of carbonization.[27] Honey samples that exhibit a high ash content indicate a significant presence of trace elements and minerals.[28] This indicated that FH was richer in minerals (and vitamins) than TH. The discrepancy is, nonetheless, common to observe as the ash composition and levels can vary based on the types of plants or trees from which the bees collected and hence can provide insights into the specific botanical sources of the honey, and help determine whether it is derived from nectar or honeydew.[29] Notably, the ash content for FH (0.23±0.04%) and TH (0.11±0.01%) was within the permissible range as stated by the Codex standard, which is ≤ 6% for nectar honey. These results were also corroborated by other findings from Zae *et al.* (2020) [30] with a range of 0.17% to 0.28%, and Chua and Adnan, [31] with a range of 0.19% to 0.27%.
Further, Table 1 indicated that FH (0.72±0.01%) has a higher protein percentage compared to TH (0.34±0.01%) (P<0.05). While the protein content of TH was within the range of protein content of honey as reported by Bogdanov, [32] ranging from 0.20 % to 0.50%, FH has remarkably higher protein content than that range. Nevertheless, Rajindran *et al.* (2022)[33] indicated that protein contents in honey can vary depending on their botanical or geographical origin and can also be influenced by the duration of storage. Proteins are typically found in higher quantities in raw honey and can be considered a sensitive but less commonly used marker for assessing honey quality due to their susceptibility to thermal degradation (thermolability).[34] Enzymes such as diastase, invertase, glucose oxidase, acid phosphatase, catalase, and β-glucosidase are present in honey, albeit in small amounts, and play a crucial role in contributing to its bioactivity.[35] According to the findings of Rajindran *et al*. (2022) [33], raw TH from Sabah exhibited a higher protein content percentage of 0.6% (6000 mg/kg). Another study by Lim *et al.* (2019)[36] stated that honey samples from three different sources in Sabah have a protein content of around 0.02% (0.200 g/kg). Meanwhile, Zae *et al.* (2020) [30] reported that the protein content of selected local honey in West Malaysia is between 0.24% to 0.45%.
Meanwhile, no differences were found in the fat content, pH value, and sugar content between FH and TH (Table 1, P>0.05). Similarly, Lim *et al.* (2019)[36] and Kek *et al.* (2017)[18] reported comparable fat contents in the honey used in their study. Noteworthy, the fat content of FH and TH used in this study was in the range of fat content reported by Zae *et al.* (2020) [30], which ranged from 0.08% to 0.36%. In addition, both honey samples have pH values ranging from 3.16 to 3.58, leading to their acidic properties and sour taste. Suto *et al.* (2020) [37] proposed that the low pH value of honey is contributed by the naturally occurring organic acids in honey. As stated by Julika *et al.* (2019) [38], honey originating from tropical and humid regions naturally exhibits a lower pH owing to its elevated water content, with a lower pH serving as an indicator of the honey's purity. In addition, sugar content for both samples, as shown in Table 1, meets the Codex standard,[22] which requires a sugar content of above 60%.
Interestingly, Table 1 showed that HMF content of both honey was significantly different (P<0.05), in which TH (2.86± 0.04 mg/kg) has higher HMF content compared to FH (1.78 ± 0.12 mg/kg), yet both fall below the International Honey Commission (IHC) limit for tropical honey, which is < 80 g/kg.[22] Rajindran *et al.* (2022) [33] indicated that HMF is an indicator of the freshness of honey. HMF is generated through the Maillard reaction when fructose breaks down in an acidic environment. The amount of HMF tends to increase during storage as the breakdown of fructose might occur more intensely as compared to fresh honey.[33] In the study of Khalil *et al.* (2010) [39], they revealed that Malaysian honey stored for 3–6 months stayed within the IHC limit while samples stored for 12–24 months exceeded this recommended threshold in terms of HMF concentrations. Indeed, the HMF value serves as a parameter to determine the permissible storage duration of honey before it reaches a state of being unsuitable for consumption.[33]
Furthermore, this study also reported the Total Plate Count (TPC) of both honeys were significantly different (P<0.05), where FH has a higher TPC than TH (Table 1). Lani *et al.* (2017) [40] indicated that TPC of honey provides an insight into the overall microbial population within honey samples. Even though both samples have different TPC values, both samples, were within the safe limits of microbial counts, which is < 6.00 log CFU/g, as stated by the Ministry of Health, Malaysia.[41] The health risks posed by the microbes found in honey are generally minimal, while the quality of honey is known to be influenced by factors like seasonal variations, postharvest handling, and storage conditions.[40] These results have affirmed the connection between lower pH values and the antibacterial properties of honey, as described by Almasaudi,[42] where honey's typical acidic pH range of 3.2 to 5.4 acts as a natural inhibitor of bacterial growth, contrasting with the preferred pH range of 7.2 to 7.4 for bacterial thriving. In addition, Hanifah *et al.* (2017)[43] indicated that acidic conditions in food products provide significant advantages by preventing the survival of pathogenic and spoilage bacteria

LC-MS/MS Analysis
Further, metabolite compounds of both honey samples were then investigated using LC-MS/MS. The chromatogram of FH obtained from LC-MS/MS is shown in Figure 1a and shows the presence of 10 peaks, with a retention time of 0.3 – 10.5 min. Further compound

identification under the peaks using the HR_msms_nist and plant metabolites databases revealed 16 compounds in FH, as detailed in Table 2. On the other hand, only 8 peaks were observed in the chromatogram of TH under LC-MS/MS (Figure 1b). From the 8 peaks, 20 compounds were identified (Table 2), which implied that FH has fewer metabolite compounds as compared to TH.

These differences are deemed acceptable, given that the honey compounds vary from one source to another, depending on factors such as botanical sources, geographical origins, climatic conditions, types of bees, and even the handling treatment applied.[35] It is worth noting that, the compounds profiling in this study was performed using LC-MS/MS and was limited to non-volatile compounds. In addition, the column used in this experiment was C18 column which was also limited to non-polar compounds to profile. Accordingly, while the number of identified compounds from both samples was different (Table 2), this does not necessarily imply that the total metabolites compounds were also different, as the current study did not include volatile and polar compounds.

All of the identified compounds listed in Table 2 represented a diverse range of chemical compounds with various functional groups and structural features. These compounds include amines, ketones, sugars, carboxylic acids, phenyl groups, halogens (bromine, chlorine), and other functional groups. These functional groups impart specific chemical properties and can be important for reactivity and biological activity. There is no reported study on all the compounds in the honey samples except for lactulose, 1,6-anhydro-*β*-D-glucopyranose, and arbutin. In this study, lactulose (peak 6, RT 1.9 minutes) was present in FH. Lactulose was also found in multi-floral, acacia, dandelion, rhododendron, and honeydew honey samples as reported by Tedesco *et al*.(2020).[44] It is a disaccharide present in honey at low concentrations, while carbohydrates account for 80% of the total solid contents in honey.[45] Notably, lactulose has been shown to enhance gut health and boost the absorption of crucial minerals like calcium and magnesium in the gastrointestinal tract, which could hold significant implications for overall bone health.[46] 1,6-anhydro-*β*-D-glucopyranose (peak 5 and 10, RT 1.5 minutes) was detected in both samples. It is also known as levoglucosan (LG), a product of fast pyrolysis of glucose/cellulose.[47] Approximately 10% of LG was present in the Kelulut honey sample from Perak, Malaysia in the study of Akmar *et al*. (2022).[48] The detection of levoglucosan in honey samples suggests that the honey originated from a region characterized by warmer weather, a higher risk of fires, or proximity to urban areas with air pollution.[49] This compound is seen as an indicator of biomass burning, including both natural and human-related activities like burning agricultural waste and wood for energy, which is known to affect air quality and human health, without posing a direct threat to humans.[50]

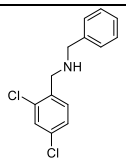
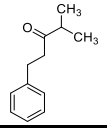
Interestingly, arbutin (peak 4 and 9, RT 1.5 minutes) was detected in both honey samples. Arbutin is a chemical compound that consists of a molecule of D-glucose bound to hydroquinone and is used in cosmetics for skin lightening.[51] Moreover, arbutin has displayed biological activities such as antioxidant [52] and anti-inflammatory effects,[53] along with its antibacterial properties within the urinary tract.[54] To date, there have been no reports of arbutin in Malaysian honey. However, arbutin has been identified in honey samples from Sardinia, Italy,[55] as well as in strawberry tree (*Arbutus unedo* L.) honey from southern Europe.[56]

Table 1: The physicochemical and microbiological properties

Properties	Forest Honey	Tualang Honey
Moisture (%)	10.79 ± 0.03	10.69 ± 0.42
Ash (% DM)	0.23 ± 0.04b	0.11 ± 0.01a
Protein (% DM)	0.72 ± 0.01b	0.34 ± 0.01a
Fat(% DM)	0.49 ± 0.27	0.50 ± 0.16
pH	3.58 ± 0.42	3.16 ± 0.90
Sugar content (%)	82.78 ± 3.19	81.07 ± 4.01
HMF (mg/kg)	1.78 ± 0.12a	2.86 ± 0.04b
Total plate count (log CFU/g)	1.02 ± 0.03a	0.41 ± 0.02b

*Means followed by different letters within a row are significantly different at $p<0.05$.
DM = dry matter

Table 2: Compounds identified in the honeys

Peak Number	RT (Min)	Chemical Formula	Compound Name	Molecular Weight	Structure
			Forest Honey		
1	0.3	$C_{14}H_{13}Cl_2N$	N-Benzyl-1-(2,4-dichlorophenyl) methanamine	266.1650	
2	0.3	$C_{12}H_{16}O$	4-Methyl-1-phenylpentan-3-one	176.1201	

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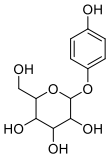
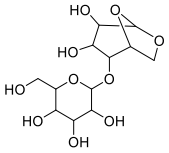
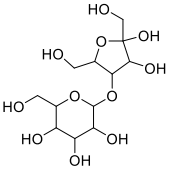
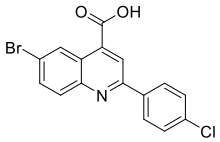
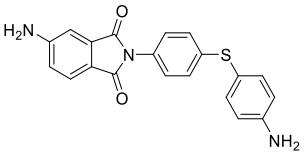
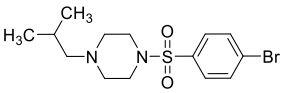
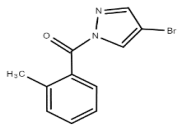
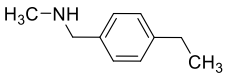
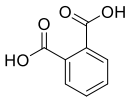
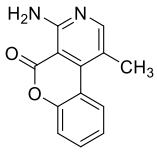
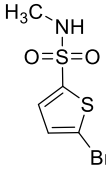
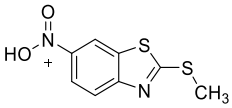
4	1.5	$C_{12}H_{16}O_7$	Arbutin	272.0896	
5	1.5	$C_6H_{10}O_5$	1,6-anhydro-β-D-glucopyranose	126.0314	
6	1.9	$C_{12}H_{22}O_{11}$	Lactulose	342.1162	
9	8.3	$C_{16}H_9BrClNO_2$	6-Bromo-2-(4-chlorophenyl)quinoline-4-carboxylic acid	360.9505	
10	8.3	$C_{20}H_{15}N_3O_2S$	5-Amino-2-{4-[(4-aminophenyl) sulfanyl]phenyl}-1H-isoindole-1,3(2H)-dione	361.4.	
11	8.3	$C_{18}H_{20}BrNO_2$	N-(4-Bromo-2-methylphenyl)-2-[4-(propan-2-yl)phenoxy]acetamide	n.d.	n.d.
12	8.3	$C_{14}H_{21}BrN_2O_2S$	1-(4-Bromophenylsulfonyl)-4 isobutylpiperazine	360.0507	
13	8.3	$C_{15}H_{12}ClN_5O4$	Terasil Yellow 4G	n.d	n.d.
14	8.7	$C_{11}H_9BrN_2O$	(4-Bromo-1H-pyrazol-1-yl)(2-methylphenyl)methanone	414.2043	
15	8.7	$C_{10}H_{15}N$	N-(4-Ethylbenzyl)-N-methylamine	149.23	
16	9.0	$C_8H_6O_4$	Phthalic acid	166.0266	
			Tualang Honey		
1	0.3	$C_{13}H_{10}N_2O_2$	4-Amino-1-methyl-5Hchromeno [3,4-c]pyridin-5-one	226.0742	
2	0.3	$C_5H_6BrNO_2S_2$	5-Bromo-N-methylthiophene-2-sulfonamide	256.1320	
3	0.3	$C_{14}H_{10}OS$	Dye X-27237-62	n.d.	n.d.
4	0.3	$C_8H_7N_2O_2S_2$	2-(Methylthio)-6-nitrobenzothiazole	227.2755	

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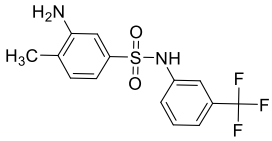
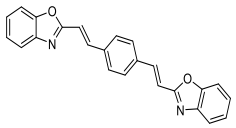
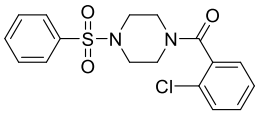
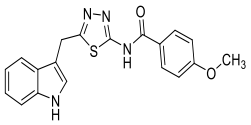
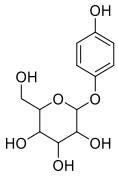
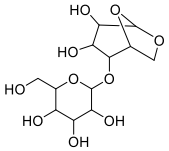
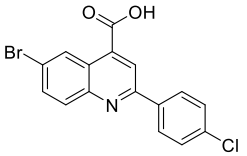
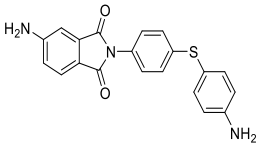
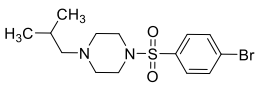
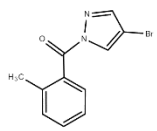
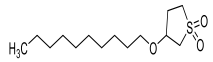
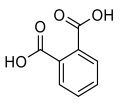
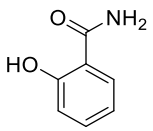
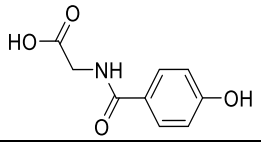
5	0.3	$C_{14}H_{13}F_3N_2O_2S$	3-Amino-4-methyl-N-[3-(trifluoromethyl) phenyl]benzenesulfonamide	330.0650	
6	1.4	$C_{24}H_{16}N_2O_2$	2-(2-{4-[2-(1,3-Benzoxazol-2-yl) ethenyl]phenyl}ethenyl)-1,3-benzoxazole	364.1212	
7	1.4	$C_{17}H_{17}ClN_2O_3S$	(2-Chlorophenyl)[4-(phenylsulfonyl)piperazin-1-yl]methanone	364.0648	
8	1.4	$C_{19}H_{16}N_4O_2S$	N-[5-(1H-Indol-3-ylmethyl)-1,3,4-thiadiazol-2-yl]-4-methoxybenzamide	364.4230	
9	1.5	$C_{12}H_{16}O_7$	Arbutin	272.0896	
10	1.5	$C_6H_{10}O_5$	1,6-anhydro-β-D glucopyranose	126.0314	
11	8.3	$C_{16}H_9BrClNO_2$	6-Bromo-2-(4-chlorophenyl)quinoline-4-carboxylic acid	360.9505	
12		$C_{20}H_{15}N_3O_2S$	5-Amino-2-{4-[(4-aminophenyl) sulfanyl]phenyl}-1H-isoindole-1,3(2H)-dione	361.4	
13		$C_{18}H_{20}BrNO_2$	N-(4-Bromo-2-methylphenyl)-2-[4-(propan-2-yl)phenoxy]acetamide	n.d.	n.d.
14		$C_{14}H_{21}BrN_2O_2S$	1-(4-Bromophenylsulfonyl)-4 isobutylpiperazine	360.0507	
15		$C_{15}H_{12}ClN_5O_4$	Terasil Yellow 4G	n.d.	n.d.
16	8.4	$C_{11}H_9BrN_2O$	(4-Bromo-1H-pyrazol-1-yl)(2-methylphenyl)methanone	361.1708	
17	8.7	$C_{14}H_{28}O_3S$	3(Decyloxy)tetrahydrothiophne 1,1-dioxide	276.4350	
18	9.0	$C_8H_6O_4$	Phthalic acid	166.0266	
19	9.0	$C_7H_7NO_2$	Salicylamide	137.1380	

20	9.0	$C_9H_9NO_4$	4-Hydroxyhippuric acid	195.17	

a.

b.

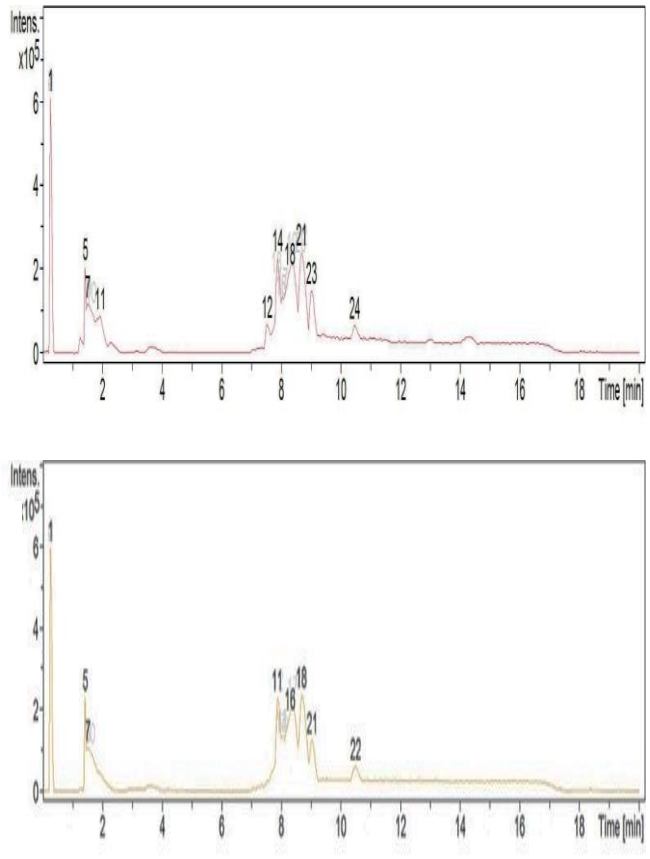


Figure 1: Chromatogram of a) FH and b) TH by LC-QTOF-MS analysis

Conclusion

Physicochemical analysis indicated that the contents of moisture, fat, sugar content, and pH values of FH and TH used in this study were considerably comparable and fell within the standard range for honey. Discrepancies were found in protein, ash and HMF contents of both honeys. In addition, microbial analysis under total plate count indicated a significant difference between the honeys. Further, the analysis of biochemical metabolites revealed the presence of numerous compounds that have not been extensively studied. These compounds may have biological significance and could serve as biomarkers for environmental studies.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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