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RESEARCH

Evaluation of stingless bee (*Tetragonula pagdeni*) honey properties and melissopalynological analysis from different geographical origins in Thailand

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Background: Honey from different geographical origins can have distinct characteristics due to variations in the floral sources available to stingless bees in different regions. The most abundant stingless bee for meliponiculture in Thailand is *Tetragonula pagdeni*. However, only a few studies about the properties of honey from a different origin were carried out. The objective of this study was focused on a comparative study to evaluate the melissopalynological, physicochemical, antioxidant activities, and total phenolic contents (TPCs) of stingless bee honey produced by *T. pagdeni* from different parts of Thailand.
Results: Fifty honey samples were collected from five locations, and the physicochemical properties of *T. pagdeni* honey samples are acidic (pH 3.02–4.15) and have a high water content (18.42–25.06 %w/w), which is related to the regions of meliponary. Melisopalynological analysis reveals the predominant pollen from *Melaleuca quinquenervia*, *Cocus nuciferca*, *Nephelium lappaceum*, *Salacca wallichiana*, and multiflora honey. All honey samples were analyzed for their TPC and 2,2-diphenyl1-picrylhydrazyl radical scavenging activity. The results show that all samples had high TPC and antioxidant activities with a strong correlation ($p < 0.05$).
Conclusions: The data from this study indicates the importance of geographical origin, which links physicochemical properties, phenolic compounds, and functional characteristics to their floral. Besides, the floral sources and harvesting location affected the properties of stingless bee honey. Our results identify *Melaleuca* honey as a promising source of phenolic content and antioxidant activity that can be used as a functional food, as well as multiflora and *Cocus* honey. However, further studies are required to characterize the phenolic compound and its biological potential, which could be a stingless bee honey biomarker and quality control, simultaneously with the physicochemical analysis.

Keywords: antioxidant activity, honey, melisopalynological analysis, stingless bee, *Tetragonula pagdeni*

Introduction

A stingless bee is a highly eusocial insect as well as a honey bee. These bees are inhabitants of tropical areas around the world. Accordingly, meliponiculture is a well-known tradition in several countries, such as Malaysia, Thailand, Mexico, Venezuela, Brazil, and Australia. Moreover, the managed stingless bees are manageable and become popular. Because stingless bees do not sting, it is easier to collect and extract honey than honey bees, requiring more tools and experience (Abd et al. 2017; Nordin et al. 2018). In Thailand, the stingless bee is distributed in all areas. However, only a few species can be cultured in the artificial wooden hive for meliponiculture. The most managed stingless bee for meliponiculture is *Tetragonula pagdeni*, which is widely distributed nationwide. However, the meliponiculture industry in Thailand is still in the infant phase but is expanding. The Department of Agricultural Extension (DOAE) estimated that the number of meliponiculturists in Thailand jumped from 700 in 2014 to 3,890 in 2022, with over 27,900 colonies (Chuttong et al. 2014).

Studies also report that stingless bee honey contains high phenolic compounds and flavonoids, which reflect the significant antioxidant and antimicrobial activities due to phenolic compounds. However, few studies reported that stingless bee honey's antioxidant and antimicrobial activity is slightly stronger. The comparative studies of therapeutic properties between honey from stingless bee honey and honey bees found that stingless bee honey has exceptional potential to be developed for common medicinal uses due to the varieties of bioactive components as a therapeutic agent over honey from honey bees such as anticancer, antidiabetic and wound healing (Abd et al. 2017; Zulkhairi et al. 2018).

Honey from different botanical and geographical origins can be determined based on the compositional data of honey physicochemical profiles, phenolic acids, flavonoids, carbohydrates, and other constituents. Moreover, the honey's colors, moisture content, and viscosities are naturally diverse due to the multi-floral origin of nectars and season, which change during the year (do Nascimento et al. 2015; Shamsudin et al. 2019). Several countries, including Guatemala, Mexico, Venezuela, and Malaysia, are attempting to establish standards for stingless bee honey. Malaysia is the first country to establish the stingless bee honey standard (Nordin et al. 2018; Vit et al. 2004). Regrettably, the definition and criteria for honey standards by the International Codex Alimentarius Commission (CODEX) need to cover the honey from stingless bees because the honey is stored in honey potsinstead of honeycomb. Again, the standard values for moisture and pH also do not acclimatize the stingless bee honey, which is naturally high in moisture content with lower pH (Zawawi et al. 2022). However, Thailand still needs more information and research to set the national standard for stingless bee honey regarding the physicochemical characteristics, melissopalynology, phenolic content, antioxidant, and antimicrobial properties of honey from stingless bees, particularly *T. pagdeni*, the most common meliponiculture in Thailand.

The objective of this study was focused on a comparative study to evaluate the melissopalynological analysis, physicochemical, antioxidant activities, and total phenolic contents (TPCs) of stingless bee honey produced by *T. pagdeni* from different locations in Thailand.

Materials and Methods

Study area

The study was carried out with the main species of managed stingless bee (*T. pagdeni*) from three main areas of meliponiculture, including the Northern part (Chiang Mai province), the Eastern part (Chanthaburi and Rayong provinces), and the Southern part (Phatthalung and Songkhla provinces) (Fig. 1). Only queenright colonies were selected for study, and the honey samples were collected from 10 colonies/meliponary.

Honey samples

Honey samples were harvested between December 2022 and the end of February 2023. The stingless bee honey samples (25 g/hive) were obtained directly from the hive using a sterilized knife to gently cut the honey pot and put it in the sterilized sampling bag. The honey was extracted from the honey pot using a sterilized spatula to squeeze the honey pot gently. The honey was filtered through the cheesecloth. All filtered honey samples were kept at 4°C.

Physicochemical analysis

Each honey sample was diluted in deionized water at different concentrations if required. All assays were measured in parallel three times.

Color

According to Zarei et al. (2019), the color of honey samples was determined. Honey samples were diluted to 50% with deionized water and filtrated through the Whatman no.1 filter. The A_{635} was measured using a NanoDrop™ UV-Vis Spectrophotometer (Thermo Fisher Scientific Inc., Cleveland, OH, USA). The following equation determined the stingless bee honey color according to the Pfund scale.

$$\text{Pfund} = -38.70 + 371.39 \times \text{Abs}$$

where Pfund represents the honey color value in the Pfund scale (mm), and Abs is the absorbance at 635 nm.

Total soluble solid content

The total soluble solid content in stingless bee honey was determined by refractometer using the digital refractometer model PR-201α (Atago Co. Ltd., Tokyo, Japan)

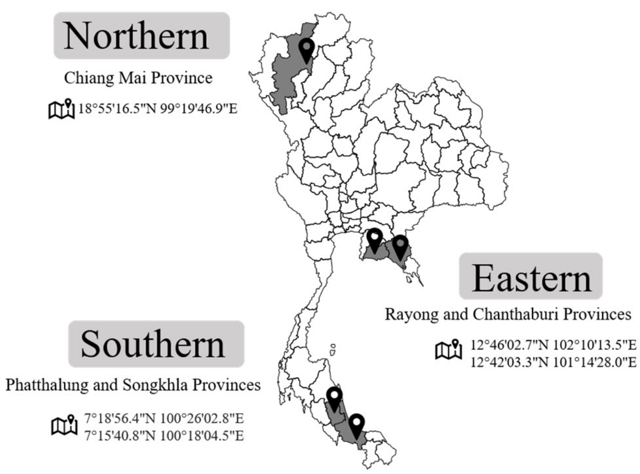

Fig. 1 Map showing the locations of meliponinaries in different geographical origins.

(Khongkwanmueang et al. 2020).

Moisture

The Halogen Moisture Analyzer HC103 (Mettler Toledo®) was used to determine the moisture content (%w/w).

pH

The pH values of the honey samples were measured with a pH pen meter model ST20 (Ohaus, Parsippany, NJ, USA) according to the International Honey Commission (IHC).

Palynological analysis

Ten grams of honey were weighed and fully dissolved in 20 mL of distilled water and then centrifuged for 10 minutes at 4,500 rpm, and the supernatant was discarded. Another 20 mL of distilled water was added before centrifugation for 5 minutes. The supernatant was discarded, and the pellet was subjected to acetolysis (Thakodee et al. 2018). The representation percentage for each pollen type was calculated by counting at least 300 pollen grains per sample. The pollen grains were classified as pollen type, genus, or a single species when possible. The results are represented as the frequency class, using the criteria suggested by Louveaux et al. (1978).

Total phenolic contains

The total phenolic compounds in honey samples were determined by the Foiln–Ciocalteu colorimetric method, according to Ávila et al. (2019). The TPC was performed in a 96-well plate by adding 80 μL of diluted honey samples (0.062–1 μg/mL) to 100 μL of Foiln–Ciocalteu followed by 80 μL of 14% sodium carbonate (Na_2CO_3) solution and incubated at room temperature in the dark for 2 hours. A_{760} reading was performed by a microplate reader (Metertech, Taipei, Taiwan). Gallic acid (0.4–16 μg/mL) was used as a standard. The TPCs in honey were expressed in mg of GAE/g of the honey sample.

DPPH assay

For antioxidant activities, the 2,2-diphenyl1-picrylhydrazyl (DPPH) assay was performed according to Jantakee and Tragoolpua (2015) by adding 20 μL of honey samples at different concentrations (0.062–1 μg/mL) was mixed with 180 μL of 0.2 mM DPPH radical solution in methanol. The mixtures were incubated in the dark at room temperature for 30 minutes. A microplate reader observed A_{517}. Ascorbic acid was used as a reference. The corresponding blank readings were also taken, and the percent of radical scavenging activity (RSA) was then calculated as follows:

$$\%RSA = (Abs._{DPPH} - Abs._{Sample}/Abs._{DPPH}) \times 100.$$

Statistical analysis

The data are presented as mean values and standard deviations of triplicate measurements. The differences between the samples were analyzed by a one-way ANOVA with Tukey's multiple comparison test ($p < 0.05$) using SPSS version 28.0.0.0 (190) (IBM Co., Armonk, NY, USA).

Results

Physicochemical analysis

In this study, we collected stingless bee honey from 5 different meliponaries in three regions across Thailand. The physicochemical parameters of the honey from stingless bees (*T. pagdeni*) from different geographical origins are presented in Table 1.

Color, total soluble solid, pH, and moisture

The Pfund unit is a scale of honey color analysis with a scale range from 1 to 140 mm (Ratiu et al. 2019). The Pfund unit can classify honey color into seven categories, from water white (< 8 mm Pfund) to dark amber (> 144 mm Pfund). The honey color from the *T. pagdeni* in this analysis ranges from 78.96–180.05 mm Pfund. The highest Pfund unit was honey from Songkhla (180.05 ± 8.86 mm Pfund), followed by Phatthalung and Chanthaburi (156.95 ± 10.23 and 125.75 ± 13.96 mm Pfund, respectively), Rayong (92.59 ± 5.38 mm Pfund), and the lowest was honey from Chiang Mai (78.96 ± 12.03 mm Pfund). Based on the Pfund scale results, honey from the *T. pagdeni* would grade in the color range from light amber to dark amber. The values of total soluble solids in *T. pagdeni* honey ranged from 67.6–75.6 °Brix. The pH of the honey ranged from 3.02 to 4.15. The highest pH was honey from Chiang Mai (4.15 ± 0.14), followed by Rayong (3.81 ± 0.12), Chanthaburi (3.38 ± 0.87), Songkhla (3.24 ± 0.67), and Phatthalung

Table 1 Physicochemical parameters of stingless bee (*Tetragonula pagdeni*) honey from 5 meliponaries

Location	mm Pfund	Color	Total soluble solid (°Brix)	pH	Moisture (%w/w)
Chiang Mai	78.96 ± 12.03^{a}	Light amber	75.6 ± 3.7^{a}	4.15 ± 0.14^{a}	18.42 ± 3.22^{a}
Rayong	92.59 ± 5.38^{ab}	Amber	68.2 ± 6.2^{b}	3.81 ± 0.12^{b}	20.65 ± 3.37^{b}
Chanthaburi	125.75 ± 13.96^{b}	Amber-dark amber	70.4 ± 4.3^{b}	3.38 ± 0.87^{c}	21.25 ± 2.83^{b}
Phatthalung	156.95 ± 10.23^{b}	Dark amber	70.4 ± 3.5^{b}	3.02 ± 0.15^{c}	22.93 ± 2.10^{bc}
Songkhla	180.05 ± 8.86^{c}	Dark amber	67.6 ± 5.5^{b}	3.24 ± 0.67^{c}	25.06 ± 2.75^{c}

Values are presented as mean ± standard error.
The values with different superscript letters in a column are significantly different ($p < 0.05$).

(3.02 ± 0.15), respectively. Moisture of honey (%w/w) was observed by moisture analyzer. The highest water content in honey was honey from Songkhla (25.06 ± 2.75), Phatthalung (22.93 ± 2.10), Chanthaburi (21.25 ± 2.83), Rayong (20.65 ± 3.37), and Chiang Mai (18.42 ± 3.22), respectively.

Palynological analysis

The melisopalynological analysis is shown in Table 2; the pollen grain ratio varied among samples of different geographical origins. The pollen types were classified as predominant pollen (> 45%), secondary pollen (16%–45%), important minor pollen (3%–15%), and minor pollen (< 3%) of isolated pollen. The most common pollen grains were *Nephelium lappaceum*, *Salacca wallichiana, Cocus nuciferca*, and *Melaleuca quinquenervia*, followed by numerous others (Figs. 2 and 3).

Table 2 Palynological characteristics of stingless bee (*Tetragonula pagdeni*) honey according to geographical origin

Origins	Plant community	Predominant pollen (> 45%)	Secondary pollen (16%–45%)	Important minor pollen (3%–15%)	Minor pollen (< 3%)
Chiang Mai	Hill Evergreen Forest	None	*Chromolaena odorata* *Muntingia calabura*	*Bidens palosa*	*Coccinia geandis* *Pennisetum pedicellatum*
Rayong	Mixed fruit orchard	*Nephelium lappaceum* (45.7 ± 1.3)	*Cocus nuciferca*	*Salacca wallichiana* *Ziziphus mauritiana*	*Amaranthus viridis* *Bidens palosa*
Chanthaburi	Mixed fruit orchard	*Salacca wallichiana* (49.7 ± 2.3)	*Acacia mangium*	*Cocus nuciferca*	*Bidens palosa* *Pennisetum pedicellarum*
Phatthalung	Mixed fruit orchard and palm oil fields	*Cocus nuciferca* (47.1 ± 5.8)	*Elaeis guineensis* Jacq	*Acacia mangium*	*Salacca wallichiana*
Songkhla	Wetlands	*Melaleuca quinquenervia* (62.9 ± 2.3)	*Salacca wallichiana*	*Elaeis guineensis* Jacq	*Cocus nuciferca*

Values are presented as mean ± standard error.

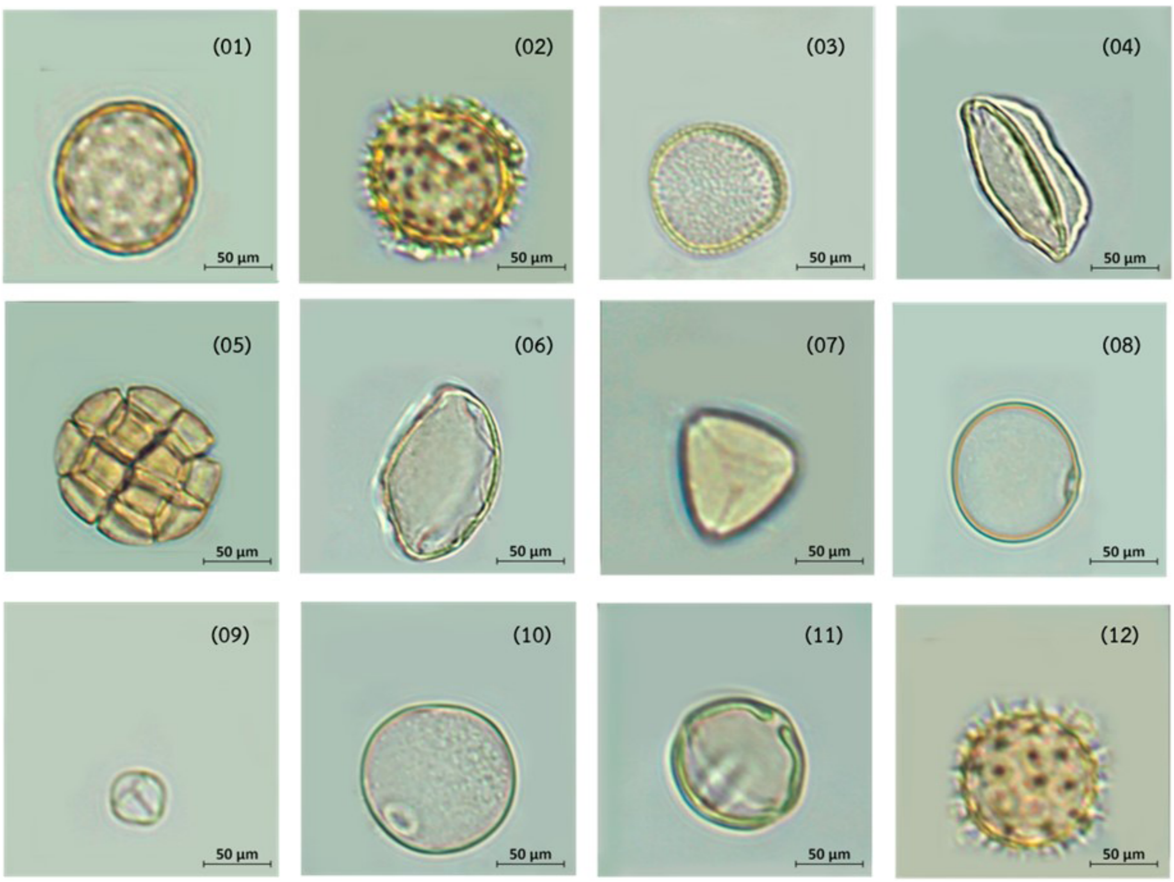

Fig. 2 Pollen grains from *Tetragonula pagdeni* honey under light microscope. (1) *Amaranthus viridis*, (2) *Bidens pilosa*, (3) *Salacca wallichiana*, (4) *Elaeis guineensis* Jacq, (5) *Acacia mangium*, (6) *Cocos nucifera*, (7) *Melaleuca quinquenervia*, (8) *Mimosa pudica*, (9) *Pennisetum pedicellatum*, (10) *Zea mays*, (11) *Citrus aurabtufolia*, (12) *Chromolaena odorata*.

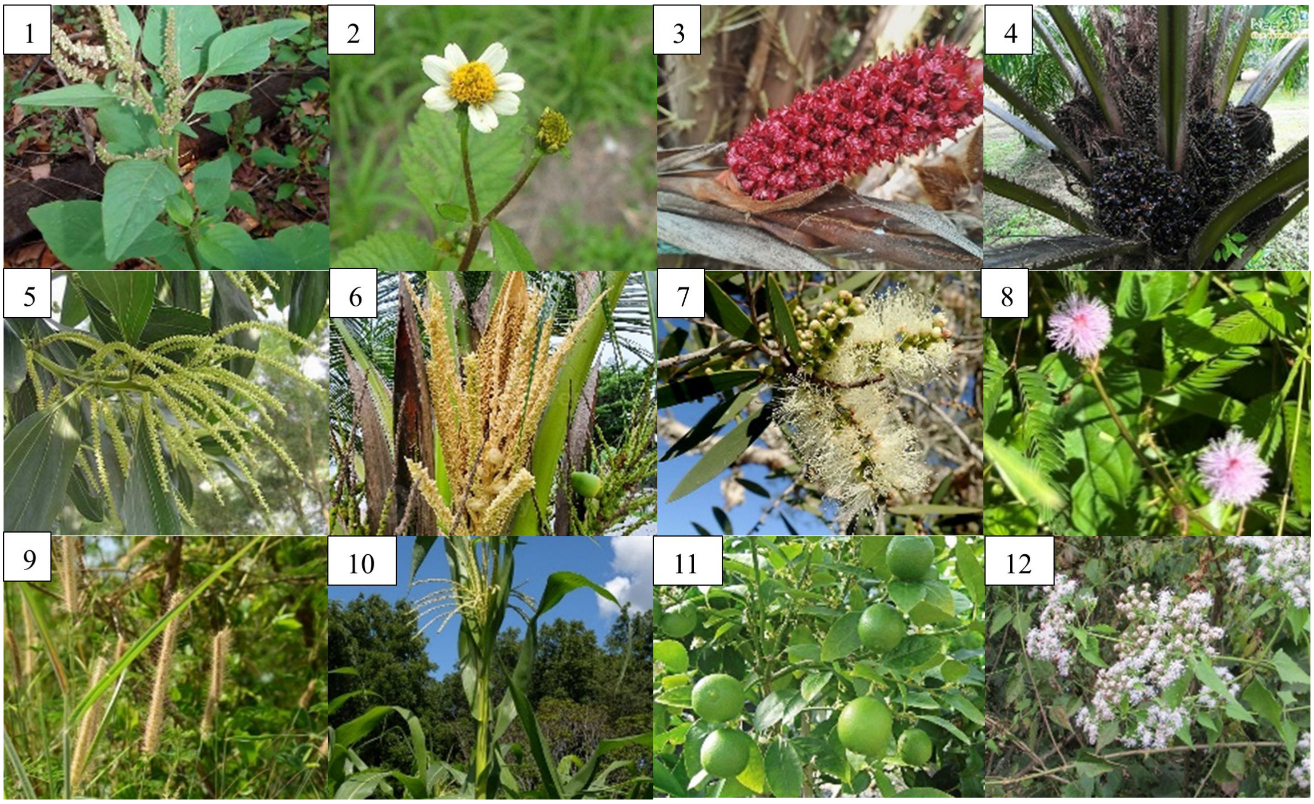

Fig. 3 The botanical origin of *Tetragonula pagdeni* honey in this study (Photo by Tanatip Sittisorn and Amonwit Polgate). The number in Fig. 3 is related to the number in Fig. 2.

Table 3 Total phenolic contains and antioxidant activity of stingless bee (*Tetragonula pagdeni*) honey from different locations

Origins	Predominant pollen (> 45%)	Total phenolic content (mg GAE/100 g)	DPPH activity (% inhibition)
Chiang Mai	None	95.28 ± 3.62^{b}	46.38 ± 1.02^{b}
Rayong	*Nephelium lappaceum*	61.70 ± 1.21^{a}	34.51 ± 0.71^{a}
Chanthaburi	*Salacca wallichiana*	59.09 ± 2.05^{a}	32.48 ± 1.74^{a}
Phatthalung	*Cocus nuciferca*	109.78 ± 2.69^{b}	50.65 ± 0.89^{b}
Songkhla	*Melaleuca quinquenervia*	139.77 ± 16.36^{c}	70.69 ± 0.44^{c}

Values are presented as mean ± standard error.
DPPH: 2,2-diphenyl1-picrylhydrazyl.
The values with different superscript letters in a column are significantly different ($p < 0.05$).

The TPC determined by the Folin–Ciocalteu method varied greatly among the honey types, as shown in Table 3. The stingless bee honey from Songkhla was characterized by a significantly higher content of phenolic compounds (on average, 139.77 ± 16.36 mg GAE/100 g) compared to the other tested varieties ($p < 0.05$). The TPC of stingless bee honey from Phatthalung, Chiang Mai, Rayong, and Chanthaburi were determined to be 109.78 ± 2.69, 95.28 ± 3.62, 61.70 ± 1.21, and 59.09 ± 2.05 mg GAE/100 g, respectively. The antioxidant activities of honey samples are presented in Table 3. Antioxidant activity was determined using the DPPH method. The stingless bee honey from Songkhla had significantly higher antioxidant activity according to the DPPH method ($p < 0.05$), followed by honey samples from Phatthalung, Chiang Mai, Rayong, and Chanthaburi, respectively.

Discussion

Our results show the difference between stingless bee honey produced by monospecies (*T. pagdeni*) from five meliponinaries in three regions across Thailand. The honey samples were collected freshly from the hive and kept in the refrigerator before analysis. Water content is one of the most important features of honey as it affects several properties, including viscosity, color, flavor, and especially microorganisms, which may affect the gases in the honey. The mean moisture content for the stingless bee honey from the South was significantly higher than honey from the East and Southern Thailand (Table 1). The difference in the moisture contents ofstingless bee honey may be due to the different preferred food sources used by the stingless bee and the season. In this study, the honey samples were collected between December 2022 and the end of February

2023, a dry season for Northern Thailand but a Rainy season in Eastern Thailand, particularly Southern Thailand. Thus, the humid climate directly affected the moisture content in stingless bee honey (Ramón-Sierra et al. 2015). Besides, diverse floral behaviors and floral origins might affect the moisture content and decrease the shelf life of the honey due to microorganisms (Shamsudin et al. 2019). However, the moisture content of stingless bee honey from this study is correlated with the moisture content reported by Nordin et al. (2018), which is from as low as 13.26 g/100 g to as high as 45.8 g/100 g, with a mean of 28.6 g/100 g.

The moisture content of stingless bee honey from this study is correlated with the moisture content reported by Nordin et al. (2018), which is from as low as 13.26 g/100 g to as high as 45.8 g/100 g, with a mean of 28.6 g/100 g. In this study, the pH of *T. pagdeni* honey from five different geographical origins ranges from 3.02 to 4.15. (Table 1). The lowest pH value, 3.02, was detected from the honey of *T. pagdeni* from Phatthalung, whereas the highest pH value was detected from honey from Chiang Mai, the Northern part of Thailand. A strong correlation between low pH and high moisture content in stingless bee honey samples was observed in this study. Similarly, a study conducted by Brown et al. (2020) reported that stingless bee honey with high acidity and moisture has great bactericidal activity against all strains of pathogenic bacteria due to high phenolic contents in stingless bee honey samples (Pimentel et al. 2013). Naturally, stingless bee honey is acidic. Solayman et al. (2016) report that the pH of stingless bees around the globe ranges from 3.2 to 4.5. Besides, the pH of a stingless bee can be used to determine its geographical origin (Acquarone et al. 2007).

The soluble solids (TSS) in honey include sugars, organic acids, and minerals. The value of this parameter reveals the relationship between the water and sugar content (Biluca et al. 2016). Generally, TSS value in stingless bee honey is lower than in honey from honey bees due to the higher water and sugar content. In this work, TSS values of *T. pagdeni* honey from five locations range from 67.6 to 75.6 (Table 1). The lowest TSS value, 67.6, was detected in the honey of *T. pagdeni* from Songkhla, whereas the highest TSS value, 75.6, was detected in honey from Chiang Mai, the Northern part of Thailand. A strong correlation between low pH and high moisture content in stingless bee honey samples was observed in this study. However, this parameter is not regulated in any of the honey standards.

Our results also demonstrated that the geographical origins affect the color of stingless bee honey; the stingless bee honey from the North has a light amber color, whereas the honey from the South has a darker color (over dark amber), ranging from 78.96–180.05 mm Pfund (Table 1). The honey color is one of the important parameters that can verify the botanical origin. It indicates the presence of pigments, the content of the nectar, the condition of storage time, and aging. Based on the Pfund unit, the honey colors naturally range from a nearly colorless, water white to dark amber, passing through yellow and amber tones (Crane 1980; White 1957). However, the Pfund scale does not detect slight differences in color for each sample. Nevertheless, color is one of the physical properties the consumer observes. It can be influential or unpersuasive at first impression before making a purchase decision, as most consumers prefer light tones as they believe it has a mild flavor. Generally, the darkening of honey is temperature sensitive and occurs more rapidly when honey is stored at high temperatures or exposed to light. Nevertheless, dark honeys are especially appreciated in some European and Asian countries. Moreover, numerous studies reported that dark honey contains more phenolic acid with higher antioxidant activity than light-colored honey (Beretta et al. 2005; Karabagias et al. 2016, 2020).

The melissopalynological analysis delivers information about the plant origins where the honey was harvested. At the same time, the variation in the pollen spectrum from stingless bee honey samples from different geographical origins shows the sophistication of honey as a natural product with geographical indication. The botanical origin of stingless bee honey is similar in the same region. For example, the pollen spectrum of honey from Songkhla and Phatthalung is quite similar to Rayong and Chanthaburi as they have mixed fruit orchards, which are similar in plant species. Unlike others, the stingless bee honey from Chiang Mai has no predominant pollen as the melipony is located in the middle of the wild forest with numerous plant species. The melissopalynological analysis confirms this, as it lacks predominant pollen. The Folin–Ciocalteu method is routinely used to evaluate the TPC. The lowest TPC was obtained for stingless bee honey samples from Eastern Thailand, Rayong, and Chanthaburi (61.70 ± 1.21 and 59.09 ± 2.05 mg GAE/100 g, respectively), and the highest in samples from Southern Thailand, Phatthalung and Songkhla (109.78 ± 2.69 and 139.77 ± 16.36 mg GAE/100 g, respectively). The phenolic content and antioxidant activity in this study are similar to the study by Pham et al. (2022), which reported that melaleuca honey from Vietnam contains high phenolic content (63.32 mg GAE/100 g). In contrast, melaleuca honey from Songkhla contains 139.77 mg GAE/100 g of phenolic content. Furthermore, correlation analysis indicated that TPC and antioxidant activities are moderately correlated with the type and amount of botanical origins.

Conclusions

This study stands out as the first report to demonstrate the physicochemical properties, phenolic content, and antioxidant activity of stingless bee (*T. pagdeni*) honey from five different locations across three regions of Thailand. The results demonstrated that the different plant origins affect the phenolic content and antioxidant activity. Based on the correlation between melisopalynological analysis, antioxidant activity, and phenolic content, honey from Melaleuca is the most promising source of phenolic compounds. Thus, the melisopalynological analysis can be used to identify the origin of stingless bee honey from Thailand. Besides, the results in all parameters indicated that stingless bee honey from *T. pagdeni* across Thailand is still in Malaysia's range of stingless bee honey standards. However, a more comprehensive study with a significant number of samples must be carried out to obtain more insights into the impact of botanical origin and stingless bee species on the parameters studied. However, other factors were not considered in this study but may influence the composition of each honey studied. The properties of honey may be affected by another factor, such as the climate. Monitoring the stingless bee honey properties all year round would benefit more and reveal the suitability of honey harvesting for the best properties.

Abbreviations

TPC: Total phenolic content
DPPH: 2,2-diphenyl1-picrylhydrazyl
RSA: Radical scavenging activity
TSS: The soluble solids

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Authors' contributions

JM was responsible for conception and design, research organization, laboratory material, and infrastructure. JM, AP, WP, AR, TS, and JB conducted the laboratory research and analyzed the data. SJ and PK collected the samples. JM wrote the first draft of the manuscript, and all authors commented on previous versions. The manuscript was reviewed and edited by JM and WC. All authors read and approved the final manuscript.

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Availability of data materials

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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