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Chemical profile and antidiabetic activity of raw and polyphenol extracts of Malaysian honey

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Abstract

High amount of carbohydrates present in honey poses a challenge for identifying specific phenolics and flavonoids, as these bioactive compounds are typically present in lower concentrations. Therefore, the present work aimed to investigate the chemical profiles of two Malaysian honey types, Gelam and Tualang, including their raw and polyphenol extracts. The present work also evaluated the antidiabetic property of the raw and polyphenol extracts *via* α-glucosidase inhibitory activity. The ^{1}H NMR metabolomics successfully differentiated between the raw and polyphenol extracts of Gelam, Tualang, and Manuka honey. A total of 18 metabolites, including phenolics/flavonoids, carbohydrates, amino acids, and organic acids were responsible for the separation of raw and polyphenol extracts of honey samples. The principal component analysis (PCA) loadings plot showed signals in the aromatic region of polyphenol extracts, potentially belonging to phenolics and flavonoids. HPLC analysis confirmed the presence of kaempferol, luteolin, isorhamnetin, hesperetin, chlorogenic acid, naringenin, ferulic acid, sinapic acid, and quercetin in honey samples. The results indicated that Gelam honey had higher total phenolic and flavonoid contents than Tualang honey. The α-glucosidase inhibitory activity results revealed that the raw honey of Gelam, Tualang, and Manuka exhibited a higher percentage of inhibition at the concentration of 500 µg/mL (52.58 ± 2.43%, 39.03 ± 3.47%, and 50.54 ± 0.23%, respectively) compared to the polyphenol extracts (0.57 ± 0.05 - 1.09 ± 0.09%). The antidiabetic mechanisms of the polyphenols towards *in silico* molecular docking revealed rutin having the highest binding affinity (-10.1 kcal/mol) compared to other compounds. Although phenolics and flavonoids contribute to antidiabetic activity, results obtained in the present work indicated that other metabolites, such as amino acids and organic acids, were mainly responsible for the antidiabetic activity of Gelam and Tualang honey. The present work provided important information on the chemical profile and antidiabetic activity of Malaysian honey.

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Introduction

Malaysia is a Southeast Asian country with abundant forest resources, including honey, which is a natural food containing carbohydrates and other constituents with a broad range of biological activities (Muhammad and Sarbon, 2021). In addition to carbohydrates, honey contains enzymes and other metabolites such as ascorbic acid, organic acids, amino acids, proteins, and a large number of polyphenols. Phenolic acids and flavonoids are considered potential markers of honey. Phenolic acids are divided into two subclasses, substituted benzoic acid and cinnamic acid. Flavonoids are classified as flavanols, flavones, and flavanones, which have a similar structure to phenolic acids (Khalil *et al.*,

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2011). Polyphenols are one of the most important groups of secondary metabolites in plants. Honey polyphenols originate from plants mixed with the nectar, and become enriched when added to the propolis in the beehive. The main phenolic acids reported in honey are caffeic, chlorogenic, coumaric, ellagic, ferulic, homogentisic, phenyllactic, protocatechuic, syringic, and vanillic acids. Flavonoid compounds such as apigenin, chrysin, galangin, pinocembrin, quercetin, and tricetin have also been reported in honey (Chew *et al.*, 2018). As the composition of active compounds in honey differs based on its geological environment, the biological properties of honey are also expected to differ (Devarajan and Venugopal, 2012).

Diabetes mellitus is a chronic disease that occurs when a person's blood contains high levels of glucose (Peláez-Acero *et al.*, 2022). The effects of diabetes mellitus include long-term damage, dysfunction, and failure of various organs in the body (Rahmawati *et al.*, 2019). Obese individuals are five times more likely to develop insulin resistance, which can lead to type 2 diabetes. When the pancreatic beta cells can no longer compensate for the insulin resistance, type 2 diabetes develops (Terzo *et al.*, 2020). Type 2 diabetes is a condition in which long-chain carbohydrates are hydrolysed by pancreatic α-amylase and α-glucosidase enzymes (Peláez-Acero *et al.*, 2022). The inhibition of these two enzymes is considered an effective treatment for type 2 diabetes as it can delay the breakdown of carbohydrates after the formation and absorption of glucose following meals. Acarbose, miglitol, and voglibose are the current antihyperglycemic drugs used to treat type 2 diabetes. While acarbose inhibits both α-glucosidase and α-amylase, miglitol and voglibose only inhibit α-glucosidase (Uddin *et al.*, 2022).

Tualang and Gelam honey are common types of honey found in Malaysia, produced by native wild bees (*Apis dorsata*). Both have unique botanical origins, with Gelam honey deriving from *Melaleuca* spp. and Tualang honey from *Koompassia excelsa* (Azman *et al*., 2024; Boruah *et al*., 2024). They are reported to contain bioactive compounds such as flavonoids and phenolic acids, which may influence their antidiabetic activity (Kishore *et al.*, 2011; Mohd Kamal *et al.*, 2021). Despite the availability of α-glucosidase inhibitor drugs such as acarbose, miglitol, and voglibose, bioactive compounds from Malaysian honeys might also be linked to antidiabetic mechanisms, particularly as α-glucosidase inhibitory agents. In the present work, we compared the α-glucosidase inhibitory activity of Gelam, Tualang, and Manuka honey samples. Manuka honey, known for its potent antimicrobial properties due to its high methylglyoxal (MGO) content, also demonstrates mild α-glucosidase inhibition and antioxidant effects (Mavric *et al.*, 2008; Atrott and Henle, 2009; Alvarez-Suarez *et al*., 2014). Thus, studying the antidiabetic potential of Malaysian honeys in comparison with well-established honeys like Manuka would emphasise the therapeutic potential of these tropical honeys as antidiabetic agents. Although previous studies have reported on the antidiabetic activity of Tualang honey (Erejuwa *et al*., 2011; Ahmed and Othman, 2013), no research has explored the potential antidiabetic activity of polyphenols specifically in Malaysian honeys, such as Tualang and Gelam honeys. To the best of our knowledge, this would be the first study to report on the antidiabetic activity of Gelam honey.

One of the major challenges in honey analysis is the presence of high levels of carbohydrates, such as glucose and fructose. Therefore, it is necessary to find an effective method to identify the polyphenols present in honey to determine its potential as an antidiabetic agent. Solid phase extraction (SPE) has been used to extract the polyphenols in honey (Khalil *et al*., 2011). One effective and comprehensive analytical approach that has been widely used for chemical profiling and classification of biological samples is ^{1}H nuclear magnetic resonance (NMR) metabolomics (Lajis *et al.*, 2017). In the present work, ^{1}H NMR metabolomics was employed to monitor the effectiveness of polyphenol extraction using the SPE method.

The main objectives of the present work were to determine the chemical profile and antidiabetic properties of two Malaysian honeys, namely Gelam and Tualang, as well as the Manuka honey, including their raw and polyphenol extracts, using ^{1}H NMR metabolomics. Additionally, quantitative analysis through high-performance liquid chromatography (HPLC) was conducted to confirm the identification of phenolics and flavonoids in Gelam and Tualang honey samples. Lastly, the antidiabetic mechanisms of the bioactive compounds identified in Malaysian honey were elucidated through *in silico* molecular docking.

Materials and methods

Chemicals and reagents

The following chemicals were used in this study, including C18 solid phase extraction (SPE) cartridge (Supelclean ENVI-18), hydrochloric acid, distilled water (dH_2O), analytical methanol, sodium hydroxide, Carrez solution I (15 g potassium hexacyanoferrate(II) trihydrate in 100 mL dH_2O), Carrez solution II (30 g zinc acetate dihydrate in 100 mL dH_2O), sodium bisulphate, olive oil, glycine, phosphate buffer (pH 6.9), *p*-nitrophenyl-α-D-glucopyranoside (PNPG), α-glucosidase enzyme (Megazyme, Bray, Ireland), deuterium oxide (D_2O, 99.9%), 3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid (TMSP-2,2,3,3-d_4, 98%) sodium salt, KH_2PO_4, and deuterated methanol (CD_3OD). HPLC-grade formic acid, methanol, and acetonitrile (ACN) were used for quantitative analysis.

Honey collection

Five samples of each Tualang and Gelam honey were collected from different hives and obtained from a local farmer in Tasik Kenyir, Terengganu. Both types of honey are produced by the *Apis dorsata* bee species. In addition, five samples of commercial Manuka honey, obtained from the local market, were also used.

Physicochemical properties

Moisture content

The digital refractometer was calibrated using distilled water. Honey samples were then added to the refractometer using a disposable pipette, and the lid was closed. The reading was recorded, and the well was cleaned with rubbing alcohol after each use. Five replicates were performed, and the average value was calculated.

pH and free acidity

To measure the free acidity of the honey samples, 5 g of honey was diluted with 37.5 mL of distilled water, and mixed thoroughly using a magnetic stirrer. The initial pH of the mixture was recorded using a pH meter. A burette was filled with 0.1 M NaOH, and used to titrate the mixture drop by drop until the pH reached 8.30, at which point the volume of NaOH used was recorded.

Hydroxymethylfurfural

Approximately 25 g of honey was dissolved in 25 mL of distilled water, followed by the addition of Carrez I and Carrez II solutions. The mixture was then thoroughly mixed, and two sets of readings were taken: the sample (honey + water) and reference (honey + sodium bisulphite). The mixture was filtered and prepared for HMF analysis. Next, 1 mL of filtrate was pipetted and mixed with 1 mL of distilled water in a cuvette, and the absorbance at 284 and 336 nm was recorded. The same steps were repeated with 1 mL of sodium bisulphite replacing the distilled water. Three replicates were performed, and the average value was calculated.

Insoluble matter

The filter paper was heated in an oven at 100°C for 1 h, and then allowed to cool in a desiccator before being weighed (M2). Approximately, 20 g of honey was dissolved in 200 mL of water that had been boiled at 80°C, and the mixture was thoroughly mixed. The mixture was then filtered through the pre-weighed filter paper using vacuum filtration. The filtrate was dried in the oven for 1 h, cooled in a desiccator, and weighed (M1). Three replicates were performed, and the average value was calculated.

Ash

The crucible was heated at the ashing temperature of 600°C for 25 min. After that, it was cooled in a desiccator and weighed. Approximately 5 g of honey was weighed in the crucible, and two drops of olive oil were added to the sample. The crucible with the sample was heated for 1 h at 600°C in a furnace. After the temperature of the furnace dropped to around 250°C, the crucible was removed and cooled in a desiccator. The sample was then weighed.

Polyphenol extraction

The reverse phase C18 cartridges were utilised for extracting phenolic acid and flavonoid compounds from raw honey. Firstly, approximately 20 g of honey was mixed with 75 mL of acidified water. The cartridge was rinsed with methanol to ensure that no contaminants were present. Next, conditioning was performed by introducing 3 mL of acidified water and 3 mL of distilled water into the cartridge, followed by 30 mL of the diluted honey sample. The mixture was then washed with 5 mL of

acidified water to eliminate carbohydrates, followed by 5 mL of distilled water to remove all acids. A clean bottle was placed in the tank to collect the elute. Elution was conducted using 20 mL of methanol to extract the polyphenols. The obtained polyphenol filtrate was evaporated using a rotary vacuum evaporator at 45°C, followed by freeze-drying to remove excess water. All polyphenol extracts were stored in the refrigerator (4°C) until further analysis.

α-Glucosidase inhibition

The α-glucosidase inhibitory assay was conducted following the procedure outlined by Abd Ghafar *et al.* (2018) with some modifications. The α-glucosidase enzyme was diluted to a final concentration of 2 U/well in 30 mM sodium phosphate buffer, and a solution of 1 mM ρ-nitrophenyl-ρ-D-glucopyranosidase (PNPG) substrate was prepared in the same buffer solution. In each well, 10 µL of the serially diluted extract (concentrations ranging from 16.5 to 500 g/mL) was added, followed by the addition of 130 µL of 30 mM sodium phosphate buffer and 10 µL of α-glucosidase. The solution was incubated for 5 min at room temperature (25°C). Next, 50 µL of PNPG solution was added and incubated for 15 min at room temperature. The reaction was stopped by adding 50 µL of 2 M glycine (pH 10). The absorbance was measured at 405 nm using a microplate reader (Abd Ghafar *et al.*, 2018). The percentage inhibition of the sample was calculated, and the results are presented as mean ± SD.

^{1}H-NMR data acquisition and multivariate data analysis

The procedure for preparing the samples for ^{1}H NMR measurement was as follows: 100 mg of honey or 5 mg of polyphenol extract was dissolved in 300 µL of deuterated methanol, followed by the addition of 300 µL of D_2O (pH 6.0) containing 0.1% TMSP. The solution was sonicated for 15 min at room temperature, and then centrifuged at 10,000 rpm for 5 min. Next, 600 µL of the supernatant was carefully pipetted into an NMR tube (4.97 × 4.2 mm, 178 mm) for analysis.

The ^{1}H NMR analysis was conducted using a 500 MHz Varian INOVA NMR spectrometer at a frequency of 499.887 MHz and a temperature of 26°C. Each spectrum was obtained using the PRESAT experiment, with a duration of 3.54 min, consisting of 64 scans, and a spectral width of 20 ppm. Spectra pre-processing and data extraction were performed using Chenomx Processor and Profiler software v. 8.1, respectively. Metabolite identification was accomplished by comparing the NMR signals of interest observed in the sample spectrum with the NMR data of standard compounds available in the Chenomx library and literature. The binned data (bin size of 0.04 ppm) consisting of 30 samples of raw and polyphenol extracts of honey samples were subjected to principal component analysis (PCA) to evaluate their chemical profile, with Pareto scaling applied as a data pre-treatment step prior to multivariate analysis. The region from 4.68 to 4.90 ppm, corresponding to the water peak, was excluded, and the TMSP signal was used for spectral alignment.

Quantitative HPLC analysis

To analyse the presence of phenolic and flavonoid compounds in Gelam and Tualang honey samples, approximately 2 mg/mL of each sample (in triplicate) was subjected to HPLC. The analysis was conducted using a Shimadzu HPLC machine equipped with a diode array detector. An aliquot of 10 µL of each sample was injected onto a Waters Xbridge C18 column (4.6 × 150 mm, 3.4 µm), and eluted using a gradient solvent system consisting of water and ACN (90:10) containing 0.1% formic acid. The flow rate was set at 0.7 mL/min. The quantitative analysis was performed for 60 min at a wavelength of 271 nm. To quantify the compounds, various standards from phenolics and flavonoids, including gallic acid, chlorogenic acid, mandelic acid, rutin, *p*-coumaric acid, sinapic acid, *trans*-ferulic acid, luteolin, quercetin, genistein, apigenin, naringenin, kaempferol, isorhamnetin, and hesperetin, were used. Standard calibration curves were generated using various concentrations (15.625 - 1000 µg/mL) of the standards (R^2 = 0.99), and the resulting equations were applied to estimate the amounts of bioactive compounds in the honey samples. The results are presented as mean ± SD.

In silico molecular docking

The interaction between the target protein α-glucosidase and bioactive compounds from both honey samples was evaluated using molecular docking. The crystal structure of α-glucosidase, isomaltase from *Saccharomyces cerevisiae* (PDB ID: 3A4A), was retrieved from the RCSB Protein Data Bank. The protein structure was processed using

UCSF Chimera (version 1.16) by removing water molecules, irrelevant atoms, and non-standard residues, and separating the receptor and ligand into individual structures. AutoDockTools further refined the protein by adding Kollman charges, and converting the structures into PDBQT format. Ligands, including flavonoid compounds, were designed in ChemDraw (version 12.0), converted to PDB format using Chem3D, and prepared for docking. The binding site was defined based on the native ligand located in the crystal structure's active site. Molecular docking was performed using AutoDock Vina (version 1.5.7) to predict binding affinities and interactions. The methodology was validated by redocking the native inhibitor, and comparing it with the original crystal structure. Docking results were analysed using PyMOL, and visualised in Biovia Discovery Studio. Final docking conformations were validated to confirm the accuracy of protein-ligand interactions and their relevance to the study.

Statistical analysis

To evaluate the significant differences between the samples, a statistical analysis using analysis of variance (ANOVA) was performed. The analysis was conducted using SPSS software version 20.0. Subsequently, independent *t*-tests and Tukey's Honestly Significant Difference (HSD) *post hoc* test were conducted to identify significant differences between the mean values. A *p*-value of less than 0.05 was considered statistically significant.

Results and discussion

Physicochemical analysis

To assess the authenticity of the raw honey samples, various physicochemical analyses were conducted, including measurements of HMF, moisture content, pH, acidity, ash, and insoluble matter.

The moisture content of honey is a critical parameter for determining its quality, as it can affect both the shelf life and microbial stability of the product (Keng *et al.*, 2017). In the present work, the moisture content of Gelam honey sample was found to be 29.30 ± 0.12%, while Tualang and Manuka honey samples had moisture contents of 29.02 ± 0.12% and 20.97 ± 1.00%, respectively. The moisture contents of Gelam and Tualang samples were slightly higher compared to the moisture content reported in the literature, which ranges from 14.53% to 26.51%. It is worth noting that honey samples from tropical countries like Malaysia typically have a higher moisture content, which can be attributed to the rainy seasons (Mohd Kamal *et al*., 2021).

The pH values of the honey samples tested in the present work were determined to be 3.77 ± 0.11 for Gelam honey sample and 3.90 ± 0.04 for Tualang honey sample, indicating that Tualang honey sample was more acidic than Gelam honey sample. Manuka honey sample showed a higher pH value (pH = 4.15 ± 0.14) compared to Gelam and Tualang honey samples. According to A-Rahaman *et al.* (2013), pH values can vary significantly due to differences in the floral and geographical origin of the honey. pH values are strongly correlated with the stability, shelf life, and fermentation process of honey products.

The ash content of Gelam honey sample was found to be lower (1.47 ± 0.23 g/100 g) than Tualang honey (2.12 ± 2.37 g/100 g) and Manuka honey (5.65 ± 0.73 g/100 g) samples, while Manuka honey samples had a slightly higher insoluble matter value (0.72 ± 0.05%) compared to Gelam honey (0.61 ± 0.04%) and Tualang honey (0.49 ± 0.09%) samples. The acidity of Manuka honey sample (13.00 ± 1.73 meq/kg) was also lower than Gelam honey (26.67 ± 0.98 meq/kg) and Tualang honey (28.00 ± 3.40 meq/kg) samples.

HMF is a chemical marker used to indicate the raw and unprocessed nature of honey, derived from furan, and containing both aldehyde and alcohol functional groups (Lazim and Baharudin, 2017). The lower the HMF value, the fresher the honey, and the less likely it has undergone excessive heating during processing (Alvarez-Suarez *et al.*, 2014; Lazim and Baharudin, 2017). In the present work, both Tualang honey (1.78 ± 0.24 mg/kg) and Gelam honey (1.05 ± 0.14 mg/kg) samples had low HMF values, suggesting that they were raw and uncooked natural honey. On the other hand, Manuka honey samples showed a high HMF value of 54.61 ± 11.17 mg/kg, indicating that the honey was either processed or not raw. The international standard value for HMF content in honey is 40 mg/kg or less (Codex Alimentarius Commission, 2019).

^{1}H NMR metabolomics of raw versus polyphenol extracts

The effectiveness of removing carbohydrates from raw honey was evaluated using ^{1}H NMR analysis of both the raw honey and polyphenol

extracts. In the present work, the chemical profiles of raw and polyphenol extracts of Gelam and Tualang honey samples were also compared with the Manuka honey sample. Figure 1 shows that the major signals in the carbohydrate region (3.0 - 5.4 ppm) were observed in the raw and polyphenol extracts, while the signals at 6.0 - 8.5 ppm likely correspond to phenolic compounds and flavonoids. The raw samples of Gelam, Tualang, and Manuka honey samples contained abundant carbohydrate signals, and a lower intensity of aromatic region. Conversely, the polyphenol extracts showed higher intensity in the aromatic region, indicating that a significant amount of carbohydrates had been removed. Additionally, variations in the aromatic region of Gelam, Tualang, and Manuka honey samples were observed in their ^{1}H NMR spectra, indicating differences in their polyphenol compositions.

The identified metabolites in the raw and polyphenol extracts were mostly the same, but their concentrations varied between each honey and extract. A total of 18 metabolites were identified in both extracts, with phenolic and flavonoid compounds being more abundant in the polyphenol extract, which is known to play an important role in the biological activities of honey. Phenylalanine, 4-hydroxyphenylacetic acid, glucose, glutamic acid, acetoin, acetic acid, valine, isoleucine, and leucine were identified in the polyphenol extract, while the ^{1}H NMR spectra of the raw honey showed the presence of sucrose, glucose, maltose, trehalulose, and fructose. The polyphenol extract had a small amount of carbohydrates, possibly indicating the glycoside forms of phenolics and flavonoids. The ^{1}H NMR spectra of the raw honey also showed a significant amount of amino and organic acids.

To provide a comprehensive understanding of the metabolite profile of both raw and polyphenol extracts of Gelam, Tualang, and Manuka honey samples, multivariate data analysis was performed (Figure 2). In the present work, a PCA model was used to distinguish raw honey samples from their polyphenol extracts. The results showed that the PCA model, with R^2X cumulative value of 0.956, and Q^2 cumulative value of 0.939, was effective in differentiating the metabolites present in the raw honey and polyphenol extracts of different types of honey. A high value of R^2X (cum) indicates good model fitness, while a high value of Q^2 (cum) indicates predictivity of the PCA model (Worley and Powers, 2016). The PCA scores plot also revealed that the raw samples of Manuka honey exhibited a different chemical profile compared to the raw samples of Gelam and Tualang honey. The PCA scores plot further indicated that the polyphenol extracts of all honey samples showed similarity in terms of their chemical profiles.

The PCA loadings plot in Figure 2B provides insight into the metabolites that contribute to the separation of the samples. The loadings plot revealed that the polyphenol extracts contain a greater number of aromatic signals at 7.20 - 7.30 ppm, which were likely due to the presence of metabolites from the phenolic and flavonoid groups. Conversely, the raw honey exhibited more signals of carbohydrates at 3.14 - 5.38 ppm, as expected.

Quantification of polyphenols of honey by HPLC analysis

Due to the overlapping signals in the aromatic region of the NMR spectra, it was challenging to identify phenolics and flavonoids present in the polyphenol extracts of Gelam and Tualang honey samples. Therefore, to confirm their presence, quantitative HPLC analysis was conducted at the wavelength of 271 nm, as it is commonly used and shows good absorbance for the detection of phenolics and flavonoids. According to Khalil *et al.* (2011), Gelam honey contains catechin, benzoic acid, naringenin, luteolin, kaempferol, and apigenin. On the other hand, Tualang honey contains catechin, gallic acid, syringic acid, coumaric acid, benzoic acid, cinnamic acid, and kaempferol (Khalil *et al.*, 2011). In the present work, both Gelam and Tualang honey samples were found to contain gallic acid, chlorogenic acid, rutin, quercetin, mandelic acid, sinapic acid, luteolin, genistein, *trans*-ferulic acid, hesperetin, isorhametin, and coumaric acid (Table 1).

Both Gelam and Tualang honey samples exhibited similar levels of gallic acid ($p > 0.05$). Likewise, chlorogenic acid, mandelic acid, coumaric acid, ferulic acid, and isorhamnetin were not significantly different between Gelam and Tualang honey samples. On the other hand, sinapic acid, rutin, and quercetin were significantly higher in Gelam honey sample than in Tualang honey sample. In contrast, luteolin, genistein, and hesperetin were found to be higher ($p < 0.05$) in Tualang honey sample than in Gelam honey sample. Based on Table 1, gallic acid was the most abundant phenolic

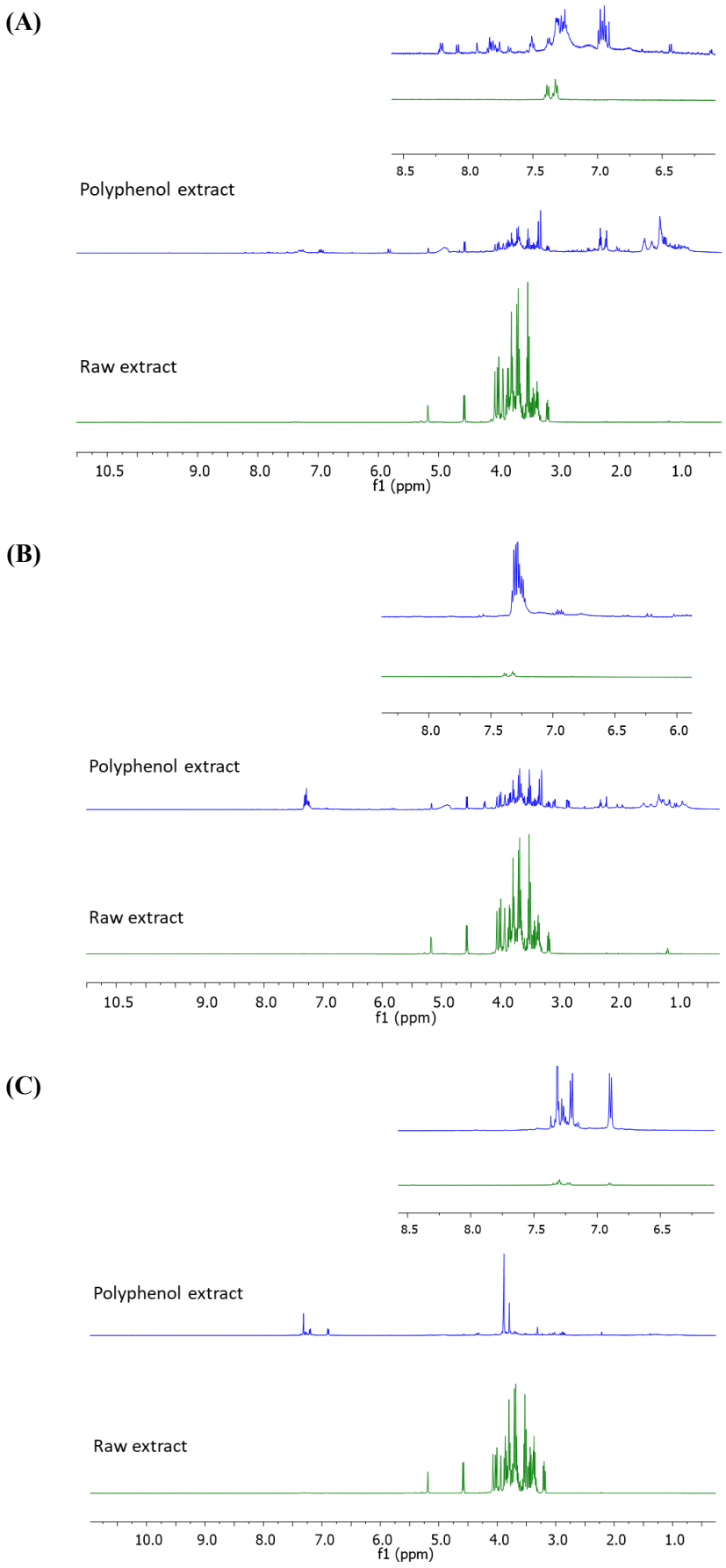

Figure 1. ^{1}H NMR spectra of raw and polyphenol extracts of Gelam **(A)**, Tualang **(B)**, and Manuka **(C)** honey samples.

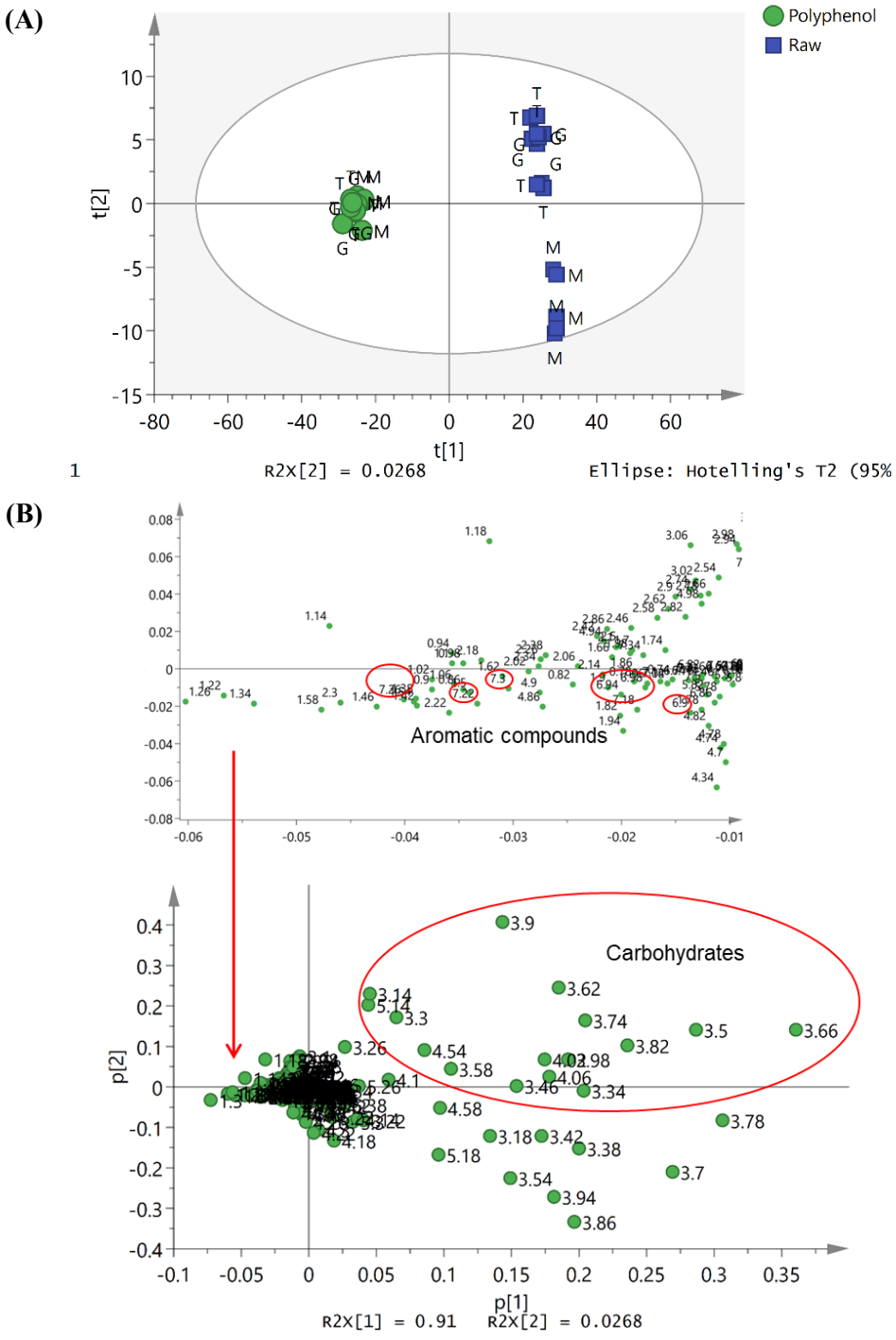

Figure 2. PCA score **(A)** and loading **(B)** plots of raw and polyphenol extracts of Gelam (G), Tualang (T), and Manuka (M) honey samples.

Table 1. Quantitative analysis of phenolics and flavonoids in Gelam and Tualang honey samples.

Compound	Amount of compound (mg/100 g extract)		*t*-test (*p*-value)
	Gelam	Tualang	
Gallic acid	52.90 ± 0.05	53.47 ± 0.55	0.568
Chlorogenic acid	10.25 ± 0.31	8.52 ± 0.05	0.065
Mandelic acid	47.90 ± 6.65	35.70 ± 4.59	0.373
Coumaric acid	1.97 ± 0.21	1.68 ± 0.28	0.576
Sinapic acid	5.33 ± 0.46	1.75 ± 0.15	0.034
trans-Ferulic acid	0.14 ± 0.01	0.13 ± 0.01	0.675
Rutin	13.96 ± 1.40	3.51 ± 0.38	0.035
Luteolin	1.55 ± 0.25	3.43 ± 0.16	0.026
Quercetin	10.63 ± 0.22	13.78 ± 0.41	0.026
Genistein	1.19 ± 0.02	4.34 ± 0.15	0.009
Naringenin	2.36 ± 0.08	nd	-
Kaempferol	nd	2.53 ± 0.05	-
Isorhamnetin	2.74 ± 0.15	2.34 ± 0.12	0.227
Hesperetin	3.08 ± 0.2	4.11 ± 0.05	0.004

nd = not detected.

compound present in both Tualang and Gelam honey samples. Overall, Gelam honey sample contained a higher amount of phenolics and flavonoids compared to Tualang honey sample, which agreed with Mohd Kamal *et al*. (2021). Botanical origin is one of the important factors that affect the polyphenol contents of honey (Shamsudin *et al*., 2022).

Antidiabetic activity

In the present work, the α-glucosidase inhibitory assay of the raw and polyphenol extracts of Gelam, Tualang, and Manuka honey samples was conducted to determine their antidiabetic activity. To monitor the performance of the α-glucosidase inhibitory assay, acarbose was used as the positive control. It was observed that the IC_{50} of acarbose was 6.75 ± 3.62 μg/mL, consistent with previous studies reporting the α-glucosidase inhibitory activity of acarbose with IC_{50} values ranging from 2.33 - 112.02 μg/mL (Elya *et al*., 2012).

The raw honey samples had significantly higher percentage inhibition towards α-glucosidase compared to polyphenol extracts ($p < 0.05$), as indicated in Table 2. The sample that exhibited the highest inhibition of diabetic activity was raw Gelam honey, while the polyphenol extract of Tualang honey sample displayed the least inhibition. Raw Gelam honey sample displayed significantly higher α-glucosidase inhibitory activity ($p < 0.05$) than raw Tualang honey sample. However, the α-glucosidase inhibitory activity of raw Gelam honey sample was not significantly different compared to raw Manuka honey sample (Table 2). The polyphenol extract of Gelam honey sample showed higher α-glucosidase inhibitory activity compared to the polyphenol extract of Tualang honey sample, although the result was not statistically significant. In addition, the polyphenol extract of Manuka honey sample showed a negative result for α-glucosidase inhibitory activity. Ali *et al.* (2020) suggested that the botanical origin of honey could affect its α-glucosidase inhibitory activity. These findings suggested that polyphenols do not entirely contribute to the antidiabetic activity of honey. Raw Gelam and Tualang honey samples were found to be rich in oligosaccharides, amino acids, and organic acids, as evidenced by their ^{1}H NMR spectra. The presence of oligosaccharides such as trehalose, turanose, panose, 6-ketose, and palatinose has been suggested to play an important role in contributing to the antidiabetic effects of honey (Erejuwa *et al.*, 2012; Pasupuleti *et al.*, 2017). Furthermore, previous studies have reported that amino acids like leucine, isoleucine, alanine, and arginine are essential for insulin secretion, and increasing glucose uptake (Newsholme *et al.*, 2006). It has also been found that the intake of weak organic acid-containing foods such as lactic acid improves insulin resistance, making it suitable for managing diabetes (Marunaka, 2018).

Table 2. Percentage of inhibition of Gelam, Tualang, and Manuka honey samples towards α-glucosidase at 500 µg/mL.

Sample	% Inhibition at 500 µg/mL
Raw Gelam	52.58 ± 2.43[a]
Raw Tualang	39.03 ± 3.47[b]
Raw Manuka	50.54 ± 0.23[a]
Polyphenol extract of Gelam	1.09 ± 0.09[c]
Polyphenol extract of Tualang	0.57 ± 0.05[c]
Polyphenol extract of Manuka	na

Lowercase superscripts indicate significant difference among samples ($p < 0.05$); na = not available.

Molecular docking study

To further understand the antidiabetic mechanisms of the identified polyphenol compounds, a molecular docking study on α-glucosidase from *S. cerevisiae* (PDB id: 3A4A) enzyme was performed. Five major bioactive compounds determined by quantitative HPLC analysis (Table 1) including gallic acid, chlorogenic acid, mandelic acid, rutin, and quercetin, were docked into the active site of α-glucosidase enzyme to evaluate their binding affinities and interactions. The binding energies, hydrogen bonds, and other interaction of the bioactive compounds are summarised in Table 3.

Table 3. Molecular docking of major phenolic acids and flavonoids of Malaysian honey towards α-glucosidase.

Compound	Bonding energy (kcal/mol)	Hydrogen bond	Other interaction residue
Gallic acid	-6.1	ASP352 (2.55 Å), GLN353 (1.80 Å)	ASP352 (3.94 Å), GLN353 (2.75 Å)
Chlorogenic acid	-8.3	LYS156 (2.65 Å), SER240 (2.76 Å), ASP242 (2.54 Å), ARG442 (3.08 Å), GLN279 (3.04 Å), ASP352 (2.79 Å), GLN353(2.54 Å), ARG315 (3.30 Å)	TYR158 (3.83 Å)
Mandelic acid	-6.1	ASP215 (2.42 Å)	VAL216 (5.36 Å), PHE178 (4.72 Å), ASP352 (4.46 Å)
Rutin	-10.1	SER241 (2.29 Å), SER311 (1.95 Å), ARG315 (3.32 Å), TYR158 (2.18 Å), GLU411 (2.61, 2.21 Å)	SER240 (3.77 Å), PHE178 (5.20 Å), VAL216 (4.52 Å), LYS156 (5.42 Å), TYR158 (4.19, 5.23 Å), ARG442 (2.06, 1.95 Å)
Quercetin	-8.7	GLU411 (2.31 Å), TYR158 (2.47 Å), GLU277 (2.71 Å), ASP352 (1.92 Å)	ARG442 (4.51 Å), TYR158 (5.46 Å), GLU277 (2.71 Å), ARG315 (4.94, 4.38 Å),

Among the tested compounds, gallic and mandelic acids exhibited the lowest binding affinity at -6.1 kcal/mol. Rutin showed the highest binding affinity (-10.1 kcal/mol) as compared to other compounds. The molecular interactions between compounds against α-glucosidase highlighted the specific contribution of hydrogen bonds and hydrophobic interaction to the stability of ligand-protein complex. The docking interaction includes the importance of hydrogen bonds and hydrophobic interaction forces to the complex stability (He *et al*., 2019; Tang *et al*., 2020). For instance, rutin formed multiple hydrogen bonds with residues such as SER241, SER311, and TYR158, alongside hydrophobic interactions involving residues like ARG442 and VAL216. Hydroxyl groups at positions C3, C6, C3', and C4' in flavonoids like rutin are crucial for increased inhibitory activity (Tang *et al*., 2020). Similarly, chlorogenic acid (-8.3 kcal/mol) and quercetin (-8.7 kcal/mol) also exhibited strong binding, involving a combination of hydrogen bonds and hydrophobic interactions. The docking results provided valuable insights into potential mechanisms of the α-glucosidase inhibition. These results emphasised the structural diversity and varied inhibitory capacities of phenolic compounds.

The results obtained in the present work agreed with previous studies that reported the enzyme inhibition properties of the respective bioactive compounds. A study by Subhan *et al*. (2025), which evaluated the effect of phenolic compounds on α-glucosidase inhibition, revealed that quercetin with a binding affinity of -7.53 kcal/mol showed significant inhibitory activity (IC_{50} = 0.85 µg/mL), whereas gallic acid with a binding affinity of -4.63 kcal/mol demonstrated moderate inhibitory potential (IC_{50} = 26.19 µg/mL). Similarly, Hyun *et al*. (2014) reported rutin as a strong α-glucosidase inhibitor, with IC_{50} values ranging from 1.0 to 84.1 µg/mL, highlighting its effectiveness in α-glucosidase inhibition.

Conclusion

The use of ^{1}H NMR metabolomics proved useful and reliable in differentiating between the raw and polyphenol extracts of Gelam and Tualang honey. The present work revealed that raw honey exhibited higher α-glucosidase inhibitory activity (39.03 ± 3.47 - 52.58 ± 2.43%) than polyphenol extracts (0.57 ± 0.05 - 1.09 ± 0.09%). Gelam honey, which had the highest amount of phenolics and flavonoids as quantified by HPLC, exhibited the most potential antidiabetic activity. Although polyphenols demonstrated α-glucosidase inhibitory activity, the activity of honey in inhibiting α-glucosidase was primarily attributed to oligosaccharides, amino acids, and organic acids. The present work provided fundamental information on the polyphenol profile and antidiabetic activity of Malaysian honey, which could be beneficial for future studies related to honey and its by-products. The methodology and approach used in the present work could be applied in the quality control of honey with antidiabetic properties. Further research is warranted to comprehensively evaluate the safety and efficacy of honey polyphenols as antidiabetic agents (*e.g*., other *in vitro* antidiabetic assays and *in vivo* animal models or clinical trials).

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References

Abd Ghafar, S. Z., Mediani, A., Maulidiani, Ramli, N. S. and Abas, F. 2018. Antioxidant, α-glucosidase, and nitric oxide inhibitory activities of *Phyllanthus acidus* and LC-MS/MS profile of the active extract. Food Bioscience 25: 134-140.

Ahmed, S. and Othman, N. H. 2013. Review of the medicinal effects of Tualang honey and a comparison with Manuka honey. Malaysian Journal of Medical Sciences 20(3): 6-13.

Ali, H., Bakar, M. F. A., Majid, M., Muhammad, N. and Lim, S. Y. 2020. *In vitro* anti-diabetic activity of stingless bee honey from different botanical origins. Food Research 4: 1421-1426.

Alvarez-Suarez, J., Gasparrini, M., Forbes-Hernández, T., Mazzoni, L. and Giampieri, F. 2014. The composition and biological activity of honey: A focus on Manuka honey. Foods 3(3): 420-432.

A-Rahaman, N. L., Lee, S. C., Sarmidi, M. R. and Aziz, R. 2013. Physicochemical and radical scavenging activities of honey samples from Malaysia. Agricultural Sciences 4(5B): 46-51.

Atrott, J. and Henle, T. 2009. Methylglyoxal in Manuka honey - Correlation with antibacterial properties. Czech Journal of Food Sciences 27(1): S163-S165.

Azman, A. N. S. S., Tan, J. J., Abdullah, M. N. H., Bahari, H., Lim, V. and Yong, Y. K. 2024. Medicinal activities of Tualang honey: A systematic review. BMC Complementary Medicine and Therapies 24(1): 358.

Boruah, T., Devi, H., Dulal, K., Namo Das, P., Devi, B., Ahmad Nayik, G. and Singh, R. 2024. Botanical (melissopalynological) and geographical analysis of honey. In Nayik, G. A., Uddin, J. and Nanda, V. (eds). Advanced Techniques of Honey Analysis, p. 39-62. United States: Elsevier.

Chew, C. Y., Chua, L. S., Soontorngun, N. and Lee, C. T. 2018. Discovering potential bioactive compounds from Tualang honey. Agriculture and Natural Resources 52(4): 361-365.

Codex Alimentarius Commission. 2019. CXS 12-1981 - Standard for honey. Rome: Food and Agriculture Organization.

Devarajan, S. and Venugopal, S. 2012. Antioxidant and α-amylase inhibition activities of phenolic compounds in the extracts of Indian honey. Chinese Journal of Natural Medicines 10(4): 255-259.

Elya, B., Basah, K., Mun'im, A., Yuliastuti, W., Bangun, A. and Septiana, E. K. 2012. Screening of α-glucosidase inhibitory activity from some plants of Apocynaceae, Clusiaceae, Euphorbiaceae, and Rubiaceae. Journal of Biomedicine and Biotechnology 2012: 281078.

Erejuwa, O. O., Sulaiman, S. A. and Ab Wahab, M. S. 2012. Oligosaccharides might contribute to the antidiabetic effect of honey: A review of the literature. Molecules 17(1): 248-266.

Erejuwa, O. O., Sulaiman, S. A., Ab Wahab, M. S., Sirajudeen, K. N. S., Md Salleh, S. and Gurtu, S. 2011. Comparison of antioxidant effects of honey, glibenclamide, metformin, and their combinations in the kidneys of streptozotocin-induced diabetic rats. International Journal of Molecular Sciences 12(1): 829-843.

He, C., Liu, X., Jiang, Z., Geng, S., Ma, H. and Liu, B. 2019. Interaction mechanism of flavonoids and α-glucosidase: Experimental and molecular modelling studies. Foods 8(9): 355.

Hyun, T. K., Eom, S. H. and Kim, J.-S. 2014. Molecular docking studies for discovery of plant-derived α-glucosidase inhibitors. In Plant Omics Journal 7(3): 166-170.

Keng, C. B., Haron, H., Abdul Talib, R. and Subramaniam, P. 2017. Physical properties, antioxidant content and anti-oxidative activities of Malaysian stingless kelulut (*Trigona* spp.) honey. Journal of Agricultural Science 9(13): 32.

Khalil, M. I., Alam, N., Moniruzzaman, M., Sulaiman, S. A. and Gan, S. H. 2011. Phenolic acid composition and antioxidant properties of Malaysian honeys. Journal of Food Science 76(6): 921-928.

Kishore, R. K., Halim, A. S., Syazana, M. S. N. and Sirajudeen, K. N. S. 2011. Tualang honey has higher phenolic content and greater radical scavenging activity compared with other honey sources. Nutrition Research 31(4): 322-325.

Lajis, N., Maulidiani, M., Abas, F. and Ismail, I. S. 2017. Metabolomics approach in pharmacognosy. In Badal, S. and Delgoda, R. (eds). Pharmacognosy: Fundamentals, Applications and Strategy, p. 597-616. United States: Elsevier.

Lazim, N. M. and Baharudin, A. 2017. Honey - A natural remedy for pain relief. In Watson, R. R. and Zibadi, S. (eds). Nutritional Modulators of Pain in the Aging Population, p. 123-134. United States: Elsevier.

Marunaka, Y. 2018. The proposal of molecular mechanisms of weak organic acids intake-induced improvement of insulin resistance in diabetes mellitus *via* elevation of interstitial fluid pH. International Journal of Molecular Sciences 19(10): 3244.

Mavric, E., Wittmann, S., Barth, G. and Henle, T. 2008. Identification and quantification of methylglyoxal as the dominant antibacterial constituent of Manuka (*Leptospermum scoparium*) honeys from New Zealand. Molecular Nutrition and Food Research 52(4): 483-489.

Mohd Kamal, D. A. M., Ibrahim, S. F., Kamal, H., Kashim, M. I. A. M. and Mokhtar, M. H. 2021.

Physicochemical and medicinal properties of Tualang, Gelam and Kelulut honeys: A comprehensive review. Nutrients 13(1): 197.

Muhammad, N. I. I. and Sarbon, N. M. 2021. Physicochemical profile, antioxidant activity and mineral contents of honey from stingless bee and honey bee species. Journal of Apicultural Research 62(2): 394-401.

Newsholme, P., Brennan, L. and Bender, K. 2006. Amino acid metabolism, β-cell function, and diabetes. Diabetes 55(2): S39-S47.

Pasupuleti, V. R., Sammugam, L., Ramesh, N. and Gan, S. H. 2017. Honey, propolis, and royal Jelly: A comprehensive review of their biological actions and health benefits. Oxidative Medicine and Cellular Longevity 2017: 259510.

Peláez-Acero, A., Garrido-Islas, D. B., Campos-Montiel, R. G., González-Montiel, L., Medina-Pérez, G., Luna-Rodríguez, L., … and Cenobio-Galindo, A. J. 2022. The application of ultrasound in honey: Antioxidant activity, inhibitory effect on α-amylase and α-glucosidase, and *in vitro* digestibility assessment. Molecules 27(18): 5825.

Rahmawati, O., Pratami, D. K., Raffiudin, R. and Sahlan, M. 2019. Alpha-glucosidase inhibitory activity of stingless bee honey from *Tetragonula biroi* and *Tetragonula laeviceps*. AIP Conference Proceedings 2092: 030001.

Shamsudin, S., Selamat, J., Abdul Shomad, M., Ab Aziz, M. F. and Haque Akanda, M. J. 2022. Antioxidant properties and characterization of *Heterotrigona itama* honey from various botanical origins according to their polyphenol compounds. Journal of Food Quality 2022: 893401.

Subhan, M., Sanachai, K., Sungthong, B., Datham, S., Ratha, J. and Puthongking, P. 2025. Comparison *in vitro* and *in silico* studies of phenolic acids and flavonoids on α-glucosidase inhibition. Journal of Pharmacy and Pharmacognosy Research 13(1): 311-323.

Tang, H., Huang, L., Sun, C. and Zhao, D. 2020. Exploring the structure–activity relationship and interaction mechanism of flavonoids and α-glucosidase based on experimental analysis and molecular docking studies. Food and Function 11(4): 3332-3350.

Terzo, S., Mulè, F. and Amato, A. 2020. Honey and obesity-related dysfunctions: A summary on health benefits. Journal of Nutritional Biochemistry 82: 1-8.

Uddin, S., Brooks, P. R. and Tran, T. D. 2022. Chemical characterization, α-glucosidase, α-amylase and lipase inhibitory properties of the Australian honey bee propolis. Foods 11(13): 1964.

Worley, B. and Powers, R. 2016. PCA as a practical indicator of OPLS-DA model reliability. Current Metabolomics 4(2): 97-103.