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*CORRESPONDENCE
Vadivelu Karuppaiah
karuppaiahv2008@gmail.com

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Biochemical, antioxidants, and mineral constituents of stingless bee honey

Chandrashekhar L. Pote[1], Dhananjay V. Shirsat[1], Payal A. Mahadule[1], Komal A. Gade[1], Trupti R. Pandit[1,2], P. S. Soumia[1], Arunachalam Thangasamy[1], Satish Kumar[1], Vijay Mahajan[1] and Vadivelu Karuppaiah[1]*

[1]ICAR-Directorate of Onion and Garlic Research, Rajgurunagar, Pune, Maharashtra, India,
[2]Department of Entomology, Lovely Professional University, Jalandhar, Punjab, India

Introduction: Floral honey has gained attention for its host-specific phytochemicals, which are associated with various health benefits, such as wound healing, reducing inflammation, and offering antioxidant protection. Stingless bee honey, in particular is renowned for its medicinal benefits.

Methods: This study compares the pH, electrical conductivity (EC), moisture content, total protein, antioxidant activity, sugar content, and mineral composition of four floral honey samples from the stingless bee, *Tetragonula iridipennis* and an Italian bee, *Apis mellifera*.

Results: The pH of *T. iridipennis* honey ranged from 3.36 to 3.46, lower than *A. mellifera* honey (4.48). The EC of *T. iridipennis* honey (1.01–1.13 mS/cm) was higher than *A. mellifera* honey (0.58 mS/cm), indicating a greater mineral content. Additionally, *T. iridipennis* honey showed higher moisture content (16.53–19.79%), protein (825–1184.33 μg/g), antioxidant activity (323.05–353.47 mg/100 g), and mineral concentrations.

Discussion: This study compares the physicochemical and mineral components of *T. iridipennis* and *A. mellifera* honey. Significant correlations were found between pH and key components, with *T. iridipennis* honey showing superior nutritional and medicinal value due to its higher biochemical and mineral composition.

KEYWORDS

stingless bee honey, antioxidant properties, physicochemical properties, mineral content, *Tetragonula iridipennis*

Introduction

Honey is a sweet, natural and viscous substance produced by honey bees from the nectar and pollen of plants (Bogdanov, 2016). It is a natural food consumed in raw form, featuring a complex nutrient composition that varies based on the storage conditions, geographical region, bee species, and floral source (Karabagias et al., 2018). Bee honey is composed of sugars and water, along with other constituents (<1%) of phenolic acids, 5-hydroxymethylfurfural (HMF), volatile compounds, flavonoids, minerals, vitamins, enzymes, and proteins, all of which play a key role in its characterization (Bogdanov, 2016; Da Silva et al., 2013; Toydemir et al., 2015; Ramón-Sierra et al., 2015; Chuttong et al., 2016a). Honey is a rich source of antioxidant compounds, including amino acids, proteins, ascorbic acid, glucose oxidase, carotenoid derivatives and organic acids (Bogdanov et al., 2002; Souza et al., 2006). Its biological properties, including antimicrobial activity, anti-inflammatory, sunburn healing effects, bacteriostatic, wound, radical scavenging activity, antioxidant activity and antibacterial properties, make it ideal for medicinal use (Souza et al., 2006; Singh et al., 2012). Minerals play a key role in the classification and characterization of honey, as they are stable and reflect the plants absorption from the soil and surrounding environment (De Alda-Garcilope et al., 2012).

Stingless bees form a significant group of eusocial bees that produce honey with unique biochemical properties (De Paula et al., 2021). Stingless bees are insect pollinators for many flowering plants and are also used in beekeeping. They pollinate native plants, cannot sting, forage year-round, small in size, have long life cycle and are easy to manage, propagate, and maintain in compact, easy-to-transport hive boxes for crop pollination (Heard, 1999; Slaa et al., 2006). Stingless beekeeping, also called meliponiculture, offers new economic opportunities. However, the large-scale farming of stingless bees remains challenging and needs to be aligned with sustainable development practices (Cortopassi-Laurino et al., 2006).

Stingless bee honey (SBH) is characterized by higher reducing sugars, acidity levels and moisture content, compared with honey from *Apis mellifera* (Souza et al., 2021). It is also known for higher electrical conductivity, lower diastase activity and a sour–sweet (acidic) taste (Kek et al., 2017). It is sweetness, flavor, texture, and aroma are also distinct from *A. mellifera* honey (Sousa et al., 2016a; Abd Jalil et al., 2017; Alvarez-Suarez et al., 2018; Avila et al., 2019a; Badrulhisham et al., 2020). SBH is characterized by lower diastase activity, higher moisture content, lower reducing sugars, higher electrical conductivity, and a sour–sweet (acidic) taste (Kek et al., 2017). The SBH have higher antioxidant and biological activities (up to 45%) such as antimicrobial and antioxidant properties, compared to traditional *Apis mellifera* honey (Suntiparapop et al., 2012; Nweze et al., 2016), similar findings have been documented in brazilian stingless bee honey through *in vitro* studies (Avila et al., 2019b). Furthermore, studies on SBH have demonstrated its anti-inflammatory (Badrulhisham et al., 2020), anti-diabetic (Ali et al., 2020) and antimicrobial properties (Batiston et al., 2020; Biluca et al., 2020). Ranneh et al. (2019) attributed a protective role of stingless bee honey against chronic lipopolysaccharide (LPS) and a reduction in lesions in the colon epithelial cells of dyslipidemic rats caused by dyslipidemia (Bezerra et al., 2018). It is also considered a health-promoting product (Vit et al., 2013). The anti-inflammatory properties of stingless bee honey may help to reduce the severity of pulmonary manifestations in COVID-19 infections (Ch'ng and Tang, 2020) and promote wound healing (Abd Jalil et al., 2017).

Although studies have summarized the Physicochemical properties and chemical profile of stingless bee honey (Nordin et al., 2018; Souza et al., 2021), research on floral honey and its properties related to SBH are limited. The characterization of the biophysical and biochemical properties of floral SBH harvested from onion-based meliponiculture could offer new insights into onion-based SBH farming.

Given these facts, the current study's objective is to assess the Physicochemical, biochemical, antioxidant properties, and mineral composition of stingless bee honey harvested from onion-based floral beekeeping. The information from these studies will provide new insights into onion honey and helps to establish national and international standards for stingless bee honey.

Materials and methods

Study site

The study was carried out at the ICAR-Directorate of Onion and Garlic Research Pune, Maharashtra, India, during the rabi season of 2022. The research site is situated at a latitude of 18.320° N and longitude of 73.510° E, with an elevation of 553.8 meter above mean sea level. The annual rainfall received in this area is 574 mm. The soil at the experimental site is well-suited for growing onion seed crop comprising 20% silt, 32–35% clay, and 40% sand. The pH of the soil is 7.9 and the bulk density is 1.4 mg/cm^3. The hives of *Tetragonula irridipennis* and *Apis mellifera* maintained at the Directorate of Onion and Garlic Research were used for this study. From this, we relocated three newly divided, honey harvested colonies of *T. irridipennis* and *A. mellifera* to the onion seed crop plots, which were covered with insect proof net cage (10 × 10 m; 100 sq. m.). One hive was placed in each 10 × 10 m cage setup when the umbel started flowering, and the bees were allowed to forage until seed setting.

Collection of honey samples

Honey samples were collected from sealed pots of *T. iridipennis* and capped cells from *A. mellifera* hives placed in onion seed production plot at the ICAR-Directorate of Onion and Garlic Research, Pune. The samples were carefully preserved in airtight containers, which were stored in dark place at room temperature for quality analysis. All honey samples were analyzed within 1 month of collection, and none of the samples exceeded 3 months. Details of the honey samples are provided in Table 1.

pH and EC estimation

The pH and electrical conductivity (EC) of the stingless bee honey samples were analyzed using the method described by Manam et al. (2021), in accordance with International Honey Commission (2009) guidelines. For pH measurement, 10 g of honey was dissolved in 75 mL of carbon dioxide-free water in a 250 mL beaker. The solution was thoroughly stirred using a magnetic stirrer, and the pH was recorded using a pH meter (Eutech pH 2,700 Meter). For EC estimation, 20 g honey was dissolved in distilled water in a 100 mL volumetric flask, and the solution was made up to volume with distilled water. A 40 mL aliquot of the solution was transferred to a beaker and placed in the thermostated water bath at 20°C. The EC was recorded using an EC meter (Thermo Scientific™ Orion™ Star A112 Conductivity Benchtop Meters) (AOAC, 2012).

TABLE 1 Honey samples.

Sr. no.	Honey bee species	Location
(1)	*T. irridipennis* hive 1	DOGR field
(2)	*T. irridipennis* hive 2	DOGR field
(3)	*T. irridipennis* hive 3	DOGR field
(4)	*A. mellifera* hive 4	DOGR field
(5)	*A. mellifera* hive 5	DOGR field
(6)	*A. mellifera* hive 6	DOGR field

Moisture

The moisture content of the honey was measured following the methodology described by Ajitha Nath et al. (2019). Five gram of honey were placed in pre-weighted crucibles and dried in a hot air oven at 105°C for 8 h. Periodically, the crucibles were weighed, and the results were noted. To reduce errors, triplicates were kept. The following formula was used to determine the moisture content.

$$Moisture\ Content\ (\%) = \frac{Loss\ in\ Weight\ (g) \times 100}{Weight\ of\ Sample\ (g)}$$

Ferric reducing antioxidant power assay

The Fe^3 + -TPTZ complex (yellow) was reduced to Fe^2 + -TPTZ (blue) by using the FRAP assay. To prepare the FRAP reagent, mix0.3 M acetate buffer having pH 3.6, 0.010 M 2,4,6- tripyridyl-S-triazine (TPTZ) (HimediaMB188) solution in HCl (0.040 M) and 0.020 M $FeCl^3{\cdot}6H_2O$ in a ratio of 50:5:5. To 2,850 µL of FRAP reagent, 150 µL of honey solution (0.1 g/mL) was added and incubated for 4 min. at 37°C. Absorption was measured at 59 nm by using a spectrophotometer, with methanol, used as blank. A calibration curve was prepared using ascorbic acid in the concentration range of 5–40 µg/mL, and the FRAP values were expressed as micromoles of ascorbic acid equivalent per gram of honey (Ahmed et al., 2016).

Total protein content

The total protein content in honey was determined using the Bradford method (1976). Five gram of honey was dissolved in 5 mL of distilled water (50% w/v). Bovine Serum Albumin was used as standard. One milliliter of Bradford reagent was mixed with 200 µL of the diluted honey solution and incubated at room temperature for 10 min. Absorbance was measured at 595 nm using a spectrophotometer. Protein content was expressed in µg per gram of honey.

Reducing sugar, total sugar, and sucrose content

The 3,5-dinitrosalicylic acid (DNSA) method, which reduces sugars to convert DNSA to 3-amino-5-nitrosalicylic acid which produces a reddish-orange coloration, was used to evaluate the total reducing sugar concentration. The measurement was taken using spectrophotometry at 540 nm. Distilled water was used to 100-fold dilute the honey solution (0.1 g/mL).

After mixing a 1 mL aliquot of this diluted solution with an equal amount of DNSA solution, the mixture was incubated for 10 min in a boiling water bath. A spectrophotometer was used to measure the absorbance at 540 nm after the mixture had been allowed to cool to room temperature and combined with 7.5 mL of distilled water. For calibration, a glucose standard solution containing 100–600 µg/mL was used.

The total sugar was estimated by inverting sucrose (a non-reducing sugar) into a reducing sugar, as described by Sawhney and Singh (2000). After diluting the honey sample (0.1 g/mL) 33-fold with deionized water, hydrochloric acid was added to 1 mL of the diluted sample to reach a final concentration of 2 N. For 8 min, the mixture was incubated at 68°C to enables the full inversion of sucrose into a reducing sugar. Following the hydrolysis of the acid, the solution was allowed to cool to room temperature before sodium hydroxide was added to neutralize it. After that, distilled water was added to bring the final volume down to 2 mL.

A 500 µL aliquot was taken out to estimate the amount of total sugar, and a spectrophotometer was used to measure the absorbance at 540 nm. Measurements were performed in triplicate. The sucrose concentration (%) in honey samples was determined using the equation given by Amin et al. (1999),

$$Sucrose\ (\%) = (\text{Total sugar} - \text{Total reducing sugar}) \times 0.95$$

Minerals content (Fe, Mn, Zn, Cu) estimation

One gram of honey was heated in a water bath at 65°C until it liquefied, making it easier to handle and ensure more uniform distribution. After cooling, 12 mL of an acid mixture (3:1 ratio of HNO3 and H_2O_2) 9 mL HNO3 + 3 mL of H_2O_2 was added in 100 mL conical flask containing 1 g of honey. The solution was evaporated to about 3–5 mL, avoiding dryness. After cooling, 10 mL of distilled water was added, and the solution was filtered through Whatman No. 1 filter paper into a 25 mL flask. The mineral content was analyzed using an Atomic Absorption Spectrophotometer (AAS) (Divakar and Vijaykumar, 2019).

Statistical analysis data were analyzed using R software (version 4.4.2). One-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test was used to compare the means, with statistical significance set at a 95% confidence level ($p < 0.05$). All experiments were performed in triplicates, and the data are presented as mean ± standard deviation (SD), with error bars representing the mean.

Results

The pH, EC and moisture content of six floral honey samples from *T. iridipennis* (Hives 1, 2, 3) and *A. mellifera* (Hives 5, 6, 7) are shown in Figure 1. In this study, the three floral honey samples from *T. iridipennis* contain pH ranges from 3.36–3.46, compared to the three sample from *A. mellifera* exhibits pH ranges from 4.44–4.53. These results indicate a decrease the pH of *T. iridipennis* honey samples compared to *A. mellifera* honey. The EC values of *T. iridipennis* honey samples range from 1.01–1.13 mS/cm, whereas the EC of *A. mellifera* honey exhibits 0.58–0.98 mS/cm. The *T. iridipennis* honey samples show higher EC values than *A. mellifera* honey. The moisture content of *T. iridipennis* honey samples range from 16.53–19.79% compared to the *A. mellifera* honey exhibits 16.07–16.87%. These results showed that *T. iridipennis* honey samples have higher moisture content than *A. mellifera* honey.

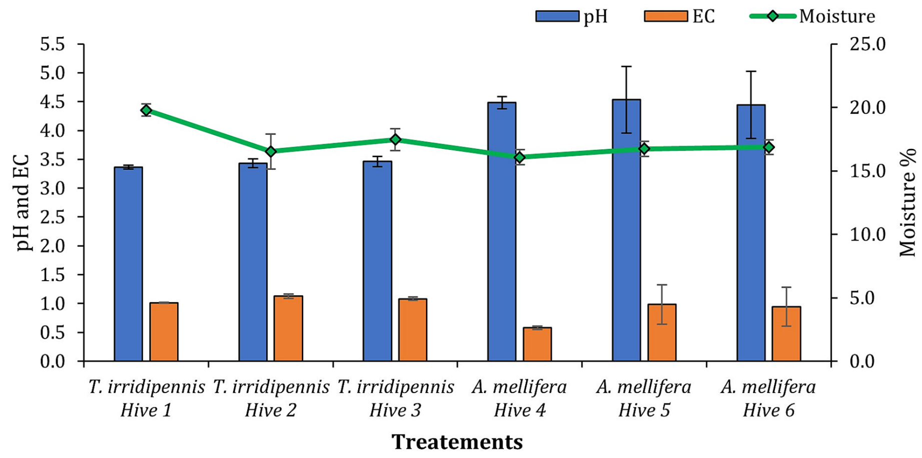

FIGURE 1
Graph of pH, EC, and moisture content in honey samples.

TABLE 2 Physico-chemical parameters [protein, antioxidant (FRAP), sucrose, reducing sugar and total sugar].

Parameters			Protein (µg/g)	Antioxidant (FRAP) (mg/100 g)	Sucrose (%)	Reducing sugar (%)	Total sugar (%)
T. irridipennis	Hive 1	Mean ± SD	1184.33 ± 64.47^{a}	323.05 ± 16.87^{ab}	3.73 ± 0.12^{abc}	78.30 ± 0.88^{a}	81.70 ± 1.45^{a}
	Hive 2	Mean ± SD	825 ± 23.07^{c}	353.47 ± 29.55^{a}	3.87 ± 0.12^{ab}	75.45 ± 1.21^{ab}	79.20 ± 1.93^{a}
	Hive 3	Mean ± SD	1030 ± 40.78^{b}	343.28 ± 10.17^{a}	4.40 ± 0.40^{a}	73.70 ± 0.95^{bc}	78.40 ± 1.02^{a}
A. mellifera	Hive 4	Mean ± SD	709.67 ± 74.33^{cd}	288.85 ± 12.42^{b}	2.17 ± 0.15^{c}	70.35 ± 1.23^{cd}	72.10 ± 2.41^{b}
	Hive 5	Mean ± SD	690.33 ± 1.53^{d}	287.87 ± 1.53^{b}	2.16 ± 1.00^{c}	70.18 ± 1.53^{d}	73.34 ± 1.53^{b}
	Hive 6	Mean ± SD	719.58 ± 1.53^{cd}	291.32 ± 1.53^{b}	2.23 ± 1.00^{bc}	71.74 ± 1.53^{cd}	73.68 ± 1.53^{b}
Mean			859.82	314.64	3.09	73.29	76.40
CV			22.62	9.69	35.03	4.32	5.12
F-value			0.64	1.55	1.94	0.13	0.28
p. value ($p > 0.05$)			0.72	0.37	0.02	0.15	0.54
LSD			79.11	27.37	1.08	2.22	3.02

Results were expressed as mean ± standard deviation of triplicate experiments. Means were compared by using One way ANOVA and Tukey's *post hoc* test. In each column, mean values with different letters (superscripts "a-d") indicate significant differences ($p < 0.05$).

The total protein content and total antioxidant (FRAP) content of the six floral honey samples from *T. iridipennis* and *A. mellifera* are shown in Table 2. The total protein content of *T. iridipennis* honey samples from 825–1184.33 µg/g, compared to 709.67–719.58 µg/g for *A. mellifera* honey. The *T. iridipennis* honey samples exhibited higher total protein content than the *A. mellifera* honey sample. Furthermore, the *T. iridipennis* honey samples showed significantly higher total antioxidant activity, ranging from 323.05–353.47 mg/100 g, compared to 287.87–291.32 mg/100 g in *A. mellifera* honey. Total antioxidant activity was significantly higher in *T. iridipennis* honey samples compared to *Apis mellifera* honey sample.

The sucrose, reducing sugar, and total sugar contents of six floral honey samples of honey bee species *viz., T. iridipennis* hives 1, 2, 3 honey samples and *A. mellifera* hive 4,5,6 honey sample are shown in Table 2. The sucrose content of *T. iridipennis* ranges from 3.73–4.40%, while reducing sugars content was 73.70–78.30% and the total sugars was 78.40–81.70%. In comparison, *A. mellifera* honey has 2.16–2.23% sucrose, 70.18–71.74% reducing sugars and 72.10–73.68% total sugars. *T. iridipennis* honey samples show significantly higher amounts of sucrose, reducing sugar, and total sugar compared to *A. mellifera* honey.

The mineral (Fe, Mn, Zn, Cu) content of the six floral honey samples of *T. iridipennis* and *A. mellifera* honey are shown in Figures 2–5. The iron (Fe) content in *T. iridipennis* ranges from 0.146–0.178 µg/g, compared to 0.135–0.144 µg/g in *A. mellifera* honey. *T. iridipennis* honey samples recorded a higher amount of Fe content than *A. mellifera* honey. The manganese (Mn) content in *T. iridipennis* honey samples ranges from 0.028 to 0.031 µg/g, compared to 0.022–0.026 µg/g in *A. mellifera* honey. Similarly, *T. iridipennis* honey samples showed increased Mn content compared to *A. mellifera* honey. The zinc (Zn) content in *T. iridipennis* honey samples ranges from 0.077 to 0.079 µg/g, compared to 0.031–0.040 µg/g in *A. mellifera* honey. *T. iridipennis* honey samples exhibited higher Zn content than *A. mellifera* honey. The Cu content in *T. iridipennis* honey samples ranges from

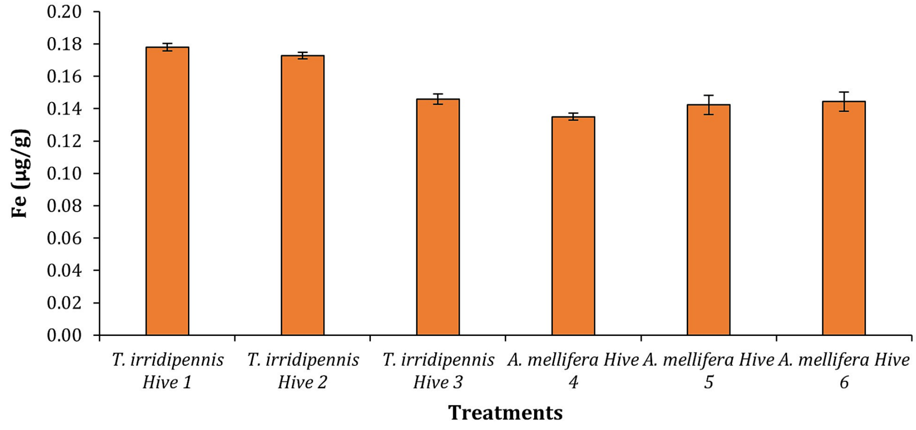

FIGURE 2
Graph of Fe content in honey samples.

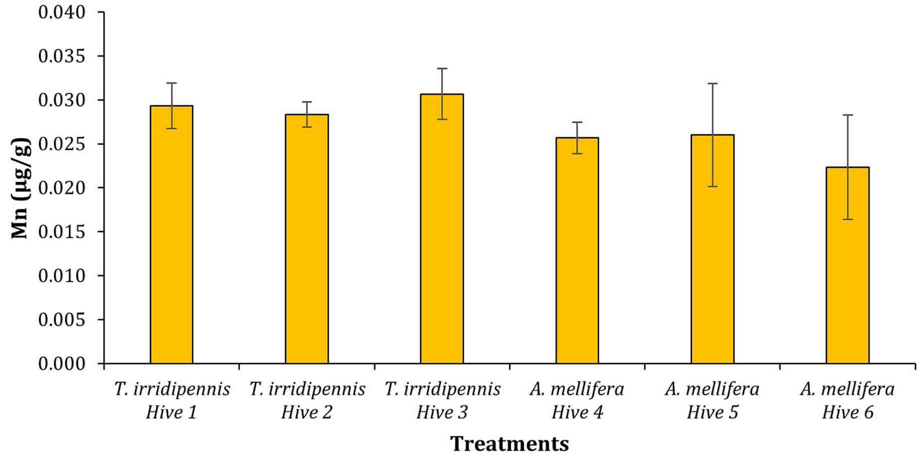

FIGURE 3
Graph of Mn content in honey samples.

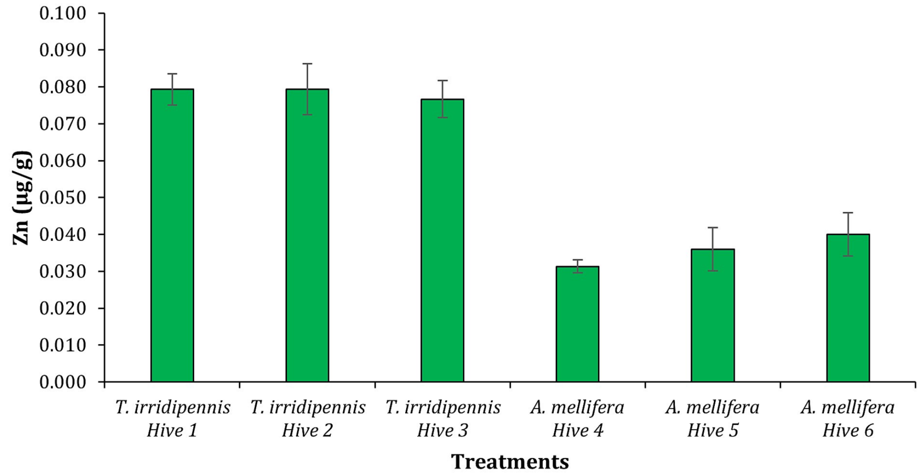

FIGURE 4
Graph of Zn content in honey samples.

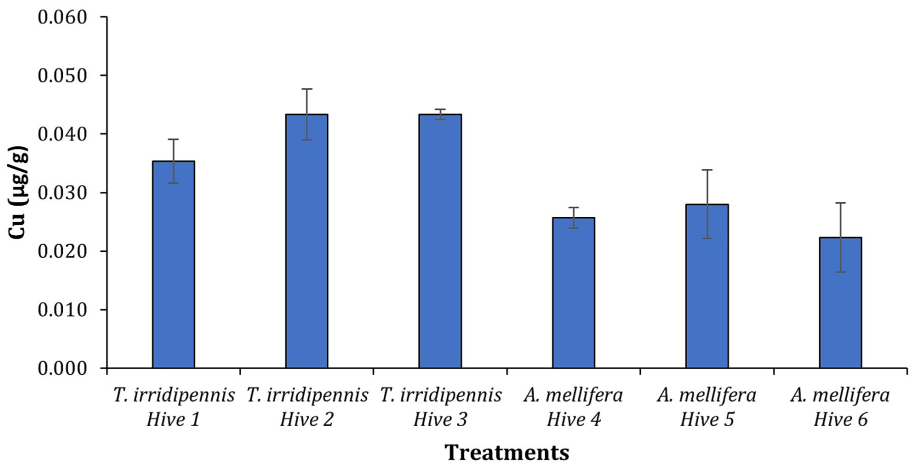

FIGURE 5
Graph of Cu content in honey samples.

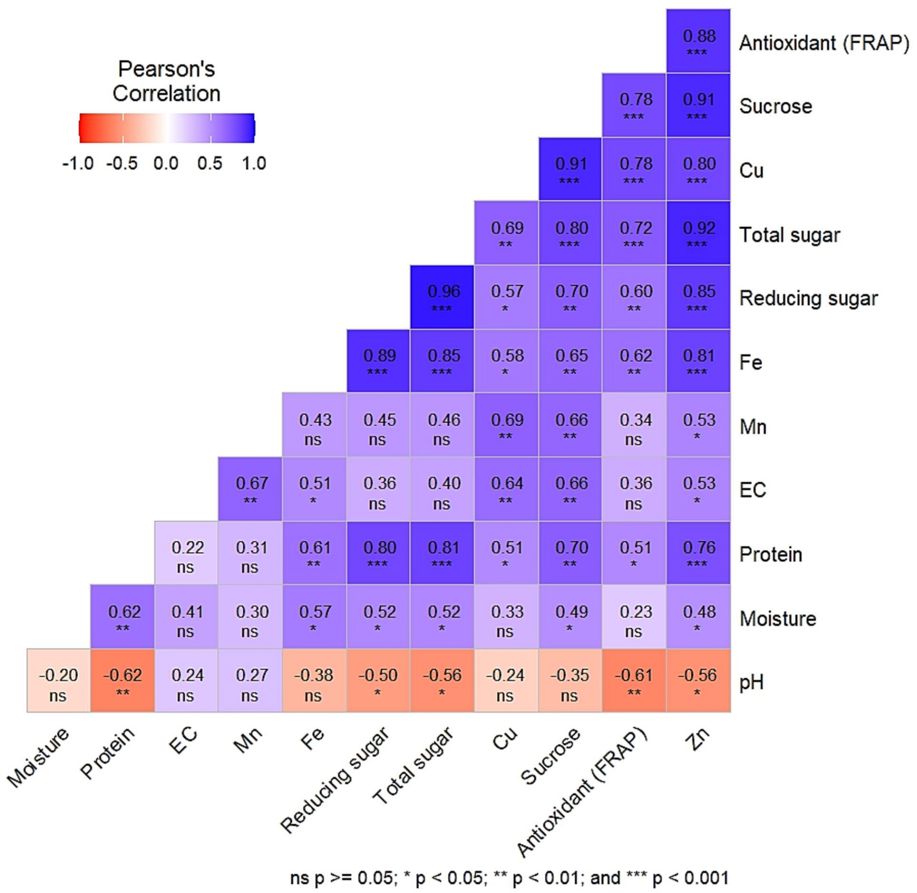

FIGURE 6
Pearson correlation heatmap of physico-chemical and mineral components of *T. iridipennis* and *A. mellifera* honey.

0.035 to 0.043 µg/g, compared to 0.022–0.028 µg/g in *A. mellifera* honey. *T. iridipennis* honey samples showed higher Cu content than *A. mellifera* honey.

The heatmap (Figure 6) presents Pearson's correlation coefficients for various parameters of physicochemical and mineral components of *T. iridipennis* and *A. mellifera* honey using a color gradient. Statistical significance is also represented through *p*-values.

The pH exhibited a significantly negative correlated with Zn (−0.56*), antioxidant (−0.61**), total sugar (−0.56*, ns), reducing sugar (−0.50*, ns), protein (−0.62**). The pH negatively correlated with sucrose (−0.35, ns), Cu (−0.24, ns), Fe (−0.38, ns), and moisture (−0.20, ns). Also, pH positively correlated with EC (0.24, ns) and Mn (0.27, ns). Strong positive correlations were observed between reducing sugar and total sugar (0.96 ***), sucrose and Zn (0.91 ***), Cu and sucrose (0.91***), total sugar and Zn (0.92***). Some correlations, such as moisture and antioxidant (0.23, ns), EC and protein (0.22, ns), and Mn and antioxidant (0.34), moisture and Cu (0.33, ns), were found to be weak or statistically insignificant.

Discussion

Floral honey contains host-specific phytochemicals that are directly associated with health benefits such as anticancer, antioxidant, anti-inflammatory and wound healing properties (Mărgăoan et al., 2021). It has been reported that the SBH is well known for its numerous medicinal, health properties, whether used alone or combined with different ingredients (Reyes-González et al., 2014; Rosales, 2012). SBH demonstrate excellent potential and shows beneficial effects as an anticancer agent, antimicrobial and in improving hypertension, wound healing, lipid profiles, the treatment of eye diseases, fertility and even in some studies showing higher antidiabetic effects than the EBH (Zulkhairi Amin et al., 2018).

In the current study, the pH of *T. iridipennis* honey was found to be more acidic compared to *A. mellifera* honey. The acidic pH of honey is attributed to an important factor that contributes to its antibacterial properties (Tan et al., 2009) and help prolong its shelf life by providing stability against microbial spoilage (Moniruzzaman et al., 2013). Additionally, factors such as harvest and storage conditions may cause variations in its acidic levels, which can differ between bee species (Terrab et al., 2002). Similarly, a study by Ismail et al. (2021) reported that *Trigona* honey had a lower pH (3.03) than compared to *Apis* honey (3.37). Furthermore, Shamsudin et al. (2019) found that *Trigona* honey had a lower pH (3.00–3.27) than *A. mellifera* honey (3.56).

The electrical conductivity (EC) of honey reflects the concentration of mineral elements, which possess electrical conductivity properties (Yadata, 2014). EC is also used as an indicator of honey quality (Karabagias et al., 2014). In general, honey is naturally acidic (Alvarez-Suarez et al., 2018); however, as well as the floral source and the amounts of organic acids and proteins, can influence the electrical conductivity of honey samples (Karabagias et al., 2014). In this study, the EC of *T. iridipennis* honey was found to be higher than that of *A. mellifera* honey. This finding is consistent with the previous report by Ismail et al. (2021), indicated that *Trigona* honey exhibited a higher EC (1.05 mS/cm) compared to *Apis* honey (0.62 mS/cm). Elevated level of EC values, ranging from 1.07 to 1.80 mS/cm, as well as values exceeding 2.0 mS/cm, have been earlier reported in Thai stingless bee honey as well as in Malaysian *Apis* honey (Chuttong et al., 2016a; Chua et al., 2012).

Acidity has been reported to be linked to the moisture content of honey. Stingless bee honey naturally has high moisture content, which is a crucial parameter in the determining honey quality. The moisture content of the tested honey may be influenced by the harvest season and the hive's maturity level (AOAC, 1990). According to Codex Alimentarius (2001), honey with high moisture is vulnerable to fermentation, which results in high free acidity and low pH values. In the present study, SBH contained higher moisture content than Apis honey, which may explain its low pH compared to *Apis* honey. However, the estimated moisture content was within the limit of international standards (Codex Alimentarius, 2002). Similarly, higher moisture, low pH and free acidity in stingless bee honey were reported earlier by Shamsudin et al. (2019).

In the current study, stingless bee honey exhibited higher total protein and antioxidant content followed by *A. mellifera* honey. This elevated level of antioxidant and protein in SBH can be attributed to the unique botanical resources, specific processing method employed. Stingless bee honey is generally richer in proteins compared to *A. mellifera* honey. This is attributed to higher pollen content due to their foraging behavior, as they prefer protein rich pollens during foraging (Ghramh and Khan, 2023). The types and concentrations of polyphenols found in each sample is probably what causes the variations in antioxidant activity among honey samples. Polyphenols, particularly flavonoids, are known to be vital for antioxidant activity in honey (Ismail et al., 2016; Ismail et al., 2018). Total antioxidant activity is not entirely contributed by polyphenols, other compounds such as E (α-tocopherol), vitamin C (ascorbic acid) and carotenoids may also contribute to the antioxidant activity of honey (Gheldof et al., 2002; Al-Mamary et al., 2002). The specific type of phenol compounds and reducing potential of honey is the key factors influencing antioxidant activity (Divya et al., 2018; Can et al., 2015). This indicates that the higher antioxidant activity in SBH is due to its significantly enhanced FRAP value. Similar findings were reported by Shamsudin et al. (2019) and Ismail et al. (2021), observed that Trigona (stingless bee) honey generally exhibits greater antioxidant activity than in *A. mellifera* honey. Furthermore, protein content in *T. iridipennis* honey was also found to be elevated relative to *Apis* honey, which is consistent with a previous study by Nweze et al. (2017), Sousa et al. (2016b) and Fahim et al. (2014), which reported higher protein content in *Melipona* spp. honey compared to *A. mellifera*. The protein content in honey is largely attributed to the presence of enzymes, either introduced by the bees or derived from the plant nectar (Saxena et al., 2010; Moniruzzaman et al., 2013). Additionally, honey's botanical and geographic origins, as well as its storage duration, might affect its total protein and amino acid content (Moniruzzaman et al., 2013), suggesting that different honey have varying protein concentration based on the pollen grains received from the plants they are derived from and the climate under which they are produced. Honey typically contains <5 mg protein per gram, with proline being the predominant amino acid (Anklam, 1998). Floral resources play a crucial role in determining protein content of honey, with variations occurring of protein in nectar and enzymes introduced by the bees (Habib et al., 2014). The higher protein and antioxidant levels observed in stingless bee honey suggest that these honey, particularly from species like *T. iridipennis*, may offer superior nutritional and health benefits compared to *Apis mellifera* honey, which can be attributed to differences in the floral and environmental influences on honey production.

The total sugar and reducing sugar concentrations in honey samples from *T. iridipennis* and *A. mellifera* were found to be elevated relative to those in Apis honey. Notably, the sucrose content was higher in SBH compared to *A. mellifera* honey. The observed sugar content in SBH attributed to a variety of factors, including nectar source, chemical composition of the nectars, geographical origin, climate conditions, processing and storage. In contrast to previous studies that reported low sugar levels of SBH compared to *A. mellifera* honey, the current study reveals that SBH contains higher levels of both reducing and total sugars. This disparity emphasizes the complex interplay of factors influencing honey composition and suggests that regional and environmental variation may contribute to the observed difference in sugars. Onion nectar contains fructose and glucose in varying ratios depending on environmental conditions (Silva et al., 2004). Warmer temperatures and lower humidity levels tend to enhance nectar sugar concentration, influencing honey sweetness (Nicolson et al., 2013). Since stingless bees prefer concentrated nectar due to their smaller body size and shorter foraging range (Kuhn-Neto et al., 2009), their honey may reflect a more refined sugar balance compared to *A. mellifera* honey. Likewise, a study by Nweze et al. (2017) reported that a higher amount of total and reducing sugar and sucrose in the honey of stingless bee species, *Melipona* compared to *A. mellifera* honey. Another, study by Pimentel et al. (2022) estimated the total reducing sugar of stingless bee honey ranged from 13 to 97.10 (g/100 g) and sucrose from 0.07 to 5.14 (g/100 g). The sugar content values observed in the honey samples fall within the acceptable limits. According to these standards, the reduced sugar content should not be less than 60% (g/100 g), and the sucrose content should not exceed 5% (g/100 g). Our findings indicate that both the reducing sugar and sucrose levels in honey samples are in accordance with the quality standards of *T. iridipennis* and *Apis mellifera* honey.

The study of minerals in honey has attracted considerable attention due to their nutritional and potential therapeutic properties. Minerals like iron (Fe), manganese (Mn), zinc (Zn), and copper (Cu) are key constituents in honey, and their concentrations are primarily determined by the source of the pollen collected by bees, as well as the uptake of these minerals from the surrounding soil (Taha et al., 2018). In the current study, among the four minerals (Fe, Mn, Zn, and Cu) analyzed, iron was found to be the most abundant mineral in *T. iridipennis* honey, followed by copper, zinc, and manganese. Furthermore, *T. iridipennis* honey contained greater mineral content than those in *Apis mellifera* honey. This finding is consistent with previous studies by Avila et al. (2019a) and Biluca et al. (2017), who reported the concentrations of iron (Fe) and manganese (Mn) in SBH, confirming that the concentrations of these minerals may vary based on the type of bee and the plant sources used. The presence of iron in stingless bee honey, noting that 88.6% of their samples contained measurable concentrations of Fe, which ranged from 0.2 μg/g to 123.9 μg/g was reported by Pucholobek et al. (2022). Zawawi et al. (2022) reported that Fe, Mn, Zn, and Cu in SBH. This suggests that the stingless bee honey contains significant amount of essential trace minerals, which could contribute to its nutritional value. Like-wise, studies by Nascimento et al. (2018), Veleminsky et al. (1990), Boussaid et al. (2018), Oliveira et al. (2019), Altun et al. (2017), Karabagias et al. (2018), and Dżugan et al. (2018) have reported the presence of various minerals in the honey of different bee species, including both stingless bees and the more common *A. mellifera*. Significant levels of macro-minerals such as potassium (K), calcium (Ca), sodium (Na), and magnesium (Mg), along with phenolic compounds, further enhancing its potential health benefits (Biluca et al., 2017) The higher mineral levels in *T. iridipennis* honey than in *A. mellifera* honey, as observed in this study, may reflect differences in foraging behavior, pollen sources, and soil composition, emphasizing the importance of considering these factors in studies of honey's nutritional value.

The correlation analysis of *T. iridipennis* and *A. mellifera* honey revealed several significant relationships between physico-chemical and mineral components of floral honey. The observed negative correlation between pH with various parameters, including EC, moisture, protein, antioxidant activity, sucrose, reducing sugar, total sugar, Fe, Mn, Zn, and Cu aligns with previous studies on floral stingless bee honey. Study found that floral stingless bee honey typically has a lower pH than *A. mellifera* honey, which is attributed to the presence of organic acids such as gluconic and citric acids (Biluca et al., 2016). This acidity plays important role in its antimicrobial properties and preservation characteristics of honey (Chuttong et al., 2016b).

The significant positive correlation between EC with antioxidant activity, sucrose and Cu each (0.96, *) is in found with earlier findings that report higher EC values in stingless bee honey compared to *A. mellifera* honey (Khalil et al., 2010). Higher EC values in honey are often associated with greater mineral content, particularly elements such as potassium, sodium, and minerals like Cu and Zn (da Silva et al., 2016).

Similarly, the strong correlation between total sugar and reducing sugar (0.97, *) reflects the dominance of monosaccharides like glucose and fructose in honey composition. Previous studies have documented that stingless bee honey contains a higher moisture content and a more complex sugar profile than *A. mellifera* honey (Oddo et al., 2008), which influences its physico-chemical properties.

The strong positive correlation between antioxidant activity and Cu (0.99, *) further supports the role of minerals in the antioxidant potential of honey. Stingless bee honey has been reported to have higher antioxidant activity due to its rich phenolic and flavonoid content (Tuksitha et al., 2018). The presence of Cu, Zn, and Mn may contribute to enzymatic antioxidant mechanisms, enhancing the overall bioactive properties of the honey (Sant'Ana et al., 2012). Stingless bees also play a vital role in biodiversity conservation, sustainable agriculture, and ecosystem resilience by pollinating diverse plant species, boosting crop yields, and supporting natural ecosystems (Singh and Singh, 2024; Slaa et al., 2006; Patel et al., 2021). Their minimal reliance on intensive agricultural practices makes them a more sustainable choice for beekeeping, contributing to pollinator biodiversity and ecosystem stability. Stingless bee farming offers multiple advantages for sustainable beekeeping, requiring little intervention and reducing the dependence on artificial feed, pesticides, and chemicals commonly used in conventional beekeeping. Bees thrive in natural habitats, support agroforestry and conservation efforts. Additionally, their small-scale honey production aligns with sustainable agricultural practices, benefiting rural communities while maintaining ecological balance (Aldasoro Maya et al., 2023). Acknowledging their ecological significance and integrating stingless bees into sustainable agricultural systems can promote environmentally friendly beekeeping that supports both ecosystems and local communities (Singh and Singh, 2024; Aldasoro Maya et al., 2023).

Conclusion

The study highlights that *T. iridipennis* honey has distinct physicochemical and nutritional advantages over *A. mellifera* honey. Its lower pH, attributed to organic acids like gluconic and citric acids, enhances antibacterial properties and shelf life. The higher electrical conductivity (EC), linked to increased mineral content, supports its bioactive potential. Additionally *T. iridipennis* honey contains greater moisture, protein, and antioxidant levels, largely due to its unique botanical sources and processing methods. The higher concentrations of minerals, particularly iron, copper, zinc, and manganese, further contribute to its nutritional value. Its rich flavonoid and antioxidant content plays a crucial role in neutralizing oxidative stress, potentially slowing aging and aiding in wound healing. The Pearson correlation heatmap analysis highlights several significant differences between *T. iridipennis* and *A. mellifera* honey, emphasizing the superior physicochemical and mineral composition of floral stingless bee honey. Floral stingless bee honey exhibits superior biochemical and mineral composition compared to *A. mellifera* honey. Overall, floral stingless bee honey stands out as a nutritionally rich, functional, and medicinally valuable honey.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

CP: Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. DS: Formal analysis, Investigation, Methodology, Validation, Writing – original draft, Writing – review & editing. PM: Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. KG: Formal analysis, Methodology, Writing – original draft, Writing – review & editing. TP: Data curation, Formal analysis, Methodology, Software, Visualization, Writing – original draft, Writing – review & editing. PS: Conceptualization, Investigation, Resources, Supervision, Writing – original draft, Writing – review & editing. AT: Methodology, Resources, Supervision, Writing – original draft, Writing – review & editing. SK: Writing – original draft, Writing – review & editing. VM: Funding acquisition, Resources, Supervision, Writing – original draft, Writing – review & editing. VK: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The authors declare that no Gen AI was used in the creation of this manuscript.

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