Current **A**pplied **S**cience and **T**echnology e-ISSN: 2586-9396

Curr. Appl. Sci. Technol. 2027, Vol. 27 (No. 1), e0268683

Research article

Phytochemicals, Anti-Amylase and Anti-Acetylcholinesterase Activities of Honey and Bee Bread of Stingless Bees

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Received: 5 August 2025, Revised: 22 November 2025, Accepted: 27 December 2025, Published: 10 April 2026

Abstract

Honey and bee bread from stingless bees are widely recognized for their health benefits and are considered promising sources of bioactive compounds for medicinal applications. Inhibition for amylase and acetylcholinesterase activities is an important medicinal property involved in the prevention of type 2 diabetes and Alzheimer's disease, respectively. In this study, the anti-amylase and anti-acetylcholinesterase activities of stingless bee honey and bee bread, flower extracts of *Clitoria ternatea* L., and a honey-flower drink were evaluated using colorimetric assays. The phytochemical profiles of these samples were further analyzed by Fourier Transform Infrared (FTIR) spectroscopy and Gas Chromatography-Mass Spectrometry (GC-MS). The findings of this study indicated the presence of anti-amylase and anti-acetylcholinesterase activities in the honey and bee bread, flower extracts of *C. ternatea* L., and the formulated flower and honey drink. The flower and honey drink exhibited the strongest anti-acetylcholinesterase activity, while ethanol extracts from bee bread demonstrated the highest anti-amylase activity. In addition, these findings indicated that the FTIR showed functional groups of flavonoids, while GC–MS confirmed the presence of several phytochemicals with biological activities. Interestingly, a siginifcant positive correlation was found between anti-acetylcholinesterase activity and FTIR data (β=0.427, p-value <0.05), while a significant negative correlation was found between anti-amylase activity and FTIR data (β=-0.307, p-value<0.05). Honey and bee bread can be developed as healthy drinks.

Keywords: stingless bees; honey; bee bread; anti-amylase activity; anti-acetylcholinesterase activity

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https://doi.org/10.55003/cast.2026.268683
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1. Introduction

Stingless bees (*Meliponinae*) are eusocial insects that lack functional stingers and serve as important pollinators in subtropical and tropical regions (Rasmussen, 2008). Stingless bees are widely applied for agricultural and commercial objectives because they can act as effective pollinators that can produce honey from flower nectar and collect pollen similar to other honeybees. Several studies reported that stingless bee honey contained a range of bioactive compounds such as p-coumaric acid, quercetin, and hesperetin, various nutrients including vitamin C, protein and disaccharide, and possessed several biological activities (i.e. antimicrobial, antioxidant, anti-inflammatory, anti-diabetic, skin aging delay, and hypolipidemic activities) (Ranneh et al., 2018; Pimentel et al., 2022). Stingless bees provide various natural products such as honey, propolis, pollen, and bee bread that are used in folk medicine due to their medicinal properties and bioactive molecules. Bee bread is a mixture of pollen, salivary enzymes, regurgitated honey and lactic acid bacteria, known as fermented pollen which are kept in their pots as major foods for bees (Kieliszek et al., 2018). Bee bread consists of several phytochemicals, nutrients (i.e. sugar, vitamins, amino acids, and fatty acids) and microbes that are useful for health product applications (Kostić et al., 2015).

However, insecticides and environmental pollution have negative effects on the stingless bees (Piovesan et al., 2020). Insecticides can disrupt cholinergic neurotransmission, causing cognitive disorders such as learning and memory problems (Beizunces et al., 2012). The existence of insecticides in honey and bee bread occurs from contaminated plants, which lead to destroying consumer health (Zafeiraki et al., 2022). Generally, the determination of acetylcholinesterase inhibition is used as a bioindicator to monitor human health and pesticides contaminated in food and environments (Thummajitsakul et al., 2020a).

Acetylcholinesterase is an important enzyme responsible for hydrolysis of neurotransmitter acetylcholine at postsynaptic neuromuscular junctions. High levels of acetylcholine are linked to Alzheimer's disease, a chronic neurodegenerative disease involving disorders of thinking and memory and commonly found in elderly people (Fiest et al., 2016). Hence, inhibition of acetylcholinesterase enzyme is a key strategy involved in the prevention or treatment of Alzheimer's disease (dos Santos et al., 2018). Plant extracts with anti-acetylcholinesterase activity showed potential in the treatment of Alzheimer's disease (Taqui et al., 2022). Generally, reversible inhibitors of acetylcholinesterase enzyme are mostly used to treat Alzheimer's disease, while irreversible inhibitors (i.e. organophosphorus compounds) and some reversible inhibitors (i.e. carbamates) show toxicity to human and animals (Colović et al., 2013). Previously, honey from honeybees (*Apis cerana*, *Apis andreniformis*, *Apis koschevnikovi* and *Apis nuluensis*) showed antioxidant and anti-cholinesterase activities (Philip et al., 2015).

Moreover, patients with type 2 diabetes mellitus have a greater risk of developing Alzheimer's disease (Han & Li, 2010). Type 2 diabetes mellitus and Alzheimer's disease are non-communicable diseases that are frequently found in an aging population. Treatment of type 2 diabetes mellitus involves the inhibition of amylase enzyme. The enzyme is responsible for the breakdown of complex carbohydrates into glucose and maltose, which can lead to high blood glucose levels after a meal (Devarajan & Venugopal, 2012). Currently, there is a limited number of research studies focusing on the identification of natural sources with dual therapeutic potential against both type 2 diabetes and Alzheimer's disease. Besides the identification of significant natural sources of bioactive components, the formulation and development of functional health beverages have

become increasingly popular, with a focus on enhancing nutritional value and delivering bioactive benefits for disease prevention and well-being.

Therefore, developing functional health beverages derived from honey of stingless bees and flower extract of *Clitoria ternatea* L. was emphasized in the current study. *Clitoria ternatea* L. (Fabaceae family) is a perennial medicinal plant in tropical Asia (Gupta et al., 2010). *Clitoria ternatea* L. consists of several phytochemicals (i**.**e**.** phenols, terpenoids, glycosides, coumarins, catechols, proteins, anthocyanins, and carbohydrates) with medicinal properties (i.e. nootropic, anxiolytic, anticonvulsant, sedative, antipyretic, anti-proliferative, anti-inflammatory, anti-diabetic, and antioxidant activities) (Gupta et al., 2010; Divya et al., 2018). Furthermore, the useful analytical tools Fourier Transform Infrared Spectra (FTIR) and Gas Chromatography–Mass Spectrometry (GC–MS) were used in this research. FTIR was commonly used to indentify the functional groups of chemical compounds, while GC**-**MS was qualitatively used to identify volatile metabolites found in ethanol extracts. Volatile organic metabolites are commonly found in both honey and bee bread samples, contributing to the aroma and flavor of these bee products, and serving as biomarkers to identify their quality and geographical origins (Montaser et al., 2023; Pokajewicz et al., 2024; Almeida-Dias et al., 2025). FTIR spectroscopy is an analytical technique that is commonly used to identify functional groups of chemical components and to estimate qualities of herbals and foods (Wongsa et al., 2022).

In this study, anti-amylase and anti-acetylcholinesterase activities of natural products derived from stingless bees and *C. ternatea* L. were focused, which have not been comprehensively examined in combination before. Furthermore, the findings from this study could be used to confirm the existence of several bioactive compounds and emphasize the potential of these natural products for pharmaceutical and functional food applications.

2. Materials and Methods

2.1 Chemicals

Acetylthiocholine iodide, 3,5-dinitrosalicylic acid, DTNB (5,5′-dithiobis, 2-nitrobenzoic acid), sodium potassium tartrate and acetylcholinesterase enzyme from *Electrophorus electricus* (electric eel) type V-S were obtained from Sigma (USA). Absolute ethanol solvent and alpha-amylase enzyme from porcine pancreas were obtained from Merck (USA). A GT-pesticide residual test kit was purchased from Higher Enterprises Co., LTD. (Thailand).

2.2 Sample preparation and extraction

Honey (honey 1 and honey 2) and bee bread (bee bread 1, 2 and 3) of stingless bees (*Tetragonula pagdeni*) were collected from different nest boxes from Chanthaburi province, Thailand. Dried flowers of *Clitoria ternatea* L. were purchased from Punsuk Farm, Chaiyaphum province of Thailand. The dried flowers of *Clitoria ternatea* L. (10 g) were finely homogenized and extracted with 70% ethanol and deionized water at 45^{o}C for 48 h and sieved by a filer cloth. For bee bread, the samples were dried at 50^{o}C for 48 h and finely ground with a pestle and mortar. The powdered bee bread (2 g) was then extracted with 15 mL of 70% ethanol and deionized water at 70^{o}C for 30 min. Each extract was concentrated by a rotary evaporator (IKAa RV10) at 45^{o}C for 20-38 min (Thummajitsakul et al., 2020b). Each concentrated extract was adjusted using 70% ethanol or deionized water to obtain the final concentration for analysis.

For honey preparation, honey (2 mL) was diluted with deionized water (10 mL) and 70% ethanol (10 mL), while the flower and honey drink were prepared by mixing the diluted honey with aqueous extract of *C. ternatea* flowers in a ratio of 19:1 mL, 18:2 mL and 16:4 mL.

2.3 Analysis by GT-pesticide residue test kit

Determination of organophosphate and carbamate compounds was performed by A GT-pesticide residue test kit (Higher Enterprises Co., LTD. Thailand), according to the protocol of Thoophom (2025). Each sample (5 mL) of honey, bee bread extract, or flower extract of *C. ternatea* and the flower and honey drink was mixed with extraction solvent-1 (dichloromethane) with stirring for 1 min and left for 15 min, and then 1 mL of the mixture was extracted with extraction solvent-2 (5% ethanol) (1 mL) and stirred until extraction solvent-2 had disappearance. After that, each sample extract (1 mL) was added to a new sample tube, while critical and control tubes were prepared by using 1 mL of extraction solvent-2, followed by the addition of acetylcholinesterase enzyme (GT-1) (0.5 mL), and reacted at 37°C for 10 min. Then, the critical tube was added with a GT-2 mixed reagent (acetylcholine) (375 µL), while the control and sample tubes were added with 250 µL of GT-2 mixed reagent, and allowed to react at 37°C for 30 min. Moreover, a GT-3 mixed reagent (hydroxylamine) was added for 1 mL, followed by a 500 µL of GT-4 mixed reagent (hydrochloric acid) and a 500 µL of GT-5 mixed reagent (ferric chloride), respectively. The reaction color of each sample tube was compared with the color of the critical and control tubes. If the reaction color of the sample tube was lighter than the control tube, it indicated that the insecticides were not found in the sample. If the reaction color of the sample tube was darker than the control tube but lighter than the critical tube, it indicated that the insecticides were found in the sample, but they were at a safe level. If the reaction color of the sample tube was darker than the critical tube, it indicated that the insecticides were found in the sample at an unsafe level.

2.4 Anti-acetylcholinesterase activity

Anti-acetylcholinesterase activity was evaluated by reacting 200 µL of each sample with 1.5 mM acetylthiocholine iodide (200 uL), 3 mM DTNB (1000 µL) and 0.3 U/mL of acetylcholinesterase (200 µL) at 37°C for 15 min (Thummajitsakul et al., 2020a). The absorbance of the reaction mixture was read at 410 nm by a spectrophotometer (Model T60UV). Water was used as negative control. In the absence of anti-acetylcholinesterase activity, the reaction developed a darker color. Inhibition potential of acetylcholinesterase was calculated from an absorbance of enzyme-combining-sample and water, and an absorbance of sample and water without enzyme. The inhibitory percentage was used to calculate 50% effective concentration (EC_{50}) or sample concentration for 50% acetylcholinesterase inhibition. Lower EC_{50} indicates higher inhibitory activity.

2.5 Anti-amylase activity

Anti-amylase activity was evaluated by reacting to each sample (100 µL) with 1.5 units/mL alpha-amylase (100 µL) and 1% starch solution (100 µL) at 37°C for 20 min. Then, color reagent solution (100 µL) was added to react at 85°C for 15 min. Finally, deionized water (900 µL) was added to the sample before measuring its absorbance at 540 nm. Water was used as negative control. In the absence of anti-amylase activity, the reaction developed a

darker color. Acarbose (6.25, 12.5, 25 and 50 mg/mL) was used as a positive control and inhibition potential of amylase was calculated. The EC_{50} of each sample was determined (Thummajitsakul et al., 2020b).

2.6 FTIR analysis

Each sample was analyzed by Fourier transform infrared (FTIR) spectroscopy (Spectrum Two™, Perkin Elmer, USA) on the crystal plate center across a wavenumber range of 550 to 4000 cm^{-1} using a resolution of 4 cm^{-1}. The FTIR spectra were analyzed according to Caunii et al. (2012) and Topalăa et al. (2017). FTIR data was obtained by scoring the presence or absence of FTIR peaks as binary data (1/0).

2.7 GC-MS analysis

Ethanol extract of each sample was used for the detection of chemical components. A Shimadzu gas chromatograph (a DB-5MS capillary column, 30 m × 0.25 mm × 0.25 µm) and a Shimadzu mass spectrometer (Shimadzu, Kyoto, Japan) were used to detect bioactive compounds. The GC-MS analysis was carried out under the conditions as follows: at 40°C for 1 min, 40-150°C at 8°C/min, 150-200°C at 15°C/min, and temperatures for injector, transfer and ion source were performed at 250°C, 250°C, and 200°C, respectively. For carrier gas, helium was used in this analysis at a flow rate of 1 mL/min. Each mass spectrum peak of bioactive compounds was aligned with NIST14 data at least 90% similarity index.

2.8 Data analysis

Anti-acetylcholinesterase activity, anti-amylase activity, and insecticide contamination were expressed as mean, SD, and percentage. One-way ANOVA was used to test hypothesis of the differentiation of anti-acetylcholinesterase activity and anti-amylase activity among extracts of *C. ternatea* L., bee bread and honey, and the flower extract-containing honey. All statistical analyses were performed using PSPP program (version 0.10.5), and significance was considered at $p<0.05$ (Pfaff et al., 2013). Principal component analysis (PCA) and the unweighted pair group method (UPGMA) were generated with Paleontological Statistic program version 3.16 (Hammer et al., 2001). Path coefficients and P-values from partial least squares structural equation modeling (PLS-SEM) were generated from SmartPLS version 3 (Ringle et al., 2015).

3. Results and Discussion

As shown in Table 1, the ethanol and aqueous extracts of *C. ternatea* flowers, the honey and bee bread of stingless bees, and the flower extract-containing honey demonstrated a high level of anti-amylase and anti-acetylcholinesterase activities. The highest anti-amylase activity was found in ethanol extracts of bee bread (EC_{50} = 0.22 to 348.68 mg/mL), followed by water extracts of honey (EC_{50} =23.59 to 33.62 mg/mL), the flower extract-containing honey (EC_{50} =50.32 to 157.66 mg/mL), ethanol extracts of honey (EC_{50} = 68.69 to 111.31 mg/mL), aqueous extracts of *C. ternatea* flowers (EC_{50} =0.0112), ethanol extracts of *C. ternatea* flowers (EC_{50} =101.96 mg/mL), and aqueous extracts of bee bread (EC_{50} =108.27 to 312.46 mg/mL), respectively. For anti-acetylcholinesterase activity, the flower extract-containing honey (EC_{50} = 0.05 to 103.56 mg/mL) showed the highest acetylcholinesterase inhibitory

Table 1. Anti-acetylcholinesterase and anti-amylase activities of flower extract of *C. ternatea* L, honey and bee bread of stingless bee, and flower extract-containing honey

Samples	Solvent	Anti-acetylcholinesterase activity (EC_{50}) Mean±SD (mg/mL)	Anti-amylase activity (EC_{50}) Mean±SD (mg/mL)
C. ternatea flower	Aqueous	6.29±0.48	101.96±49.62
	Ethanol	3.43±2.80	186.12±80.03
Honey 1	Aqueous	8.22±0.29	23.59±14.64
	Ethanol	192.08±10.86	111.31±57.21
Honey 2	Aqueous	8.92±0.84	33.62± 21.07
	Ethanol	145.91±9.52	68.69±2.02
Bee bread 1	Aqueous	10.15±0.58	277.31±80.50
	Ethanol	3.65±0.54	348.68±171.81
Bee bread 2	Aqueous	13.48±0.16	108.27±29.94
	Ethanol	0.06±0.00	0.22±0.02
Bee bread 3	Aqueous	9.90±0.72	312.46±132.20
	Ethanol	7.02±0.10	296.99±108.43
Flower extract-containing honey	Formula 1 (18:2 mL)	0.05±0.01	50.32±15.27
	Formula 2 (19:1 mL)	0.08±0.00	157.66±41.25
	Formula 3 (16:4 mL)	103.56±4.35	51.90±6.92

*Note: Formulas 1, 2 and 3 were flower extract-containing honey samples obtained from mixing honey with flower aqueous extract at the ratios of 18:2 mL, 19:1 mL and 16:4 mL, respectively.

activity, followed by ethanol extracts of bee bread (EC_{50} = 0.06 to 7.02 mg/mL), ethanol extracts of *C. ternatea* flowers (EC_{50} = 3.43 mg/mL), aqueous extracts of *C. ternatea* flowers (EC_{50} = 6.29 mg/mL), water extracts of honey (EC_{50} =8.22 to 8.92 mg/mL), aqueous extracts of bee bread (EC_{50} = 9.90 to 13.48 mg/mL), and ethanol extracts of honey (EC_{50} =145.91 to 192.08 mg/mL), respectively. However, the reported EC_{50} values coverd wide ranges.

This variation in the high level of anti-amylase and anti-acetylcholinesterase activity likely arose from sample heterogeneity in the honey and bee bread of the stingless bees and in the flower extract-containing honey. Additionally, organophosphorus and carbamate insecticides were detected in all samples using a pesticide test kit based on anti-acetylcholinesterase activity. Preliminarily, the pesticide test kit was used qualitatively to estimate the anti-acetylcholinesterase activity of all samples. According to a previous report, a cut-off point of 50% is used to define acceptable levels of enzyme inhibition. If the insecticide toxicity in the sample results in enzyme inhibition not exceeding 50%, it is regarded as a safe level (Thoophom, 2025).

The results showed that the ethanol extracts of *C. ternatea* flowers and honey contained insecticides, but they were at a safe level, while the ethanol extract of bee bread

showed insecticide contamination at an unsafe level. These findings were consistent with a previous report indicating that pesticide residues accumulated predominantly in beebread, leading to higher hazard quotients and thus greater risk to honey bee health (Mahdavi et al., 2025). However, pesticide contamination also reveales significant variability in both the concentration and types of pesticide residues among individual colonies, even when sampled at the same location and time (Schaad et al., 2023). In the current study, pesticide residues were determined in natural products from stingless bees collected from different nest boxes in Chanthaburi province. Pesticide contamination in honey and bee bread may be associated with surrounding areas where chemical substances are used, as well as from other environmental factors even if no chemicals are applied within the beekeeping area. However, insecticide contamination was not found in flower aqueous extract-containing honey. Organophosphorus compounds have functional groups (i.e. amides, esters, thiol and phosphoric acids) and carbamate compounds have methyl group, carbonyl group, and aromaticity (Mdeni et al., 2022). However, it is possible that each sample consisted of chemical components that showed reversible anti-acetylcholinesterase activity that were useful for Alzheimer's disease treatment. In case of toxic organophosphorus and carbamate compounds used as insecticides, they act as irreversible acetylcholinesterase inhibitors, leading to death in insects (Colović et al., 2013).

The study showed strong anti-amylase and anti-acetylcholinesterase activities, particularly for bee bread ethanol extract and flower extract-containing honey. Additionally, the greater inhibition of enzyme activities was shown at higher concentrations of the samples and standard inhibitors such as acarbose for anti-amylase activity. Corresponding to previous report, acarbose at higher concentrations shows greater inhibition of amylase activity (Kumar et al., 2024). For anti-acetylcholinesterase activity, standard inhibitors were not used in this study. However, several standard inhibitors of acetylcholinesterase have been reported that the presence of different features of inhibitors depends on enzyme specificity such as donepezil and galantamine (Grossberg, 2003). They are both standard inhibitors for acetylcholinesterase used to treat Alzheimer's disease symptoms. Previous data from in vivo tests demonstrated that it was necessary to use galantamine at doses 3-15 times greater than donepezil in order to achieve the same level of acetylcholinesterase inhibition in the brain (Geerts et al., 2005). Interestingly, bee bread ethanol extract and flower extract-containing honey shows strong anti-amylase and anti-acetylcholinesterase activities in this study. Bee bread is a natural bee product for which limited information is available regarding its acetylcholinesterase inhibitory effects and is unknown to treat Alzheimer's disease symptoms. However, bee bread contains various components such as protein, phenol and flavonoid that also demonstrate anti-amylase activity in vitro (Hashemirad et al., 2024). Moreover, honey contains high total polyphenol content and can act as natural amylase and acetylcholinesterase inhibitors (Zakaria et al., 2020; Fratianni et al., 2025). Additionally, *C. ternatea* flowers showed cognitive enhancing effect, oxidative stress status and acetylcholinesterase inhibition in prefrontal cortex of normal male mice (Yimdee et al., 2014) and showed anti-amylase activity in vitro test (Hungerford et al., 2019). Thus, the potential of combining bee bread, *C. ternatea* flower extract, and stingless bee honey as natural inhibitors for amylase and acetylcholinesterase may provide additive or synergistic effects.

Moreover, the bioactive components in the ethanol and aqueous extracts of *C. ternatea* L. flowers, bee bread, honey and flower extract-containing honey were preliminary screened by detecting functional groups by FTIR analysis. The FTIR results of the ethanol and aqueous extracts of *C. ternatea* L. flowers, bee bread, honey and flower extract-containing honey demonstrated FTIR spectrum absorptions that appeared in ten different wavenumber ranges which indicated the presence of functional groups, as shown in Table

2. The honey samples showed the greatest number of FTIR peaks, which were found at all ten wavenumber ranges, followed by ethanol extracts of bee bread, ethanol and aqueous extracts of *C. ternatea* flower, and the flower extract-containing honey. Interestingly, all sample types consisted of functional groups similar to flavonoid compounds at wavenumber ranges of 3263.29-3307.56, 1627.29-1644.28 and 1603.35-1644.28 cm^{-1}, indicating the presence of hydroxyl groups, unsaturated bonds and conjugated bonds, respectively. Corresponding to the previous report, the wavenumber ranges of 4000-3125, 3140-3000, 1670-1620, 1650-1600 and 1600-1500 cm^{-1} were identified as being to those of the hydroxyl groups, di-substituted heterocyclic ring carbons, unsaturated bonds, conjugated bonds, and aromaticity of flavonoids, respectively (Noh et al., 2017). Furthermore, all flower extract-containing honey samples showed the same patterns of FTIR spectra, while they were different from the FTIR patterns of the ethanol and aqueous extracts of *C. ternatea* L. flower, bee bread, and the honey of stingless bees at wavenumber ranges of 2926.32-2932.53, 1343.65-1417.08 and 1232.33-1258.21 cm^{-1} (Figure 1), indicating the presence of CH_2 and CH_3 groups, phenyl groups, and acid or ester groups, respectively.

Interestingly, a significant positive correlation was found between anti-acetylcholinesterase activity and FTIR data (β=0.427, p-value=0.016), while a low negative correlation was significantly found between anti-amylase activity and FTIR data (β=-0.307, p-value=0.015) (Figure 2). This implied that some bioactive compounds consisted of functional groups involved in anti-acetylcholinesterase activity. Corresponding to previous reports, honey from Apis *cerana*, *Apis andreniformis*, *Apis koschevnikovi* and *Apis nuluensis* displayed high anti-acetylcholinesterase activity (Philip et al., 2015). Moreover, commercial honey samples from different 19 regions demonstrated anti-acetylcholinesterase activity that significantly correlated with total phenolic content (Szwajgier et al., 2022).

Table 2. Functional groups of chemical components of bee bread and honey of stingless bees, the ethanol and aqueous extracts of C. *ternatea* L. flower, and different flower extract-containing honey samples

Number	FTIR Peaks in Samples (cm^{-1})	Functional Groups
1	3263.29-3307.56	hydroxyl groups
2	2926.32-2932.53	CH_2 and CH_3
3	1627.29-1644.28	unsaturated bonds
4	1603.35-1644.28	conjugated bonds
5	1343.65-1417.08	phenyl groups
6	1232.33-1258.21	acid or ester
7	1024.39-1258.21	acid or ester
8	1024.39-1148.5	C–O stretch
9	1034.79-1148.5	C-O
10	502.98-924.39	C-H

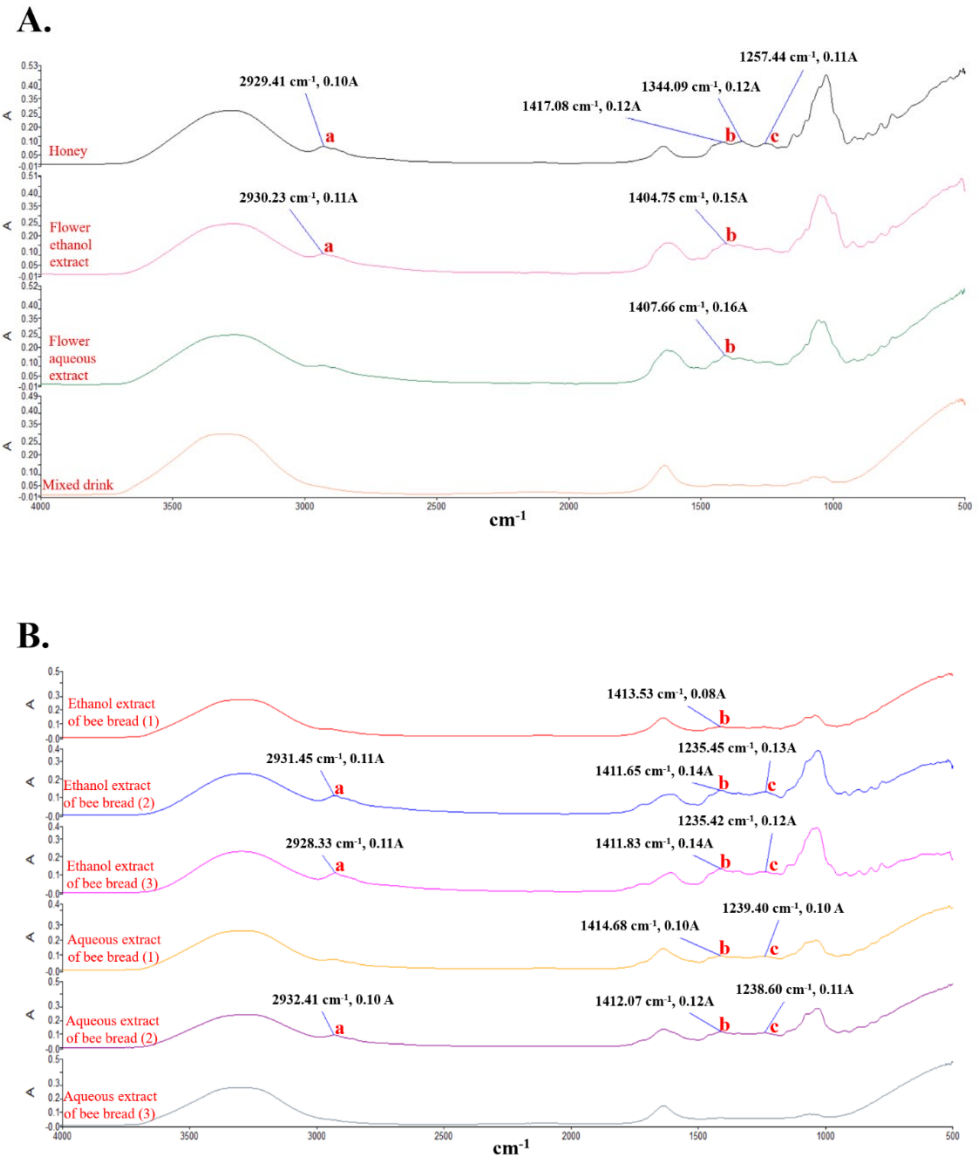


Figure 1. FTIR spectra of ethanol and aqueous extracts of C. *ternatea* L. flower, bee bread, honey of stingless bees, and flower extract-containing honey. A is FTIR spectra of flower sample, flower ethanol and aqueous extracts and mixed drink, while B is FTIR of ethanol and aqueous extracts of bee bread. Different FTIR peaks of honey, mixed drink, the ethanol and aqueous extracts of *C. ternatea* L. flower and bee bread were at wavenumber ranges of 2926.32-2932.53 (a), 1343.65-1417.08 (b) and 1232.33-1258.21 cm^{-1} (c) indicating the presence of CH_2 and CH_3 groups, phenyl groups, and acid or ester groups, respectively, as shown in Table 2.

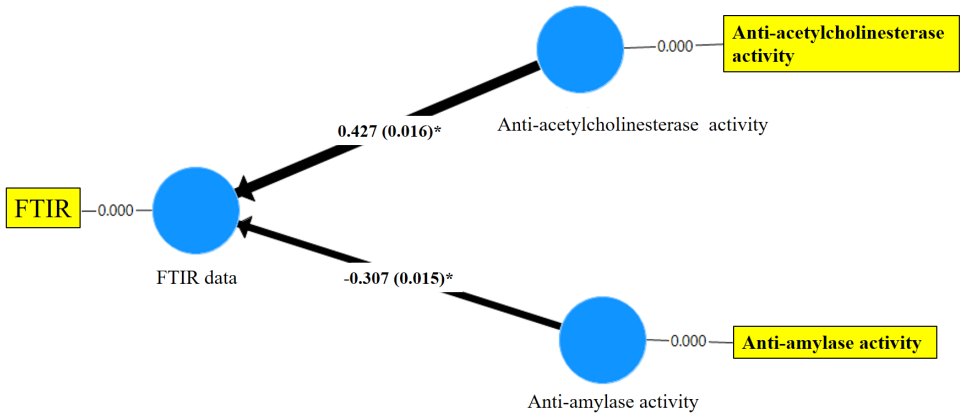


Figure 2. Path coefficients and P-values from partial least squares structural equation modeling (PLS-SEM) between anti-acetylcholinesterase activity and FTIR data, and between anti-amylase activity and FTIR data. The (*) asterisk indicates a significant level of P-value < 0.05, demonstrated in parentheses. Correlation between anti-acetylcholinesterase activity and FTIR data and between anti-amylase activity and FTIR data were demonstrated with path coefficients (β) at 0.427 and -0.307, respectively.

However, a weak negative correlation was found between anti-amylase activity and FTIR data. This indicated that the presence of some functional groups on bioactive compounds may decrease the inhibition of amylase. Interestingly, amylase enzyme is commonly found in honey where it acts as a freshness and adulteration marker in honey and is related to the viscosity loss of starchy food (Babacan et al., 2002; Nagai et al., 2009; Pasias et al., 2017). Moreover, honey that was processed by ultrasonic treatment had a high level of bioactive agents and amylase inhibitory potential (Peláez-Acero et al., 2022). For stingless bees, honey samples from stingless bee consisted of high total phenolic content, total flavonoid content and anti-amylase activity, and honey from different regions vary with the concentrations of bioactive compounds and enzyme inhibition potentials (Ali et al., 2020).

Moreover, bee bread showed several bioactive compounds (fatty acids and flavonoids) with anti-amylase activity (Sobral et al., 2017; Keskin & Özkök, 2020). Furthermore, extracts of *Clitoria ternatea* petals showed inhibition of oxidants, α-amylase and α-glucosidase (Escher et al., 2020), and root extracts of *Clitoria ternatea* were able to inhibit acetylcholinesterase activity in rat models (Damodaran et al., 2020).

To simplify data analysis, principal component analysis (PCA) was used to observe correlation and reduce data dimensions in this study without affecting the data, while cluster analysis was used to explain the similarity of the samples. Scatter plots from the PCA results for anti-amylase and anti-acetylcholinesterase activities showed that the first and second principal components were 70.80% and 25.20% of the overall variation. The mixed drinks showed the highest anti-acetylcholinesterase activity, while extracts from bee bread showed the highest anti-amylase activity (Figure 3A). Moreover, the PCA analysis showed that stingless bee honey, extracts of *C. ternatea* flower and bee bread consisted of several FTIR peaks (Figure 3B). Furthermore, the cluster analysis based on anti-amylase and anti-acetylcholinesterase activities revealed two groups: group 1 consisted of ethanol extracts of bee bread and the flower extract-containing honey samples (formulas

1 and 2), group 2 consisted of the ethanol and aqueous extracts of *C. ternatea* flowers, stingless bee honey, ethanol and aqueous extracts of bee bread and the flower extract-containing honey sample (formula 3). Similarly, cluster analysis of all samples based on the FTIR spectra pattern showed two groups: group 1, consisting of stingless bee honey, the ethanol extracts of *C. ternatea* flowers, ethanol extracts of bee bread, and group 2, which consisted of the aqueous extracts of *C. ternatea* flowers, the aqueous extracts of bee bread, and the flower extract-containing honey samples (formulas 1, 2 and 3). Therefore, cluster analysis based on the FTIR data was more effective in differentiating the sample groups than analyses based on anti-amylase and anti-acetylcholinesterase activities (Figures 4A and 4B). Moreover, the GC-MS results showed that the *C. ternatea* flowers, bee bread and honey consisted of compounds with various pharmaceutical properties, such as furfural, 2-furanmethanol and 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one, which were previously reported to exhibit antityrosinase, antimicrobial, anti-inflammatory, and antioxidant activities (Kumar et al., 2010; Chai et al., 2013; Yu et al., 2013). Interestingly, C. *ternatea* flower had dimethyl silane diol which is an inhibitor of angiotensin-converting enzyme and HIV protease (Chen et al., 2001; Mutahi et al., 2002), while bee breed and honey had 5-hydroxymethylfurfural and 9,12,15-octadecatrienoic acid showed antioxidant, antiproliferative activities and antibacterial activity (Zhao et al., 2013; Kusumah et al., 2020) (Table 3 and Figure 5). The results also indicated that bee bread and honey from different nest boxes showed some different bioactive compounds such as n-hexadecanoic acid, octanoic acid, 5-hydroxymethylfurfural, and 9,12,15-octadecatrienoic acid. Interestingly, 5-hydroxymethylfurfural or HMF was detected in bee bread and honey. HMF is a volatile heterocyclic compound that is generated from dehydration mechanism of reducing sugars in honey. Moreover, HMF is an indicator used to determine the freshness of honey (Alghamdi et al., 2020). A high level of HMF was detected in honey samples that had been exposed to excessive heat, food processing, and prolonged storage (Alghamdi et al., 2020). Our study indicated the presence of several components with medicinal properties namely furfural, 2-furanmethanol and 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one in the honey, bee bread and flower extract-containing honey of stingless bees. Moreover, dimethyl silane diol was found in the *C. ternatea* flowers, while 5-hydroxymethylfurfural and 9,12,15-octadecatrienoic acid were found in both bee bread and honey. The bioactive components of stingless bee products and *C. ternatea* extracts are the foundation for the development of health-promoting beverages or nutraceutical products, addressing the demands of modern consumers for health, wellness, and quality of life. Using bee bread, *C. ternatea* flower extracts, and stingless bee honey as natural inhibitors for amylase and acetylcholinesterase can contribute to reduce side effects when compared with synthetic inhibitors. However, interesting future research should be focused on the bioactive components associated with the pharmacological properties, validation of mechanisms in vivo models, comprehensive safety evaluations, and a molecular docking-based approach to simulate the molecular interaction of natural inhibitors with amylase and acetylcholinesterase. Although natural compounds are valuable sources for health-promoting beverages or nutraceutical products, ensuring their safety is nessesary. One major concern is pesticide residues in organophosphorus and cabamate groups which are commonly used in agriculture. The ethanol extracts of *C. ternatea* flowers and honey contained insecticides at a safe level, but the ethanol extract of bee bread showed insecticide contamination at unsafe level in this study. Therefore, novel health products based on natural compounds should be improved not only for functionality and efficacy but should also concern rigorous safety assessments before recommending these products for human consumption.

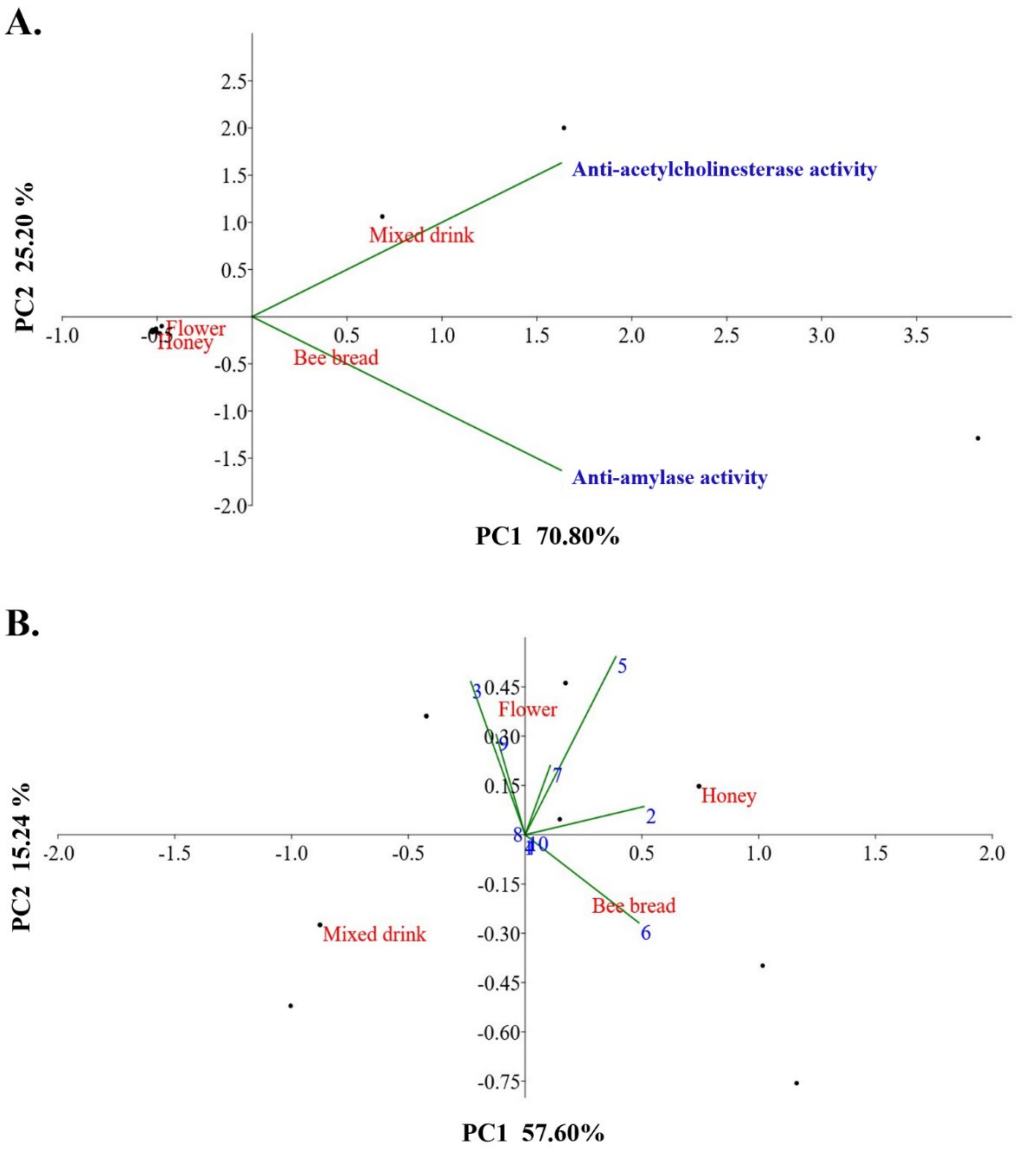


Figure 3. Scatter plots from principal component analysis (PCA) of anti-amylase activity and anti-acetylcholinesterase activity and FTIR data. A is the PCA that was generated from data of anti-amylase activity and anti-acetylcholinesterase activity, while B is the PCA that was generated from FTIR data. Flower, honey, bee bread and mixed drink that were scattered in the two plots were groups of all samples. Numbers 1-10 in the B plot were based on FTIR peaks found in the samples.

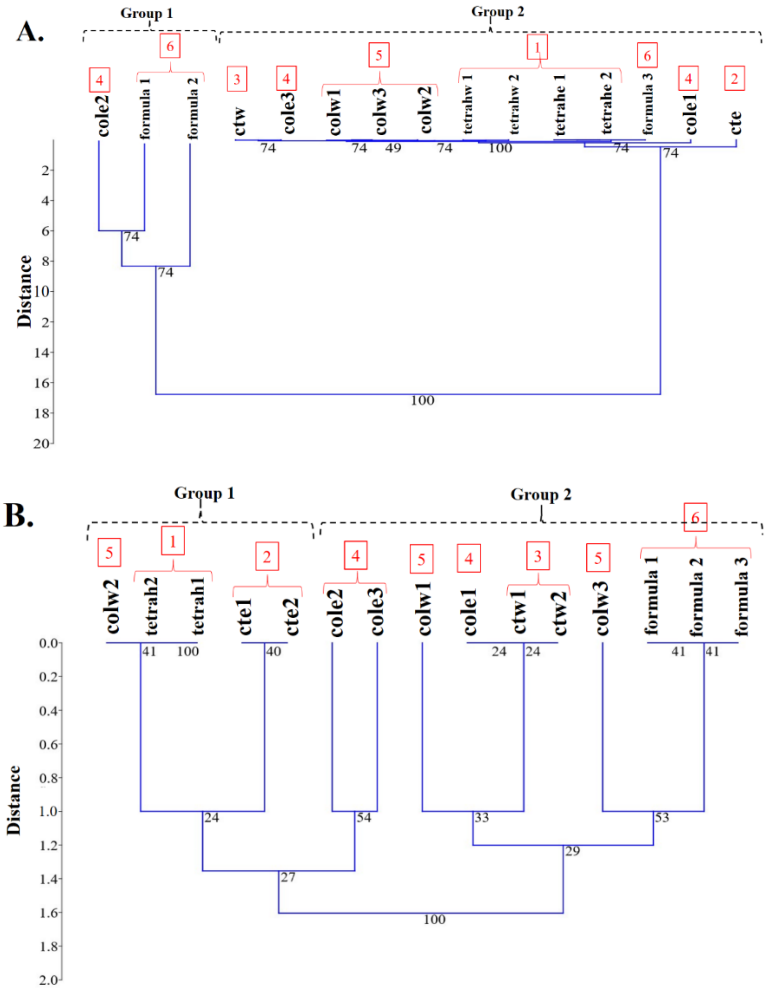


Figure 4. Cluster analysis from the unweighted pair group method (UPGMA) of anti-amylase activity and anti-acetylcholinesterase activity and FTIR data. Number 1 was samples of stingless bee honey, 2 was samples of ethanol extracts of *C. ternatea* flower, 3 was samples of aqueous extract of *C. ternatea* flower, 4 was samples of ethanol extracts of bee bread, 5 was samples of aqueous extract of bee bread and 6 was samples of flower extract-containing honey. A was cluster analysis of all samples based on data of anti-amylase activity and anti-acetylcholinesterase activity that revealed two groups: group 1 consisted of ethanol extract of bee bread and the flower extract-containing honey samples (formulas 1 and 2), while group 2 consisted of ethanol and aqueous extracts of *C. ternatea* flower, stingless bee honey, ethanol extracts and aqueous extract of bee bread and the flower extract-containing honey sample (formula 3). For B, it was cluster analysis of all samples based on FTIR data that showed two groups: group 1 consisted of stingless bee honey, ethanol extracts of C. ternatea flower, ethanol extracts of bee bread, and group 2 which consisted of aqueous extract of *C. ternatea* flower, aqueous extract of bee bread, and the flower extract-containing honey samples (formulas 1, 2 and 3).

Table 3. Bioactive compounds found in stingless bee honey, ethanol extracts of *C. ternatea* flower and bee bread, and flower extract-containing honey

Samples	Phytochemicals	% Similarity	Formula	Biological Activity	References
C. ternatea flower	Dimethyl silanediol	91	$C_2H_8O_2Si$	inhibitors of angiotensin-converting enzyme and HIV protease	Chen et al., 2001; Mutahi et al., 2002;
	Furfural	96	$C_5H_4O_2$	antityrosinase and antimicrobial activities	Chai et al., 2013
	2-Furanmethanol	94	$C_5H_6O_2$	antityrosinase and antimicrobial activities	Chai et al., 2013
	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	90	$C_6H_8O_4$	antimicrobial, anti-inflammatory, and antioxidant capacity	Kumar et al., 2010; Yu et al., 2013
Bee bread 1	furfural	97	$C_5H_4O_2$	antityrosinase and antimicrobial activities	Chai et al., 2013
	2-Furanmethanol	95	$C_5H_6O_2$	antityrosinase and antimicrobial activities	Chai et al., 2013
	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	93	$C_6H_8O_4$	gustatory activity, flavourant	Chukwu et al., 2017
	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	90	$C_6H_8O_4$	antimicrobial, anti-inflammatory, and antioxidant capacity	Zhao et al., 2013; Yu et al., 2013
Bee bread 2	Furfural	95	$C_5H_4O_2$	antityrosinase and antimicrobial activities	Chai et al., 2013
	2-Furanmethanol	92	$C_5H_6O_2$	antityrosinase and antimicrobial activities	Chai et al., 2013
	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	93	$C_6H_8O_4$	gustatory activity, flavorant	Chukwu et al., 2017
	5-Hydroxymethylfurfural	96	$C_6H_6O_3$	antioxidant and antiproliferative activities	Zhao et al., 2013
	(Z, Z, Z)-9,12,15-Octadecatrienoic acid	91	$C_{18}H_{30}O_2$	cancer preventive and antibacterial activity	Kusumah et al., 2020

Table 3. Bioactive compounds found in stingless bee honey, ethanol extracts of *C. ternatea* flower and bee bread, and flower extract-containing honey (continued)

Samples	Phytochemicals	% Similarity	Formula	Biological Activity	References
Bee bread 3	Furfural	96	C5H4O2	antityrosinase and antimicrobial activities	Chai et al., 2013
	2-Furanmethanol	91	C5H6O2	antityrosinase and antimicrobial activities	Chai et al., 2013
	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	94	C6H8O4	gustatory activity, flavourant	Chukwu et al., 2017
	5-Hydroxymethylfurfural	91	C6H6O3	antioxidant and antiproliferative activities	Zhao et al., 2013
Honey 1	n-Hexadecanoic acid	91	C16H32O2	anti-inflammatory property	Aparna et al., 2012
	Octanoic acid	91	C8H16O2	antibacterial activity	Zhang et al., 2019
Honey 2	2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	94	C6H8O4	antimicrobial, anti-inflammatory, and antioxidant capacity	Kumar et al., 2010; Yu et al., 2013
	5-Hydroxymethylfurfural	93	C6H6O3	antioxidant and antiproliferative activities	Zhao et al., 2013
	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	91	C18H30O2	cancer preventive and antibacterial activity	Kusumah et al., 2020

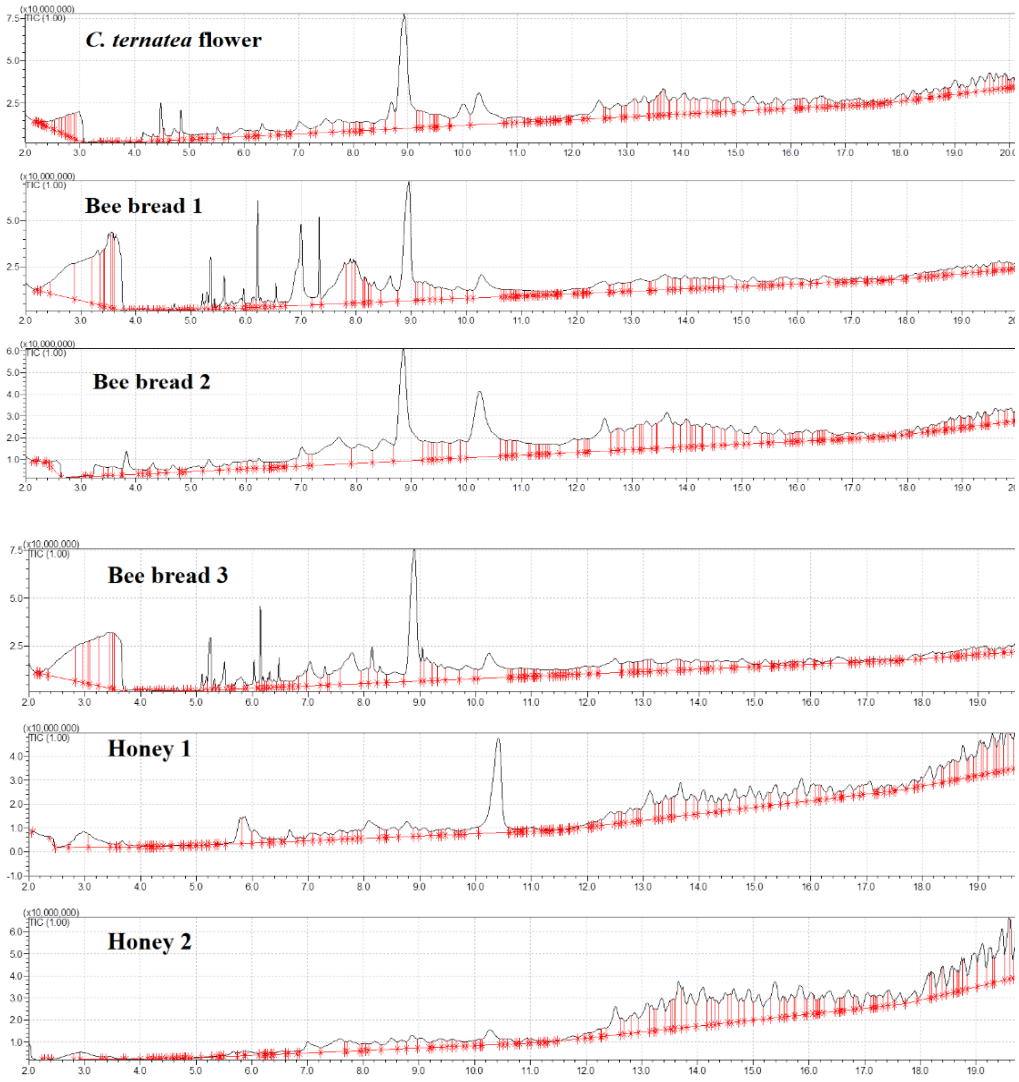


Figure 5. GC-MS chromatograms of *C. ternatea* flower, bee bread and honey samples

4. Conclusion

In conclusion, the results indicated the presence of anti-amylase and anti-acetylcholinesterase activities in the honey and bee bread of stingless bees, flower extracts of *C. ternatea* L., and the flower and honey drink. Enzyme inhibition showed an increase after mixing honey with the flower aqueous extracts. The flower and honey drink showed the highest anti-acetylcholinesterase activity, while ethanol extracts from bee bread showed the highest anti-amylase activity. The FTIR results showed the presence of functional groups in all samples that were similar to the functional groups of flavonoid compounds namely hydroxyl groups, unsaturated bonds and conjugated bonds. A significant positive correlation was found between anti-acetylcholinesterase activity and FTIR data, while a negative correlation was significantly found between anti-amylase activity and FTIR data. This study indicated that the honey and bee bread of stingless bees and the flower extracts of *C. ternatea* L. have potential as functional ingredients for developing health-promoting beverages or nutraceutical products and safety assessments should also be considered.

5. Acknowledgements

This research received a grant from Faculty of Physical Therapy, Srinakharinwirot University (grant number: 070/2566), and facillities from Faculty of Environmental Culture and Ecotourism, Srinakharinwirot University.

6. Author's Contributions

Sirikul Thummajitsakul designed research, analyzed data, wrote the paper.
Suprapada Rodtanorm performed research and analyzed data.
Nuchnapa Boonpokkhrong performed research, analyzed data.
Patcharee Pongkanphai performed research and analyzed data.
Tipwan Sappasat collecting samples.
Kun Silprasit performed research, contributed new analytical tools.

7. Conflicts of Interest

The authors declare that they have no conflicts of interest.

References

Almeida-Dias, J. M. V., Morais, M. M., Belinato, J. R., Diogo, Y. R., Miranda-Pinto, P., Batista, N. N. M., Quinete, N., Nascimento, F. S., Francoy, T. M., De Jong, D., & Cappelini, L. T. D. (2025). Botanical origin and volatile organic compounds contents of bee bread from four Brazilian regions. *Next Research, 2*(2), Article 100341. https://doi.org/10.1016/j.nexres.2025.100341

Alghamdi, B. A., Alshumrani, E. S., Saeed, M. S. B., Rawas, G. M., Alharthi, N. T., Baeshen, M. N., Helmi, N. M., Alam, M. Z., & Suhail, M. (2020). Analysis of sugar composition and pesticides using HPLC and GC-MS techniques in honey samples collected from Saudi Arabian markets. *Saudi Journal of Biological Sciences, 27*(12), 3720-3726.

Ali, H., Abu Bakar, M. F., Majid, M., Muhammad, N., & Lim, S. Y. (2020). *In vitro* anti-diabetic activity of stingless bee honey from different botanical origins. *Food Research*, *4*(5), 1421-1426.

Aparna, V., Dileep, K. V., Mandal, P. K., Karthe, P., Sadasivan, C., & Haridas, M. (2012). Anti-inflammatory property of n-hexadecanoic acid: structural evidence and kinetic assessment. *Chemical Biology and Drug Design*, *80*(3), 434-439.

Babacan, S., Pivarnik, L. F., & Rand, A. G. (2002). Honey amylase activity and food starch degradation. *Journal of Food Science*, *67*(5), 1625-1630.

Beizunces, L. P., Tchamitchian, S., & Brunet, J. L. (2012). Neural effects of insecticides in the honey bee. *Apidologie*, *43*, 348-370.

Caunii, A., Pribac, G., Grozea, I., Gaitin, D., & Samfira, I. (2012). Design of optimal solvent for extraction of bioactive ingredients from six varieties of *Medicago sativa*. *Chemistry Central Journal*, *6*, Article 123. https://doi.org/ 10.1186/1752-153X-6-123

Colović, M. B., Krstić, D. Z., Lazarević-Pašti, T. D., Bondžić, A. M., & Vasić, V. M. (2013). Acetylcholinesterase inhibitors: pharmacology and toxicology. *Current Neuropharmacology*, *11*(3), 315-335.

Chai, W. M., Liu, X., Hu, Y. H., Feng, H. L., Jia, Y. L., Guo, Y. J., Zhou, H. T., & Chen, Q. X. (2013). Antityrosinase and antimicrobial activities of furfuryl alcohol, furfural and furoic acid. *International Journal of Biological Macromolecules*, *57*, 151-155.

Chen, C. A., Sieburth, S. M., Glekas, A., Hewitt, G. W., Trainor, G. L., Erickson-Viitanen, S., Garber, S. S., Cordova, B., Jeffry, S., & Klabe, R. M. (2001). Drug design with a new transition state analog of the hydrated carbonyl: silicon-based inhibitors of the HIV protease. *Chemical Biology*, *8*(12), 1161-1166.

Chukwu, C. J., Omaka, O. N., & Aja, P. M. (2017). Characterization of 2,5-dimethyl-2,4-dihydroxy-3(2H) furanone, a flavourant principle from *Sysepalum dulcificum*. *Natural Products Chemistry and Research*, *5*(8), Article 299. https://doi.org/10.4172/2329-6836.1000299

Damodaran, T., Cheah, P. S., Murugaiyah, V. & Hassan, Z. (2020). The nootropic and anticholinesterase activities of *Clitoria ternatea* Linn. root extract: potential treatment for cognitive decline. *Neurochemistry International*, *139*, Article 104785. https://doi.org/10.1016/j.neuint.2020.104785

Devarajan, S., & 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.

Divya, A., Anbumalarmathi, J., & Sharmili, S. A. (2018). Phytochemical analysis, antimicrobial and antioxidant activity of *Clitoria ternatea* blue and white flowered leaves. *Advances in Research*, *14*(5), 1-13.

dos Santos, T. C., Gomes, T. M., Pinto, B. A. S., Camara, A. L., & Paes, A. M. D. A. (2018). Naturally occurring acetylcholinesterase inhibitors and their potential use for Alzheimer' s disease therapy. *Frontiers in Pharmacology*, *9*, Article 1192. https://doi.org/10.3389/fphar.2018.01192

Geerts, H., Guillaumat, P.-O., Grantham, C., Bode, W., Anciaux, K., & Sachak, S. (2005). Brain levels and acetylcholinesterase inhibition with galantamine and donepezil in rats, mice, and rabbits. *Brain Research*, *1033*(2), 186-193. https://doi.org/10.1016/j.brainres.2004.11.042

Grossberg, G. T. (2003). Cholinesterase inhibitors for the treatment of Alzheimer's disease: getting on and staying on. *Current Therapeutic Research, Clinical and Experimental, 64*(4), 216-235. https://doi.org/10.1016/S0011-393X(03)00059-6

Gupta, G. K., Chahal, J., & Bhatia, M. (2010). *Clitoria ternatea* (L.): Old and new aspects. *Journal of Pharmacy Research*, *3*, 2610-2614.

Escher, G. B., Marques, M. B., do Carmo, M. A. V., Azevedo, L., Furtado, M. M., Sant'Ana, A. S., da Silva, M. C., Genovese, M. I., Wen, M., Zhang, L., Oh, W. Y., Shahidi, F., Rosso, N. D., & Granato, D. (2020). *Clitoria ternatea* L. petal bioactive compounds display antioxidant, antihemolytic and antihypertensive effects, inhibit α-amylase and α-glucosidase activities and reduce human LDL cholesterol and DNA induced oxidation. *Food Research International*, *128*, Article 108763. https://doi.org/10.1016/j.foodres.2019.108763

Fiest, K. M., Roberts, J. I., Maxwell, C. J., Hogan, D. B., Smith, E. E., Frolkis, A., Cohen, A., Kirk, A., Pearson, D., Pringsheim, T., Venegas-Torres, A., & Jetté, N. (2016). The prevalence and incidence of dementia due to Alzheimer's disease: a systematic review and meta-analysis. *Canadian Journal of Neurological Sciences*, *43*, 51-82.

Fratianni, F., Ombra, M. N., Giulio, B. D., d'Acierno, A., & Nazzaro, F. (2025). Lamiaceae honey: polyphenol profile, vitamin C content, antioxidant and in vitro anti-inflammatory, cholinesterase and tyrosinase inhibitory activity. *Food Chemistry Advances*, *7*, Article 100996. https://doi.org/10.1016/j.focha.2025.100996

Hammer, Ø., Harper, D. A. T., & Ryan, P. D. (2001). Past: Paleontological statistics software package for education and data analysis. *Paleontologia Electronica*, *4*(1), 1-9.

Han, W., & Li, C. (2010). Linking type 2 diabetes and Alzheimer's disease. *PNAS*, *107*(15), 6557-6558. https://doi.org/10.1073/pnas.1002555107

Hashemirad, F.-S., Behfar, M., & Kavoosi, G. (2024). Proximate composition, physico-chemical, techno-functional, amino acid profile, fatty acid profile, nutritional quality, antioxidant, anti-amylase and anti-lipase properties of bee bread, royal jelly, and bee propolis. *LWT- Food Science and Technology*, *200*, Article 116190. https://doi.org/10.1016/j.lwt.2024.116190

Hungerford, G., Lemos, M. A., & Chu, B.-S. (2019). Binding of *Clitoria ternatea* L. flower extract with α-amylase simultaneously monitored at two wavelengths using a photon streaming time-resolved fluorescence approach. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *211*, 108-113. https://doi.org/10.1016/j.saa.2018.11.062

Keskin, M., & Özkök, A. (2020). α-Amylase inhibition properties of bee pollen and bee bread (Perga). *Hacettepe Journal of Biology and Chemistry*, *48*, 389-393.

Kieliszek, M., Piwowarek, K., Kot, A. M., Błazejak, S., Chlebowska-Smigiel, A., & Wolska, I. (2018). Pollen and bee bread as new health oriented products: A review. *Trends in Food Science & Technology*, *71*, 170-180.

Kostić, A. Ž., Barać, M. B., Stanojević, S. P., Milojković-Opsenica, D., Tešić, Ž. L., Šikoparija, B., Radišić, P., Prentović, M., & Pešić, M. B. (2015). Physicochemical composition and techno-functional properties of bee pollen collected in Serbia. *LWT - Food Science and Technology*, *62*(1), 301-309. https://doi.org/10.1016/j.lwt.2015.01.031

Kusumah, D., Wakui, M., Murakami, M., Xie, X., Yukihito, K., & Maeda, I. (2020). Linoleic acid, α-linolenic acid, and monolinolenins as antibacterial substances in the heat-processed soybean fermented with *Rhizopus oligosporus*. *Bioscience, Biotechnology, and Biochemistry*, *84*(6), 1285-1290.

Kumar, P. P., Kumaravel, S., & Lalitha, C. (2010). Screening of antioxidant activity, total phenolics and GC-MS study of *Vitex nigundo*. *African Journal of Biochemistry Research*, *4*, 191-195.

Kumar, A., Singh, V. K., & Kayastha, A. M. (2024). Studies on α-amylase inhibition by acarbose and quercetin using fluorescence, circular dichroism, docking, and dynamics simulations. *Spectrochimica Acta. Part A, Molecular and Biomolecular Spectroscopy*, *314*, Article 124160. https://doi.org/10.1016/j.saa.2024.124160

Mahdavi, A., Torabi, E., Ghasemi, V., Mahdavi, V., & Hasan, F. H. M. (2025). Pesticide residues in nectar, pollen, and beebread matrices from rapeseed (*Brassica napus* L.): Which matrix is associated with the highest risks to the honey bee (*Apis mellifera* L.). *Environmental Research*, *270*, Article 121014. https://doi.org/10.1016/j.envres.2025.121014

Mdeni, N. L., Adeniji, A. O., Okoh, A. I., & Okoh, O. O. (2022). Analytical evaluation of carbamate and organophosphate pesticides in human and environmental matrices: A review. *Molecules*, *27*, Article 618. https://doi.org/10.3390/molecules27030618

Montaser, M., Sayed, A. M., Bishr, M. M., Zidan, E. W., Zaki, M. A., Hassan, H. M., Mohammed, R., & Hifnawy, M. S. (2023). GC-MS analysis of honeybee products derived from medicinal plants. *Beni-Suef University Journal of Basic and Applied Sciences*, *12*, Article 63. https://doi.org/10.1186/s43088-023-00396-3

Mutahi, M. W., Nittoli, T., Guo, L., & Sieburth, S. M. (2002). Silicon-based metalloprotease inhibitors: synthesis and evaluation of silanol and silanediol peptide analogues as inhibitors of angiotensin-converting enzyme. *Journal of the American Chemical Society*, *124*(25), 7363-7375. https://doi.org/10.1021/ja026158w

Nagai, T., Inoue, R., Suzuki, N., & Nagashima, T. (2009). Alpha-amylase from persimmon honey: Purification and characterization. *International Journal of Food Properties*, *12*(3), 512-521.

Noh, C. H. C., Azmin, N. F. M., & Amid, A. (2017). Principal component analysis application on flavonoids characterization. *Advances in Science, Technology and Engineering Systems Journal*, *2*(3), 435-440. https://doi.org/10.25046/aj020356

Pasias, I. N., Kiriakou, I. K., & Proestos, C. (2017). HMF and diastase activity in honeys: A fully validated approach and a chemometric analysis for identification of honey freshness and adulteration. *Food Chemistry*, *229*, 425-431.

Peláez-Acero, A., Garrido-Islas, D. B., Campos-Montiel, R. G., González-Montiel, L., Medina-Pérez, G., Luna-Rodríguez, L., González-Lemus, U., & 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), Article 5825. https://doi.org/10.3390/molecules27185825

Pfaff, B., Darrington, J., Stover, J., Satman, M. H., Beckmann, F., Williams, J., Kiefte, M., Kobly, P., & van Son, R. (2013). *GNU PSPP*. https://www.gnu.org/software/pspp/

Philip, Y., & Mohd Fadzelly, A. B. (2015). Antioxidative and acetylcholinesterase inhibitor potential of selected honey of Sabah, Malaysian Borneo. *International Food Research Journal*, *22*(5), 1953-1960.

Pimentel, T. C., Rosset, M., de Sousa, J. M. B., de Oliveira, L. I. G., Mafaldo, I. M., Pintado, M. M. E., de Souza, E. L., & Magnani, M. (2022). Stingless bee honey: An overview of health benefits and main market challenges. *Journal of Food Biochemistry*, *46*(3), Article e13883. https://doi.org/10.1111/jfbc.13883

Piovesan, B., Padilha, A. C., Morais, M. C., Botton, M., Grützmacher, A. D., & Zotti, M. J. (2020). Effects of insecticides used in strawberries on stingless bees *Melipona quadrifasciata* and *Tetragonisca fiebrigi* (Hymenoptera**:** Apidae). *Environmental Science and Pollution Research*, *27*, 42472-42480.

Pokajewicz, K., Lamaka, D., Hudz, N., Adamchuk, L., & Wieczorek, P. P. (2024). Volatile profile of bee bread. *Scientific Reports*, *14*, Article 6870. https://doi.org/10.1038/s41598-024-57159-y

Ringle, C. M., Wende, S., & Becker, J. M. (2015). *SmartPLS 3*. Boenningstedt. https://www.smartpls.com

Ranneh, Y., Ali, F., Zarei, M., Akim, A. M., Hamid, H. A., & Khazaai, H. (2018). Malaysian stingless bee and Tualang honeys: A comparative characterization of total antioxidant capacity and phenolic profile using liquid chromatography-mass spectrometry. *LWT-Food Science and Technology*, *89*, 1-9. https://doi.org/10.1016/j.lwt.2017.10.020

Rasmussen, C. (2008). Catalog of the Indo-Malayan/Australasian stingless bees (Hymenoptera: Apidae: Meliponini). *Zootaxa*, *1935*, 1-80.

Schaad, E., Fracheboud, M., Droz, B., & Kast, C. (2023). Quantitation of pesticides in bee bread collected from honey bee colonies in an agricultural environment in Switzerland. *Environmental Science and Pollution Research International*, *30*(19), 56353-56367. https://doi.org/10.1007/s11356-023-26268-y

Sobral, F., Calhelha, R., Barros, L., Dueñas, M., Tomás, A., Santos-Buelga, C., Vilas-Boas, M., & Ferreira, I. C. F. R. (2017). Flavonoid composition and antitumor activity of bee bread collected in Northeast Portugal. *Molecules*, *22*, Article 248. https://doi.org/10.3390/molecules22020248

Szwajgier, D., Baranowska-Wójcik, E., Winiarska-Mieczan, A., & Gajowniczek-Ałasa, D. (2022). Honeys as possible sources of cholinesterase inhibitors. *Nutrients*, *14*(14), Article 2969. https://doi.org/10.3390/nu14142969

Taqui, R., Debnath, M., Ahmed, S., & Ghosh, A. (2022). Advances on plant extracts and phytocompounds with acetylcholinesterase inhibition activity for possible treatment of Alzheimer's disease. *Phytomedicine*, *2*(1), Article 100184. https://doi.org/10.1016/j.phyplu.2021.100184

Topalăa, C. M., Tătarua, L. D., & Ducu, C. (2017). ATR-FTIR spectra fingerprinting of medicinal herbs extracts prepared using microwave extraction. *Arabian Journal of Medicinal and Aromatic Plants*, *3*(1), 1-9.

Thoophom, G. (2025, 29 January). *GT test kit protocol*. http://www.gttestkit.com/gttestkit_eng/intro_procedure.htm

Thummajitsakul, S., Thongkerd, N., Pholmeesap, B., Phankham, P., & Silprasit, K. (2020a). Assessment of organophosphate and carbamate insecticides and heavy metal contamination in canal-grown water morning glory (*Ipomoea aquatica* forssk) in Nakhon Nayok province, Thailand. *Applied Environmental Research*, *42*(1), 26-42.

Thummajitsakul, S., Samaikam, S., Tacha, S., & Silprasit, K. (2020b). Study on FTIR Spectroscopy, total phenolic content, antioxidant activity and anti-amylase activity of extracts and different tea forms of *Garcinia schomburgkiana* leaves. *LWT - Food Science and Technology*, *134*, Article 110005. https://doi.org/10.1016/j.lwt.2020.110005

Wongsa, P., Phatikulrungsun, P., & Prathumthong, S. (2022). FT-IR characteristics, phenolic profiles and inhibitory potential against digestive enzymes of 25 herbal infusions. *Scientific Reports*, *12*(1), Article 6631. https://doi.org/10.1038/s41598-022-10669-z

Yimdee, J., Uabundit, N., Chaisiwamonkol, K., Iamsaard, S., Hipkaeo, W., & Wattanathorn, J. (2014). Evaluation of cognitive enhancing effects of *Clitoria ternatea* flowers water extract in normal male mice. *KKU Research Journal*, *4*(3), 59-66.

Yu, X., Zhao, M., Liu, F., Zeng, S., & Hu, J. (2013). Identification of 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one as a strong antioxidant in glucose-histidine maillard reaction products. *Food Research International*, *51*, 397-403.

Zafeiraki, E., Kasiotis, K. M., Nisianakis, P., Manea-Karga, E., & Macher, K. (2022). Occurrence and human health risk assessment of mineral elements and pesticides residues in bee pollen. *Food and Chemical Toxicology*, *161*, Article 112826. https://doi.org/10.1016/j.fct.2022.112826

Zakaria, N. N. A, Jaafar, N. N. M., Mad Desa N. N., & Mohamad, A. Z. (2020). Antioxidant, antibacterial and anti-diabetic activities of stingless bee honey from selected areas in Peninsular Malaysia. *IOP Conference Series: Earth and Environmental Science*, *596*, Article 012093. https://doi.org/10.1088/1755-1315/596/1/012093

Zhang, H., Dolan, H. L., Ding, Q., Wang, S., & Tikekar, R. V. (2019). Antimicrobial action of octanoic acid against *Escherichia coli* O157:H7 during washing of baby spinach and grape tomatoes. *Food Research International*, *125*, Article 108523. https://doi.org/10.1016/j.foodres.2019.108523

Zhao, L., Chen, J., Su, J., Li, L., Hu, S., Li, B., Zhang, X., Xu, Z., & Chen, T. (2013). In vitro antioxidant and antiproliferative activities of 5-hydroxymethylfurfural. *Journal of Agricultural and Food Chemistry*, *61*(44), 10604-10611.