Pertanika J. Trop. Agric. Sci. 48 (2): 603 - 620 (2025)

PERTANIKA JOURNALS

TROPICAL AGRICULTURAL SCIENCE

Journal homepage: http://www.pertanika.upm.edu.my/

# Comparative Analysis of Antioxidant Properties in Water and Ethanolic Extracts of Propolis from Two Species of Indo-Malayan Stingless Bee

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## ABSTRACT

Propolis, an organic material crafted by bees from a blend of their salivary secretions, beeswax, pollen, and resins, contains essential organic compounds. Phenolic and flavonoids, crucial for their antioxidant properties, are prominently present in this resinous substance. The antioxidant capabilities of propolis extract from *Geniotrigona thoracica* (G) and *Heterotrigona itama* (H), two species of Indo-Malayan stingless bees, were examined in this study by using different solvents (ethanol, E and water; W). The analysis involved four extracts of stingless bee propolis which included the ethanolic extract of *G. thoracica* and *H. itama* (GE and HE), and water extract of the two species (GW and HW). The Folin-Ciocalteu method evaluated total phenolic content (TPC), while colourimetric techniques were utilised for total flavonoid content (TFC) estimation. Additionally, antioxidant strength was assessed through ferric-reducing antioxidant power (FRAP) as well as $IC_{50}$ values from the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay. The findings confirmed that HE offered the most elevated values for both TPC and TFC, with 52.775 mg GAE $g^{-1}$ and 467.37 mg QE $g^{-1}$, respectively. Meanwhile, the $IC_{50}$ value was comparable among HE and GE at 0.351 and 4.536 mg/ml, respectively, compared to HW and GW (11.985 and 30.93 mg/ml). Consequently, both species are competitive natural sources of antioxidants under the same solvent. While ethanolic extract propolis demonstrates superior antioxidant properties, water extract demonstrates potential benefits, including being consumer-friendly and

ARTICLE INFO

*Article history:*
Received: 2 April 2024
Accepted: 20 September 2024
Published: 28 February 2025

DOI: https://doi.org/10.47836/pjtas.48.2.15

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ISSN: 1511-3701
e-ISSN: 2231-8542

environmentally sustainable, making it a cost-effective alternative worth exploring. The extract holds promising potential for future applications, including in the cosmeceutical, functional food, and pharmaceutical industries.

*Keywords*: DPPH, flavonoid, FRAP, *Geniotrigona thoracica*, *Heterotrigona itama*, phenolic

## INTRODUCTION

Stingless bees, characterized by reduced stinging ability (Michener, 2000), are globally represented by over 500 species in 32 genera, with over 100 species scarcely described (Abd Jalil et al., 2017; Avila et al., 2018). Stingless bees have a pivotal function in an ecosystem, with approximately 33 identified Malaysian species, notably *Geniotrigona thoracica* (*G. thoracica*) and *Heterotrigona itama* (*H. itama*) (Shamsudin et al., 2019). These species are frequently domesticated due to easy cultivation in suburban areas, and their log hives are possible to locate and collect in nature (Zullkiflee et al., 2022).

The chemical makeup of stingless bee species varies due to factors like collection timing, surrounding flora, and geographical positions (Bankova et al., 2000; Park et al., 2000). As essential pollinators, they contribute significantly to the ecosystem, propolis, wax, pollen, and honey (Bibi et al., 2008; Michener, 2012). These bee products, used in traditional medicinal practices, offer nutritional and health benefits (Maroof & Gan, 2022; Quezada-Euán, 2018).

Plant resins, pollen, beeswax, and certain essential and aromatic oils are the most common propolis compositions (Anjum et al., 2019). Abundant in phenolic compounds, esters, flavonoids, terpenes, and beta-sterols, propolis exhibits antioxidant properties primarily due to its phenolic and flavonoid constituents. Predominant phenolic compounds in stingless bee propolis encompass phenolic acids, catechins, flavonols, stilbenes, and tannins (Bonamigo et al., 2017; Cauich-Kumul & Segura Campos, 2019; Huang et al., 2014).

Propolis extracts exhibit antioxidant, antibacterial, and anti-inflammatory characteristics which can offer various health advantages (Ahmad et al.,2019; Berretta et al., 2020; Brodkiewicza et al., 2018; Junior et al., 2018; Siheri et al., 2016; Veloz et al., 2019; Vongsak et al., 2015). Widely applied in pharmaceuticals, cosmetics, and functional foods, propolis is recognized for its antioxidant potential and diverse chemical composition (da Silva et al., 2011; Santos et al., 2019; Vasilaki et al., 2019). Its antioxidant efficacy, attributed to hydroxyl groups in phenolic compounds, effectively neutralizes free radicals (Mihai et al., 2011). Similar to stingless bee honey (Mahmood et al., 2021), propolis constituents and their biological effects are contingent upon their botanical origin, geographical location, harvest season, bee species, and extraction methods (Ibrahim et al., 2016; Lim, Chua, & Soo, 2023; Shehata et al., 2020).

Simultaneously, an extraction process significantly affects the propolis molecular makeup (Kek et al., 2014; Przybylek & Karpinski, 2019). Other than maceration and Soxhlet (Rocha et al., 2023), techniques like ultrasound extraction are advantageous, with solvent choice affecting the extraction efficiency (Bankova et al., 2021; Silva-Beltran et al., 2021). Typically, a variety of solvents are used for this purpose, including water, ethanol, methanol, chloroform, dichloromethane, acetone, and ethyl ether (Martinotti & Ranzato, 2015; Wagh, 2013). Notably, Mello et al. (2010) found that the phenolic compounds are efficiently extracted when ethanol/water solvents are utilized. Additionally, propolis ethanol extracts have demonstrated superior antioxidant activity compared to aqueous extracts.

Nevertheless, Laskar et al. (2010) observed that water-extracted propolis demonstrated a greater phenolic content alongside improved reducing capability and scavenging activity in comparison to its ethanolic equivalent. It may be attributed to the efficacy of water in aiding the movement of extractable components, such as polyphenols, within plant tissue (Altiok et al., 2008; Borges et al., 2020). However, the dissolution of certain high molecular weight phenolic compounds like tannins in water may lead to the formation of colloids, a phenomenon discussed by Fraga-Corral et al. (2020) and Kusuma et al. (2022).

Despite extensive propolis research, studies on Malaysian stingless bee propolis remain limited (Lim, Chua, & Dawood, 2023). This research uses ethanol and water extraction to explores the properties of antioxidants from propolis found in *H. itama* and *G. thoracica*. Findings could potentially aid in determining the most suitable bee species and solvent for diverse applications in distinctive industries.

## MATERIALS AND METHODS

### Raw Materials Preparation

Propolis from *H. itama* and *G. thoracica* was harvested from Kuala Terengganu, Terengganu, from August until November and transported to the Postharvest Laboratory at Universiti Malaysia Terengganu (UMT). The raw propolis was meticulously purified upon collection to remove extraneous materials, including dead bees, wood fragments, and debris. Propolis was then frozen at -20ºC prior to analysis.

### Extraction of Propolis

Propolis was extracted by adapting the methodology of Omar et al. (2020) with alteration. The powdered propolis weighed 10 g in total and was homogenised via two distinct solvents: water and 95% ethanol, using a 1:10 ratio. An ultrasonic bath was used to extract the propolis for 50 minutes at 50ºC. It was then agitated in an incubator shaker for 48 hours. Subsequent to this, the propolis mixture was strained using a cloth strainer and filter paper to eliminate solid particles and waxes. The resulting propolis extract was centrifuged at

9000 rpm for 15 min. A clarified solution was pooled and evaporated before storage at -20ºC using a rotary evaporator. The concentrated extract was dried using a vacuum oven to remove excess moisture. (Figure 1).

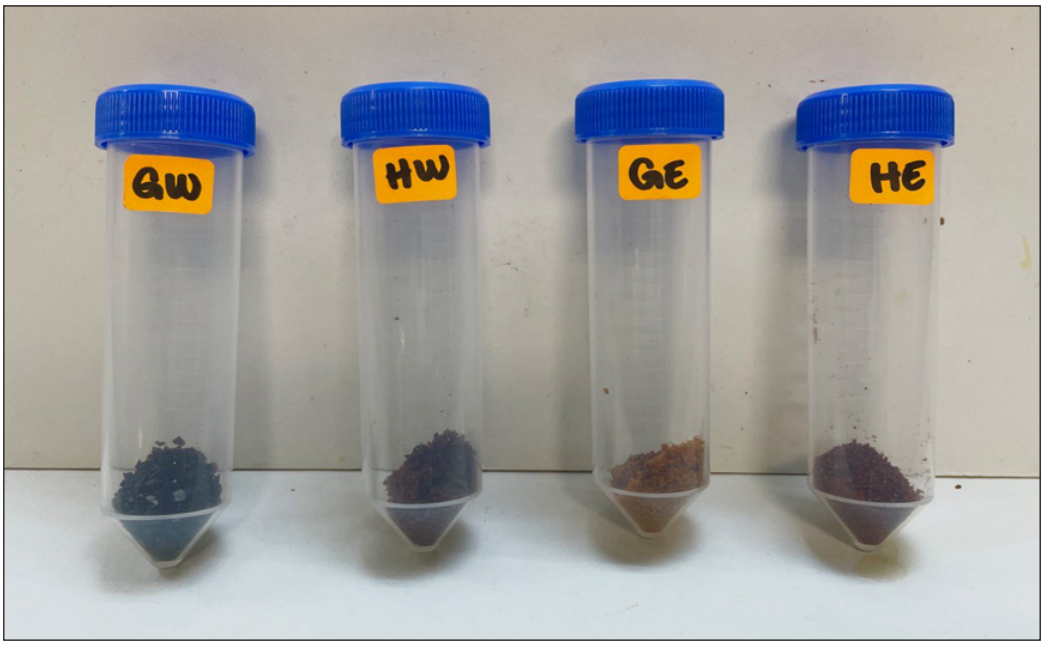


*Figure 1*. Propolis crude extract that were used for the analysis. From left to right: Water extract of *Geniotrigona thoracica* (GW), water extract of *Heterotrigona itama* (HW), ethanol extract of *G. thoracica* (GE) and ethanol extract of *H. itama* (HE)

## Yield

The yield of extraction was recorded and calculated based on a formula by Pujirahayu et al. (2014):

$$\text{Yield} = (\frac{\text{Pe}}{\text{Pm}}) \times 100$$

where,
Pe = Propolis extract weight (g)
Pm = Raw propolis weight (g)

## Phenolic Composition by HPLC

Identification of phenolic compounds of stingless bee propolis was implemented according to Sun et al. (2015) with slight modification. A diode ray detection (DAD) equipped HPLC

(Shimadzu, Japan) system was utilised for the study. A total of 10 µl of propolis extracts were injected into the HPLC coupled with Syncronis™ C18 column (250 × 46 mm, 5 µm) (Thermo Scientific™, USA). The mobile phase comprised 2% acetic acid in water (A) and 2% acetic acid in methanol (B). A constant flow rate was set at 0.75 ml/min with a 150 min gradient flow program. The gradient was set as follows: 0–25 min: 22%–36% B; 25–55 min: 36%–52% B; 55–90 min: 55%–63% B; 90–115 min: 63%–70% B; 115–135 min: 70%–75% B; and 135–150 min: 75%–80% B. Simultaneously, oven temperature was set at 35°C and the phenolic compounds of stingless bee propolis were identified based on previously reported publications.

**Total Phenolic Content (TPC)**

Total phenolic content (TPC) was implemented by the Folin-Ciocalteu colourimetric method (Syed Salleh et al., 2021). Initially, 1 ml of solution containing 5 mg crude propolis and the corresponding extraction solvent (water or 95% ethanol) was prepared. Next, 0.2 ml of sample solution was pipetted into a vial and diluted to 1 ml with 10% Folin-Ciocalteu reagent. After the solution was incubated at ambient temperature for five minutes, 1 ml of 8% sodium carbonate ($Na_2CO_3$) solution was introduced. Finally, a maximum volume of 3 ml was attained using 95% ethanol, and the mixture's absorbance was estimated at 725 nm via a UV-VIS spectrophotometer (Shimadzu, Japan). Total phenolic content was quantified and expressed in milligrams of gallic acid equivalent per gram of propolis (mg GAE $g^{-1}$).

**Total Flavonoid Content (TFC)**

The colourimetric technique involving aluminium chloride with minor adjustment was utilised to quantify the TFC within propolis (Sun et al., 2015). Crude propolis was first dissolved in its respective solvent to acquire a 5 mg/ml concentration. A volume of 0.5 ml of the prepared sample was homogenised with 0.3 ml of 5% sodium nitrite ($NaNO_2$), followed by an incubation period of 6 minutes. Subsequently, 0.3 ml of 10% aluminium nitrate ($Al[NO_3]_3$) was added to the mixture, which was incubated for another 6 minutes. Following this, 4 ml of 4.3 % sodium hydroxide (NaOH) was introduced, and the overall volume of the solution was fixed to 10 ml using the same extraction solvent. After incubation at ambient temperature for 15 minutes, a UV-VIS spectrophotometer (Shimadzu, Japan) was used to read the sample's absorbance at 510 nm. Total flavonoid content was estimated and presented as a milligram of quercetin equivalent per gram of propolis (mg QE $g^{-1}$).

**DPPH Radical-scavenging Activity**

Sun et al. (2015) outlined a technique to assess the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity. A DPPH solution of 0.2 mM concentration was prepared in 95% ethanol. Several concentrations of crude propolis extract (0.2 to 10.0 mg/ml) were

prepared using the respective extraction solvent. The test is conducted by combining a volume of 200 µL of the propolis solution with 1.8 ml of the respective solvent and 2 ml DPPH solution. This mixture was then thoroughly vortexed and incubated for 20 minutes at room temperature in darkness. A UV-VIS spectrophotometer (Shimadzu, Japan) was employed to measure the absorbance of the solution at 517 nm and quantified using $IC_{50}$ value, with ascorbic acid serving as the antioxidant standard.

### Ferric Reducing Antioxidant Power (FRAP) Assay

A modified FRAP assay was performed as per the approach outlined by Wong et al. (2006). The FRAP reagent was prepared by combining 20 mM ferric chloride ($FeCl_3$), 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ), and 300 mM sodium acetate buffer at a 10:1:1 ratio with a pH of 3.6. In the experimental procedure, a 100 µl sample was homogenised with 3 ml of FRAP reagent and incubated at 37 ºC for 4 minutes. At two time points, initially at 0 minutes (before incubation) and then at 4 minutes (after incubation), the measurement of absorbance was taken using a UV-VIS spectrophotometer (Shimadzu, Japan) at a wavelength of 593 nm. To prevent cloudiness in the solution, Tween 20 was added as a surfactant (Wojtunik-Kulesza, 2020). The FRAP value was evaluated in terms of mg Trolox equivalents antioxidant capacity per gram of propolis on a dry weight basis (mg TEAC $g^{-1}$).

### Statistical Analysis

Every analysis was implemented in three replications to ensure accuracy. A Two-Way Analysis of Variance (ANOVA) was used on the resulting data, and subsequently, a post-hoc Tukey's test was run to verify its statistical significance. These analyses were completed utilising the Statistical Analysis System (SAS) program.

## RESULTS AND DISCUSSION

### Phenolic Composition by HPLC

The expected compounds in stingless bee propolis extract are shown in Table 1 and Figure 2. The presumption of compound availability was reported by comparing the retention time with the previous study at 280 nm since most phenolic compounds showed some degree of absorption at this wavelength (Gomez-Caravaca et al., 2015). However, the reported compounds still need to be attested with an external standard calibration curve to accurately identify and quantify them in future studies. Based on Figure 2, water extract propolis (GW and HW) most probably displayed a generally lower concentration of compounds when compared with ethanolic extract propolis (GE and HE). Despite having complex HPLC profiles, GW and HW had limited peaks, contrary to GE and HE.

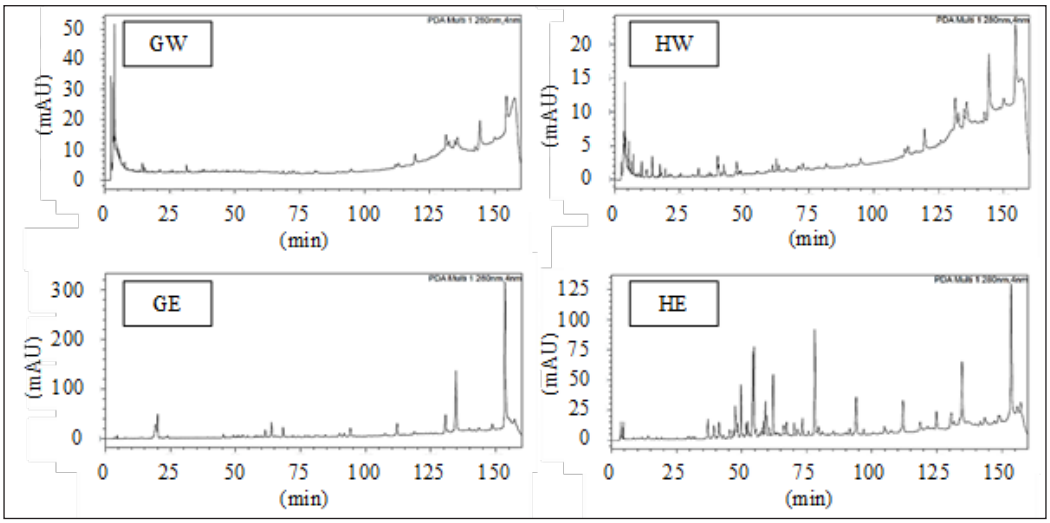


*Figure 2*. HPLC chromatograms of stingless bee propolis extracted with water and ethanol at 280 nm
Note. GW= *Geniotrigona thoracica* water extract; HW= *Heterotrigona itama* water extract; GE= *Geniotrigona thoracica* ethanol extract; HE= *Heterotrigona itama* ethanol extract

Table 1
*Retention time (min) of the presence of the expected compounds in water and ethanolic extract propolis at 280 nm based on the compounds reported by Sun et al. (2015)*

| Retention time (min) | Expected compound | Propolis samples | | | |
|---|---|---|---|---|---|
| | | GW | GE | HW | HE |
| 7.00±1.00 | 3,4-Dihydroxybenzaldehyde | + | + | + | + |
| 12.00±1.00 | Caffeic acid | + | + | + | - |
| 19.00±1.00 | Ferulic acid | + | + | + | + |
| 21.00±1.00 | Isoferulic acid | + | + | + | - |
| 24.00±1.00 | Benzoic acid | - | + | + | + |
| 33.00±1.00 | Cinnamic acid | + | + | + | + |
| 41.00±1.00 | 4-methoxycinnamic acid | + | - | + | + |
| 46.00±1.00 | 5-methoxy pinobanksin | - | + | - | + |
| 49.00±1.00 | Pinobanksin | + | + | + | + |
| 54.00±1.00 | Quercetin | - | + | - | + |
| 61.00±1.00 | Alpinetin | - | + | - | + |
| 65.00±1.00 | Kaempferol | - | + | - | + |
| 76.00±1.00 | Pinocembrin | - | + | - | + |
| 77.00±1.00 | Isorhamnetin | - | + | - | - |
| 80.00±1.00 | Benzyl caffeate | - | + | - | - |
| 82.00±1.00 | Pinobanksin-3-O-acetate | - | + | - | + |
| 96.00±1.00 | Chrysin | - | - | - | + |
| 97.00±1.00 | Benzyl-p-coumarate | - | + | - | - |
| 109.00±1.00 | Pinostrobin | - | + | - | - |
| 116.00±1.00 | Tectochrysin | - | + | - | - |

*Note*. + = Detected; - = Not detected

Nine compounds were expected to be identified in GW and HW, which include 3,4-Dihydroxybenzaldehyde, caffeic acid, ferulic acid, cinnamic acid, 4-methoxycinnamic acid, cinnamylideneacetic acid, pinobanksin, isoferulic acid, and benzoic acid. For GE and HE, 3,4-Dihydroxybenzaldehyde, caffeic acid, ferulic acid, isoferulic acid, benzoic acid, cinnamic acid, 4-methoxycinnamic acid, cinnamylideneacetic acid, 5-methoxy pinobanksin, pinobanksin, quercetin, alpinetin, kaempferol, isorhamnetin, pinocembrin, benzyl caffeate, pinobanksin-3-O-acetate, chrysin, benzyl-p-coumarate, pinostrobin, and tectochrysin were more inclined to be identified. Out of all, 3,4-Dihydroxybenzaldehyde, ferulic acid, cinnamic acid, and pinobanksin were found in all propolis samples, regardless of species and solvent used. The range of solvent polarity may cause differences in the variation of propolis's chemical profiles. More compounds tend to be characterised in GE and HE than in GW and HW, especially phenolic acids, which possess a notable antioxidant potential (Mahmad et al., 2023). The chemical makeup of propolis can also be affected by flowering plants' availability along with its resinous substance that eventually becomes the source of bee forages (Da Silva Araújo et al., 2016).

**Yield**

Based on Table 2, the present study indicated that propolis extracted with ethanol (GE and HE) has a markedly higher yield ($p<0.05$) by comparison to those extracted with water (GW and HW). GE exhibited the highest yield at 38.81%, followed by HE, GW, and HW at 23.29%, 10.36%, and 6.54%, respectively. This result is consistent with Kustiawan et al. (2022), who documented that methanolic propolis extract of *G. thoracica* produced a higher percentage of yield (33.96%) compared to *H. itama* (29.44%). Similar results were documented in earlier reports where propolis prepared with ethanol produced a higher yield than other tested solvents (Pujirahayu et al., 2017; Sambou et al., 2020). It was also mentioned that the difference was supposedly due to the organic solvent properties that can dissolve most propolis content. In addition, variations in yield and the chemical composition of each extract could be attributed to the hydroxyl groups in water, which render it a less effective solvent for many organic compounds. It suggests that solvent polarity is a significant factor influencing the differences observed in extraction yields, as Almeida et al. (2012) reported.

A study accomplished by Fikri et al. (2019) described that, on average, propolis extract prepared by ethanol had a significantly higher yield, enhanced antioxidant activity, and greater concentrations of total phenolics and flavonoids compared to those extracted with water. This finding aligns with the research on Malaysian propolis by Usman et al. (2016), which demonstrated that 70% ethanol was the most effective extraction yield, surpassing both 100% and 90% ethanol, as well as distilled water. Furthermore, the higher solubility of wax content in propolis extracted with ethanol compared to water, as noted by Fikri et

al. (2019), is likely a contributing factor to the higher yields observed in ethanolic propolis extracts.

## Total Phenolic and Flavonoid Content

While achieving the highest yield, GE exhibited lower ($p$<0.05) phenolic and flavonoid content than HE, yet it was still more potent than both GW and HW. Based on Table 2, ethanolic extracts of propolis (GE and HE) contained significantly more phenolic and flavonoid compounds than water-based extracts (GW and HW), regardless of bee species. Value of TPC ranged from 11 to 52 mg GAE $g^{-1}$. This study revealed that HE had the highest TPC value (statistically significant with $p$<0.05) at 52.775 mg GAE $g^{-1}$, followed by GE, HW, and GW with values of 24.916, 11.247, and 11.022 mg GAE $g^{-1}$, respectively. The highest TFC was observed in HE at 467.37 mg QE $g^{-1}$, while the lowest was in GW at 12.664 mg QE $g^{-1}$, which shared a comparable value to HW at 13.450 mg QE $g^{-1}$.

Table 2
*Comparison of the yield, total phenolic content (TPC), and total flavonoid content (TFC) of stingless bee propolis extract*

| Propolis | Yield (%) | TPC (mg GAE $g^{-1}$) | TFC (mg QE $g^{-1}$) |
|---|---|---|---|
| GW | 10.36$^{c}$ | 11.022$^{c}$ | 12.664$^{c}$ |
| GE | 38.81$^{a}$ | 24.916$^{b}$ | 116.46$^{b}$ |
| HW | 6.54$^{c}$ | 11.248$^{c}$ | 13.450$^{c}$ |
| HE | 23.29$^{b}$ | 52.775$^{a}$ | 467.37$^{a}$ |

*Note*. Distinct letters within rows denote statistically significant differences ($p$<0.05)

As previously mentioned, the levels of both TPC and TFC across propolis samples were congruent with one another, exhibiting the highest levels in HE and gradually decreasing through GE, HW, and GW. This pattern aligns with the findings of Sun et al. (2015), which also indicated the highest TPC and TFC values in ethanolic propolis extracts, with the lowest in water extracts. Propolis extracts are known to contain a significant number of phenolic compounds. The differing chemical characteristics and polarities of antioxidant compounds impact their solubility in specific solvents, as Turkmen et al. (2006) noted. While water is a more economical and environmentally friendly solvent, as Lim et al. (2019) highlighted, polyphenols often dissolve more readily in less polar organic solvents like ethanol (Haminiuk et al., 2012).

Various aspects, such as the plant source choices and the extraction solvent, influence the TFC, as Abdullah et al. (2019) identified. In this study, propolis from *H. itama* species demonstrated superior TPC and TFC compared to *G. thoracica,* irrespective of the solvent used. This finding concurs with the discoveries made by Ibrahim et al. (2016), which

also indicated higher levels of phenolics and flavonoids in *H. itama* propolis than in *G. thoracica.* Furthermore, Asem et al. (2019) documented that samples characterised with higher polyphenols typically demonstrate stronger antioxidant activities. However, a study by Mohd Badiazaman et al. (2018) reported that TPC of methanolic extract of *G. thoracica* propolis collected from Besut, Dungun, Lundang, Tanah Merah, and Gua Musang in Terengganu and Kelantan were ranging from 9.23 to 23.43 mg GAE per gram of extract while TFC values ranged from 9.52 to 17.22 mg QE per gram of extract. Suggesting that GE has a comparable phenolic and higher flavonoid content with other local propolis.

**Antioxidant Activities**

An $IC_{50}$ value of DPPH radical scavenging activity and FRAP assay were used for the assessment of propolis antioxidant capacity. The $IC_{50}$ value represents the sample concentration essential for neutralising 50% DPPH free radicals. A lower $IC_{50}$ value is indicative of an elevated antioxidant activity. Free radicals are reactive molecules produced by cells with the potential to cause oxidative harm to genetic information or cell membranes (Piantadosi, 2020). Table 3 shows HE and GE have the lowest ($p$<0.05) $IC_{50}$ values, followed by HW and GW at 0.351, 4.536, 11.985, and 30.93 mg/ml, respectively. In other words, the ethanolic extract of stingless bee propolis (HE and GE) demonstrated a potent scavenging effect on the DPPH free radicals, as opposed to the propolis extracted with water.

Regarding the stingless bee species, *H. itama* showed better antioxidant activity than *G. thoracica*, irrespective of their extraction solvent. The outcomes of these analyses align with the observations regarding TPC and TFC results, suggesting a direct association between the elevation of phenolic and flavonoid levels and the antioxidant potential of the extract. Adli et al. (2022) reported a slightly different opinion, claiming that only TPC may be responsible for the antioxidant activity. TFC was not significantly correlated to the $IC_{50}$ DPPH of propolis extract. Nonetheless, Barhe and Tchouya (2016) have previously observed that antioxidant activity is linked to the availability of flavonoids, phenolic compounds, and their distinct chemical structures. Additionally, research by Nafi et al. (2019) indicated that *H. itama* propolis exhibited the most potent antioxidant activity, evidenced by the lowest $IC_{50}$ values when compared to propolis from *G. thoracica* and *Lepidiotrigona terminate* at 30, 40, and 128 µg/ml, respectively. This result also aligns with Abdullah et al. (2020), revealing that *H. itama* recorded the lowest $IC_{50}$ than *G. thoracica* and *Tetrigona binghami* and eventually showed the highest total antioxidant capacity. Adli et al. (2022) revealed that the ethanolic extract of *G. thoracica* had the strongest antioxidant activity in the DPPH assay, with the lowest $IC_{50}$ at 104.2 µg/ml.

The variance seen in antioxidant activity can be related to the variations in phenolic, flavonoid, or other components present in propolis that contribute to their potential

antioxidant properties (Nafi et al., 2019). Moreover, it has been observed that the chemical composition of the ethanolic extract of *H. itama* is more intricate when considering other species of stingless bees, primarily due to the abundance of chemical compounds it contains. As concluded by Mahmad et al. (2023), chemical compounds of *H. itama* are immense that includes tannins, betalains, peptides, nucleotides, phospholipids, indoles, coumarins, quinolines, cyanogenic glycosides, isoflavonoids, pyrroles, anthocyanins, saponins, carotenoids, amino acids, glycosides, xanthones, lignans, and quinones. Despite that, the crucial components contributing to the antioxidant potential of propolis extract are phenolics and flavonoids, in general (Puspitasari et al., 2022). Additionally, the diversity of chemical compounds identified in all propolis extracts may stem from the bees' preferences for specific plants or flowers during foraging (Nafi et al., 2019).

Ferric reducing antioxidant power assay involves the reaction of electron transfer from antioxidant (as electron donor) and ferric ($Fe^{3+}$) tripyridyl triazine complex (as electron acceptor) into ferrous ($Fe^{2+}$) form (Ismail et al., 2013). According to Table 3, HE demonstrated a notably higher FRAP value (statistically significant with $p<0.05$) of 0.299 mg TEAC $g^{-1}$, while HW and GE displayed similar results at 0.039 and 0.033 mg TEAC $g^{-1}$, respectively. GW showed the lowest FRAP value at 0.009 mg TEAC $g^{-1}$. In other words, HE reduced $Fe^{3+}$ into $Fe^{2+}$, possibly owing to its higher antioxidant content than other extracts, as in Table 2. Sun et al. (2015) observed that 75% ethanolic propolis extract presented the highest FRAP value (200 µg Trolox/mg), nearly 15 times greater than a water extract, indicating a superior reducing capability. However, according to Asem et al. (2019), the antioxidant activity of the ethanolic extract obtained from *G. thoracica* was the most prominent, followed by *H. itama* and *Tetrigona apicallis*. The antioxidant activity of propolis presented in this study can be considered weaker when compared to stingless bee propolis from other locations like Hulu Bernam in Selangor at 104.2 to 332.7 ug/ml for both water and ethanolic extract of *G. thoracica* and *H. itama* propolis (Adli et al., 2022). Besides, Idris et al. (2023) reported lower $IC_{50}$ values stretching from 27 to 122.7 µg/ml for ethanolic extract of *G. thoracica* collected from Serdang, Shah Alam, and Hulu Bernam, Selangor. Despite having a lower antioxidant capacity, the propolis extract from the present study can still be potentially utilised for antimicrobial activity due to its high content of flavonoids (Anjum et al., 2019; Sforcin, 2016; Wagh, 2013).

Table 3
*Comparison of the DPPH and FRAP assay of* G. thoracica *and* H. itama *propolis extract via water and ethanol solvents*

| Propolis | DPPH $IC_{50}$ (mg/ml) | FRAP (mg TEAC $g^{-1}$) |
|---|---|---|
| GW | 30.930[a] | 0.009[c] |
| GE | 4.536[c] | 0.033[b] |
| HW | 11.985[b] | 0.040[b] |
| HE | 0.351[c] | 0.299[a] |

*Note*. Distinct letters within rows denote statistically significant differences ($p<0.05$)

## CONCLUSION

The antioxidant levels in propolis sourced from stingless bees fluctuate depending on the bee species and the type of extraction solvent employed. This investigation unveils a strong connection between the phenolic and flavonoid content and the ability to scavenge DPPH free radicals, suggesting a link between the antioxidant attributes and the efficacy of stingless bee propolis. However, a more detailed characterisation of stingless bee propolis profiles needs to be studied to identify the exact compound contributing to its antioxidant properties. Overall, ethanolic extracts exhibited elevated total phenolic and flavonoid content, along with heightened antioxidant potency in comparison to water extracts of propolis. Regarding the stingless bee propolis species, *H. itama* exhibited superior antioxidant properties and activity compared to *G. thoracica,* irrespective of the solvent used, with the order being HE>GE>HW>GW. Notably, research on Indo-Malayan stingless bees, especially in Malaysia, is limited. Therefore, this study is poised to add valuable knowledge to the relatively scarce information on stingless bee propolis in Malaysia, potentially aiding in future innovations related to stingless bee propolis.

## ACKNOWLEDGEMENTS

The authors extend their appreciation to the Faculty of Fisheries and Food Science at Universiti Malaysia Terengganu for supplying research amenities and technical assistance. This work received financial support from the Ministry of Higher Education Malaysia (MOHE) through a Talent and Publication Enhancement-Research Grant (TAPE-RG; Vot No. 55335). Additionally, gratitude is extended to En Hazian Hashim and the Society of Stingless Bee Entrepreneurs Terengganu (PELEKAT) for their contributions of raw materials to this study.

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