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DETERMINATION OF ANTIOXIDANT ACTIVITY, TOTAL PHENOLIC AND FLAVONOID CONTENTS OF MALAYSIAN STINGLESS BEE PROPOLIS EXTRACTS

MUHAMMAD AMIRUL ADLI[1,2], ROZAINI MOHD ZOHDI[2,3]*, NURUL 'AQILAH OTHMAN[2], NUR SUHAILI MOHAMED AMIN[2], SHAHIDA MUHAMAD MUKHTAR[3], ZOLKAPLI ESHAK[2], IZFA RIZA HAZMI[4] AND DZU HENDRA JA JAHRUDIN[5]

[1]*Faculty of Applied Sciences, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia.* [2]*Faculty of Pharmacy, Universiti Teknologi MARA, 42300 Puncak Alam, Selangor, Malaysia.* [3]*Atta-ur-Rahman Institute for Natural Product Discovery, Universiti Teknologi MARA Selangor Branch, Puncak Alam Campus, 42300 Puncak Alam, Selangor, Malaysia.* [4]*Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor, Malaysia.* [5]*Belantara Sr Enterprise, Kampung Simpang Empat, 35900 Tanjung Malim, Perak, Malaysia.*

**Corresponding author: rozainizohdi@uitm.edu.my*
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Abstract: This study aims to determine the total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activity of ethanolic extract propolis (EEP) and water extract propolis (WEP) derived from six Malaysian stingless bee species. The TPC and TFC were measured by Folin-Ciocalteu colorimetric and aluminium chloride methods, respectively. The antioxidant activity was determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. The results indicated that EEP of *Geniotrigona thoracica* had the highest TPC and TFC at 259.84 ± 4.97 mg/g GAE and 435.00 ± 6.57 mg/g QE, respectively. Similarly, EEP of *G. thoracica* exhibited the greatest free radical scavenging activity with the percentage of inhibition at 88.00 ± 13.34% and IC_{50} of 104.20µg/mL. Antioxidant activity was highly correlated with TPC (R^2 = -0.744, $p < 0.05$) whereas no correlation was observed between TFC and antioxidant activity. This study indicates that the antioxidant property of the propolis extract may be influenced by elevated levels of phenolic content. In conclusion, ethanolic extract of *G. thoracica* is the greatest source of antioxidants among the six tested Malaysian stingless bee propolis and this suggests that the extract may serve as a promising source of antioxidant agent.

Keywords: Propolis, stingless bee, phenolic, flavonoid, radical scavenging.
Abbreviations: Ethanolic Extract Propolis (EEP), Water Extract Propolis (WEP), Total Phenolic Content (TPC), Total Flavonoid Content (TFC), 2,2-diphenyl-1-picrylhydrazyl (DPPH).

Introduction

Stingless bees are known locally as "lebah kelulut" are members of the Apidae family and the Meliponini tribe with approximately 500 identified species from 32 genera across the globe (Sahlan *et al*., 2019). Stingless bees lack a functional sting and are of little harm to bee-keepers as opposed to honeybees (Asem *et al*., 2019). They protect their hives by covering it with wax-like substances called propolis which is composed mainly of resinous mixtures, beeswax, essential oils, bee pollen and minor components including minerals, amino acids and organic debris (Salleh *et al*., 2021). It has been reported that stingless bee propolis has higher nutritional and medicinal properties in comparison to propolis produced by honeybees (Al-Hatamleh *et al*., 2020). This can be attributed to the foraging activities of the stingless bees which originate from rich vegetation in their native environment.

Propolis represents a huge repository of chemical biodiversity which includes polyphenols, coumarins, sesquiterpene quinones, amino acids, steroids and inorganic substances (Siripatrawan *et al*., 2013). The chemical composition of propolis is highly variable depending on factors such as the geographical region, climate, botanical origin and bee species (Ahangari *et al*., 2018). Even

though propolis has a complex chemical composition, its biological activities are mainly ascribed to the polyphenol compounds such as phenolic acids, flavonoids and esters (Rosli *et al*., 2016).

A large body of evidence demonstrates that propolis possesses various biological activities such as anticancer, antioxidant, antimicrobial, anti-fungal, antiviral and anti-inflammatory properties (Ibrahim *et al*., 2016; Pasupuleti *et al*., 2017; Al-Hatamleh *et al*., 2020). Most of these studies are conducted using ethanolic extract of propolis. Ethanol is the common solvent used to obtain propolis extracts with low wax content and rich in biologically active compounds (Devequi-Nunes *et al*., 2018). However, several disadvantages have been associated with the ethanolic extract of propolis such as strong residual flavour and adverse reactions or intolerance to alcohol which limits its application in cosmetics, pharmaceutical and food industries (Kubiliene *et al*., 2015). In contrast, the water extract of propolis has been deemed to be environmentally friendlier, safer and more biocompatible for use in the pharmaceutical and health sectors (Kubiliene *et al*., 2018). Nonetheless, studies on Malaysian stingless bees, particularly using water as a solvent are still lacking and have received little attention thus far. Hence, this study aimed to compare the total phenolic and flavonoid contents as well as the antioxidant activity of ethanolic and water extracts of propolis derived from six local stingless bee species. In addition, the correlation between the phenolic and flavonoid contents with the antioxidant property was also evaluated.

Materials and Methods

Species Identification

The stingless bee species were identified according to their morphological characteristics using taxonomic key referring to Samsudin *et al.* (2018) by Izfa Riza Hazmi, an entomologist at the Centre for Insect Systematic, Universiti Kebangsaan Malaysia. The voucher accession numbers are as follows: *Geniotrigona thoracica* (CIS-TRI-2022-01), *Heterotrigona itama* (CIS-TRI-2022-02), *Tetragonula laeviceps* (CIS-TRI-2022-03), *Lepidotrigona terminata* (CIS-TRI-2022-04), *Tetrigona binghami* (CIS-TRI-2022-05) and *Tetrigona apicalis* (CIS-TRI-2022-06).

Propolis Collection

Propolis samples from the six stingless bee species with the age of colonies ranging between 1 to 2 years old were collected from local apiary of Belantara SR Enterprise (N 3° 40’ 42.1818” E 10° 31’ 14.5416”), Hulu Bernam, Selangor in December 2021. The colonies were kept in nest boxes made of wood within a 797 m^2 land area and surrounded by several species of ornamental trees (Myrtaceae, Fabaceae, Lamiaceae, Rubiaceae, Scrophulariaceae, Lythraceae, Elaeocarpaceae), fruit trees (Moraceae, Anacardiaceae, Sapindaceae) and resin-secreting trees (Melaleuca). All samples were cleaned, placed in labelled plastic bags and stored at -20ºC until further analysis.

Preparation of Ethanolic Extract Propolis

The ethanolic extract propolis (EEP) was prepared according to the method by Pobiega *et al*. (2019) with slight modifications. About 10 g of propolis sample was ground into powder and macerated in 100 mL of 70% ethanol in a 1:10 (w/v) ratio. The sample was then incubated at 25ºC while continuously shaken at 250 rpm for 48 hours. The suspensions were filtered using Whatman No. 1 filter (Millipore, USA). The filtrate was evaporated under vacuum pressure (Rotavapor R-215, Büchi, Switzerland) at 995 hPa with a temperature of 40ºC. The concentrated extracts were centrifuged for 10 minutes at 3,900 x g to eliminate any wax. The extract was then freeze dried and stored in a dark container at -20ºC prior to analysis.

Preparation of Propolis Water Extract

The water extracts propolis (WEP) was prepared using the same maceration method. Approximately 10 g of each propolis sample was ground into powder and dissolved with 50 mL distilled water (1:5 w/v). Then, the sample

was heated on a hot plate while constant stirring at 60ºC for seven hours. The suspensions were filtered using Whatman No. 1 filter paper (Millipore, USA) and the filtrate was centrifuged at 28,000 x g for 30 minutes. The supernatant was concentrated under reduced pressure to produce the water extract propolis, freeze dried and kept at -20ºC in a dark container until further use.

Determination of Total Phenolic Content

Total phenolic content (TPC) of propolis extracts was determined using the Folin-Ciocalteu colorimetric method and expressed as mg/g Gallic Acid Equivalent (GAE) (Pratami *et al*., 2018). Gallic acid was used as the standard solution. Briefly, 1.0 mL of gallic acid with eight different concentrations (5, 50, 75, 100, 250, 500, 750, 1,000 µg/mL) was prepared. About 25 µL of 1 mg/mL was extracted and the prepared standard solution was mixed with 100 µL of Folin-Ciocalteu reagent in a 96-well microplate, incubated and shaken for four minutes at room temperature. Later, 75 µL of 7.5% sodium carbonate was mixed to the reaction mixture, shaken for 60 seconds and incubated at room temperature for two hours. The absorbency of the reaction mixture was measured at 765 nm (SPECTROstar Nano, BMG Labtech, Germany) and the regression line obtained from the standard curve was used to determine the TPC value. All tests were done in triplicate.

Determination of Total Flavonoid Content

Aluminium chloride ($AlCl_3$) colorimetric technique described by Farasat *et al*. (2014) was used to measure the total flavonoid content (TFC) of propolis extract. Quercetin was prepared in various concentrations, ranging between 10 and 500 µg/mL to obtain the quercetin standard calibration curve. About 20 µL of the prepared standard and propolis extracts mixtures were added into a 96-well microplate and combined with 20 µL of 10% aluminium chloride solution, 20 µL of 1 M potassium acetate and 140 µL distilled water. The plate was shaken continuously for 60 seconds before being incubated for 30 minutes in a dark at room temperature. The absorbency readings were determined at 415 nm employing a microplate reader (SPECTROstar Nano, BMG Labtech, Germany) and TFC values were calculated using the linear regression line plotted in the standard curve. All tests were conducted in triplicate.

2,2-diphenyl-1-picrylhydrazyl (DPPH) Free Radical Scavenging Assay

DPPH free radical scavenging activity of propolis extracts was determined according to the technique by Nafi *et al*. (2019). Quercetin served as the standard reference. Briefly, a total of 1 mM DPPH solution was initially prepared by diluting 5 mg of DPPH in 100 mL of methanol. Then, the propolis extracts and quercetin were serially diluted in various concentrations of 500, 250, 125, 62.5, 31.25, 15.625 and 7.813 µg/mL. About 25 µL of the samples and standard were added into wells of a 96-well round bottomed plate. Then, 200 µL of 1 mM DPPH solution was loaded into each well and mixed well. A blank solution was prepared by mixing 25 µL of DMSO and 200 µL of 1 mM DPPH. Later, the plate was incubated in the dark at room temperature for 30 minutes. After incubation, the absorbency was at 517 nm using a microplate reader (SPECTROstar Nano, BMG Labtech, Germany). This assay was conducted in triplicate. The DPPH radical scavenging activity was calculated using Equation 1.

The DPPH scavenging activity of propolis extract was plotted against the concentration of samples and the IC_{50} value was obtained from the plots. The IC_{50} value is the concentration of the sample that can scavenge 50% of DPPH free radicals. Lower IC_{50} value indicating higher antioxidant efficiency.

$$\text{DPPH scavenging activity (\%)} = \frac{A_{blank} - A_{sample}}{A_{blank}} \text{ X } 100 \quad (1)$$

Statistical Analysis

The results were presented as mean ± standard deviation (SD) and analysed using one-way analysis of variance (ANOVA) followed by Tukey's test for variables with parametric distributions and Pearson's correlation coefficient test using GraphPad Prism version 7.0. Differences were considered significant when P value < 0.05.

Results

Total Phenolic and Flavonoid Contents

The TPC and TFC of propolis extracts are shown in Table 1. Figures 1 and 2 illustrate the TPC and TFC values of EEP and WEP. The gallic acid standard curve was used to calculate the TPC values with a regression line of $y = 0.0043x - 0.022$, ($r^2 = 0.9955$) and expressed in gallic acid equivalent (mg/mL GAE). The TPC of EEP varied in the range of 19.50 mg/mL GAE and 259.84 mg/mL GAE while WEP ranged between 18.06 mg/mL GAE and 138.91 mg/mL GAE. The results showed the highest TPC observed was from EEP of *G. thoracica* with 259.84 ± 4.97 mg/mL GAE, followed by EEP of *H. itama* with 157.91 ± 2.25 mg/mL GAE whereas WEP of *T. laeviceps* exhibits the lowest TPC with 18.06 ± 0.36 mg/mL GAE. A significant difference ($p < 0.05$) was noted in the TPC of EEP of *G. thoracica* and *H. itama* when compared with other samples.

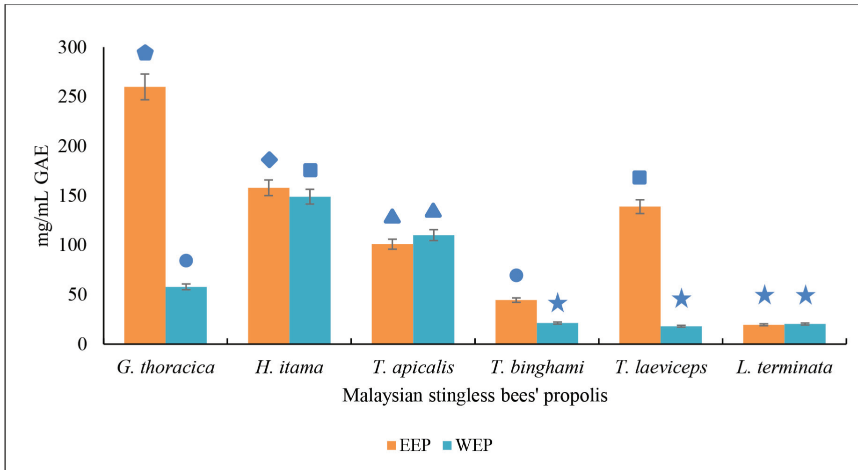
The TFC of propolis extracts was obtained from the regression equation of the standard plot ($y = 0.001x + 0.01$, $r^2 = 0.9994$) and expressed as quercetin equivalents (mg/mL QE). The TFC of EEP ranged from 15.33 to 435.00 mg/mL GAE while WEP ranged between 8.67 mg/mL GAE and 84.00 mg/mL GAE. The results showed that TFC was the highest in EEP of *G. thoracica* with 435.00 ± 6.57 mg/mL QE, followed by EEP of *H. itama* and *L. terminata* with 172.30 ± 4.56 mg/mL QE and 100.33 ± 0.51 mg/mL QE, respectively. Whereas WEP of *H. itama* exhibited the lowest TFC with 8.67 ± 0.51 mg/mL QE. A statistically significant difference ($p < 0.05$) was observed in the TFC of EEP of *G. thoracica, L. terminata* and *H. itama* when compared with other samples.

Data shown in the table below was collected from three replicates and presented as the mean ± standard deviation. Different letters in the same column indicate statistical significance at $p < 0.05$. Total phenolic content analysed as

Table 1: Total phenolic and flavonoid contents of ethanolic extract propolis (EEP) and water extract propolis (WEP) determined by Follin-Ciocalteu and aluminium nitrate colorimetric methods

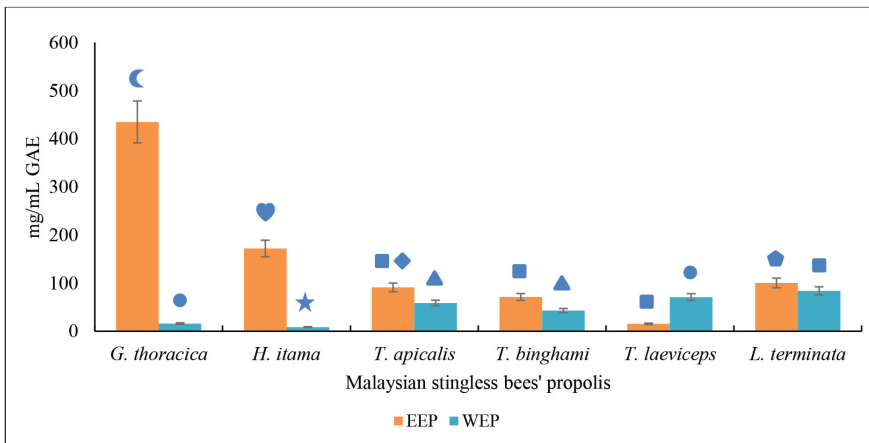
Propolis Samples		Total Phenolic Content (mg/mL GAE)	Total Flavonoid Content (mg/mL QE)
G. thoracica	EEP	259.84 ± 4.97^{f}	435.00 ± 6.57^{h}
	WEP	57.83 ± 0.32^{b}	16.00 ± 0.58^{b}
H. itama	EEP	157.91 ± 2.25^{e}	172.30 ± 4.56^{g}
	WEP	138.91 ± 2.21^{d}	8.67 ± 0.51^{a}
T. apicalis	EEP	100.93 ± 2.65^{c}	91.00 ± 3.72^{de}
	WEP	100.08 ± 0.72^{c}	58.67 ± 1.64^{c}
T. binghami	EEP	44.42 ± 1.14^{b}	71.30 ± 2.52^{d}
	WEP	21.24 ± 2.49^{a}	42.97 ± 2.22^{c}
T. laeviceps	EEP	138.84 ± 1.18^{d}	71.00 ± 3.98^{d}
	WEP	18.06 ± 0.36^{a}	15.33 ± 0.33^{b}
L. terminata	EEP	19.50 ± 0.59^{a}	100.33 ± 0.51^{f}
	WEP	19.27 ± 0.29^{a}	84.00 ± 4.48^{d}

Note: Data shown in the table were collected from three replicates and are presented as the mean ± standard deviation. Different letters in the same column indicate statistical significance at $p < 0.05$. Total phenolic content analysed as gallic acid equivalent (GAE) mg/g of extract and total flavonoid content as quercetin equivalent (QE) mg/g of extract



Note: Data shown in the graph were collected from three replicates and are presented as the mean ± standard deviation. Bars with different shapes are significant at $p < 0.05$

Figure 1: Comparison of TPC values of EEP and WEP of six different stingless bees



Note: Data shown in the graph were collected from three replicates and are presented as the mean ± standard deviation. Bars with different shapes are significant at $p < 0.05$

Figure 2: Comparison of TFC values of EEP and WEP of six different stingless bees

gallic acid equivalent (GAE) mg/g of extract, total flavonoid content as quercetin equivalent (QE) mg/g of extract.

Antioxidant Activity

The results in Table 2 demonstrate the DPPH scavenging activity and the IC_{50} values of EEP and WEP at concentrations between 7.813 and 500 µg/mL with quercetin as a standard reference. Figures 3 and 4 show the percentage of DPPH inhibition against the concentration of EEP and WEP of the stingless bees, respectively. In general, all tested extracts exhibited dose-dependent free radical scavenging activity except for WEP of *G. thoracica*, *T. binghami* and *T. laeviceps* which did not exhibit scavenging activity on DPPH assay. Among the tested samples, EEP of *G. thoracica* had the highest DPPH free radical scavenging activity (88.00 ± 13.34%) with IC_{50} of 104.20 µg/mL, followed by EEP of *H. itama* (84.00 ± 12.50%) with IC_{50}

of 159.60 µg/mL at a concentration of 250 µg/mL (Figure 3). However, the rest of the EEP including *T. apicalis, T. binghami, T. laeviceps* and *L. terminata* showed inactive antioxidant activity. As for WEP, *H. itama* showed weak antioxidant activity at IC_{50} of 332.70 µg/mL with 35.27 ± 4.42% of DPPH inhibition at a concentration of 500 µg/mL (Figure 4). Conversely, WEP of *G. thoracica*, *T. binghami* and *T. laeviceps* showed inactive antioxidant activity. The standard quercetin showed the best inhibitory effect against DPPH at 91.00 ± 13.19% with IC_{50} value of 20.51 µg/mL.

Table 2: Antioxidant activities of ethanolic extract propolis (EEP) and water extract propolis (WEP) expressed as IC_{50} values using DPPH free radical scavenging assay

Propolis Samples		**DPPH Inhibition (%)**	**IC_{50} DPPH (µg/mL)**
G. thoracica	EEP	88.00 ± 13.34	104.20
	WEP	-	-
H. itama	EEP	84.00 ± 12.50	159.60
	WEP	35.27 ± 4.42	332.70
T. apicalis	EEP	12.65 ± 1.75	850.00
	WEP	11.96 ± 1.84	882.80
T. binghami	EEP	9.64 ± 1.16	1165.00
	WEP	-	-
T. laeviceps	EEP	3.54 ± 3.54	1260.00
	WEP	-	-
L. terminata	EEP	4.20 ± 0.36	1009.00
	WEP	2.72 ± 0.34	1388.00
Quercetin		91.00 ± 13.19	20.51

Note: Data shown in the table were collected from three replicates and are presented as the mean ± standard deviation. "-" = No activity

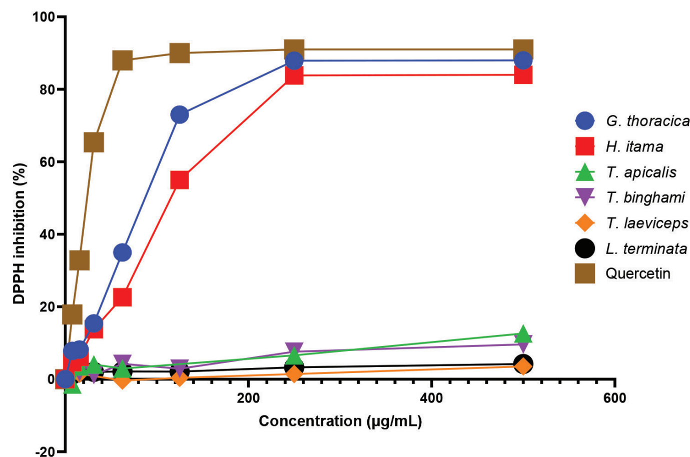


Figure 3: Percentage of DPPH inhibition against the concentration of EEP produced by different stingless bees

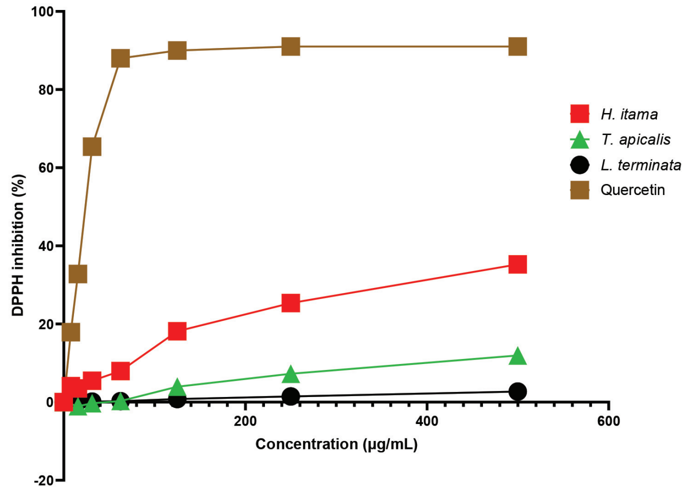


Figure 4: Percentage of DPPH inhibition against the concentration of WEP produced by different stingless bees

Correlation between Total Phenolic Content, Total Flavonoid Content and Antioxidant Activities

Correlation coefficients for TPC and TFC with DPPH assay presented as IC_{50} values are shown in Table 3, indicating flavonoids do not contribute much to the antioxidant activity. The results revealed that TPC was significantly and positively correlated to TFC ($R^2 = 0.804$). This finding clearly suggests that phenolic compounds are major constituents in the total phenolic content of propolis extracts. Furthermore, the TPC was significantly ($p < 0.05$) negatively correlated to IC_{50} DPPH with R^2 value of -0.735 indicating the TPC may be responsible for the antioxidant activity of the propolis extracts. However, there was no significant correlation between TFC and IC_{50} DPPH ($R^2 = -0.359$, $p > 0.05$), indicating flavonoids do not contribute much to the antioxidant activity.

Discussion

Propolis has been used empirically for many centuries to alleviate ailments such as gastrointestinal disorders, allergies, oral and dermatological problems (Fikri *et al.*, 2019). The medicinal properties of propolis have been ascribed to the presence of various chemical components such as flavonoids (apigenin, quercetin, pinocembrin and pinobanksin), phenolics (coumarin and scopoletol), terpenes (geraniol, nerolidol and farnisol) and ketones (acetophenone). Studies have shown that propolis is a potential source of natural antioxidants owing to its chemical components particularly phenolic acid derivatives and flavonoids (Kocot

Table 3: Pearson's correlation coefficient (R^2) of total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activity (IC_{50} of DPPH) of propolis extracts

Assays	Correlation (R^2)		
	TPC	TFC	IC_{50} DPPH
TPC	1	0.804*	- 0.735*
TFC	0.804*	1	- 0.359
IC_{50} DPPH	- 0.735*	- 0.359	1

Note: *Correlation is significant at p < 0.05

et al., 2018). Polyphenols are the major chemical compounds found in propolis which attribute to its antioxidant activity (Zulhendri *et al*., 2021).

Propolis cannot be used in its raw form and thus must be purified and extracted using suitable solvents to remove the inert substance while preserving the polyphenol compounds (Galeotti *et al*., 2018). Furthermore, extraction techniques and type of solvents used play a significant role in determining the phytochemical components responsible for the biological activities of the propolis (Yıldırım, 2022). Results from this study indicate that EEP contained higher TPC and TFC than WEP. Thus, according to the results, ethanol extraction solvents were more effective in extracting phenolics than water solvents. This was in line with the findings of Mokhtar *et al.* (2019) who demonstrated that ethanolic extracts of propolis gave the highest TPC and TFC compared with water extracts. Ethanol is an organic solvent that extracts most of the phytochemical components present in the propolis which are mostly lipophilic molecules and this could contribute to the higher TPC and TFC levels seen in the EEP as compared with WEP (Mokhtar *et al*., 2019). In contrast, water is a polar solvent that does not favour the extraction of less polar compounds such as phenolics which results in lower concentrations of phenolic compounds (Kubiliene *et al*., 2015).

The present study also demonstrated the EEP of *G. thoracica* possesses the highest TPC and TFC compared to that of the other bee species evaluated. Similar findings were reported by Asem *et al*. (2019) who demonstrated that the propolis of *G. thoracica* had the highest TPC and TFC (55.16 ± 7.52 and 326.10 ± 4.94 µM, respectively), followed by *H. itama* (34.17 ± 1.52 and 324.04 ± 5.18 µM, respectively) and *T. apicalis* (28.57 ± 3.17 and 135.93 ± 5.95 µM, respectively). The results indicated that bee species contribute to the different chemical compositions of propolis which is consistent with the report of Abdullah *et al.* (2020). In contrast, Ibrahim *et al.* (2016) showed that the propolis of the *G. thoracica* had lower TPC and TFC levels (29.1 ± 0.10 and 61.5 ± 0.15 µg/mL, respectively) compared with the propolis of *H. itama* (56.9 ± 0.12 and 163.9 ± 0.10 µg/mL, respectively). The differences in chemical constituents may be due to several factors such as origin and pollen foraging activities of the stingless bees that contribute significantly to the chemical diversity of the propolis (Nafi *et al.*, 2019).

The amount of phenolics and flavonoids in propolis extract confers a significant contribution to its antioxidant activity (Galeotti *et al*., 2018). Various classes of phenolic compounds and its derivatives were detected in propolis including phenylpropanoid, caffeic acid, isoferulic acid, chlorogenic acid derivatives, ellagic acid, ρ-coumaric acid, gallic acid, quercetin, aromadendrin, benzoic acid and anacardic acid (Huang *et al*., 2014; Al-Hatamleh *et al*., 2020). Flavonoids that are present in propolis can be classified into several categories such as flavones, flavonols, flavanonols, chalcones, flavans, isoflavans and neoflavonoids (Huang *et al*., 2014). Flavonoids mostly occur in sugar-conjugated forms, predominantly as quercetin glycoside (Zheng *et al*., 2017). These compounds are capable of scavenging free radicals by donating hydrogen ions to the free radicals such as peroxide, hydroperoxide or lipid peroxyl which are produced either during the metabolic process or external factors (Vendemiale *et al*., 1999; Lobo *et al*., 2010). Oxidative stress occurs when free radical and antioxidant levels are imbalanced eventually causing a decline in endogenous antioxidants defence and an incline in the production of reactive oxygen species (ROS) (Yusop *et al*., 2019; Seyidoglu & Aydin, 2020). High levels of free radicals in the body may attack DNA, proteins and lipids, resulting in damage to cell structures and genetic material (Yusop *et al*., 2019).

In this study, the antioxidant property of propolis extracts was determined by the DPPH free radical scavenging assay which is based on electron transfer and hydrogen atom transfer reactions (Prior *et al*., 2005). This method offers advantages of being rapid, simple inexpensive and provides initial information on the

antioxidant capacity of the test sample (Kedare & Singh, 2011). When DPPH is stable free radical appear as deep violet colour (Liang & Kitts, 2014). When DPPH reacts with an antioxidant compound, its free radical property is lost and its colour changes from violet to pale yellow (Gonçalves *et al*., 2018). The antioxidant power of a sample was measured by calculating the IC_{50} value which is described as the concentration required to inhibit and capture 50% of the DPPH free radical. The lower IC_{50} value indicates higher radical scavenging activities (Ibrahim *et al*., 2016). The present study demonstrates that the EEP of *G. thoracica* had the greatest DPPH free radical scavenging activity with percentage of scavenging of 88% and IC_{50} of 106.20 µg/mL. The results revealed that EEP of *G. thoracica* possessed the strongest antioxidant activity due to its high radical scavenging activities. Asem *et al*. (2019) reported similar findings in which ethanolic propolis extract of *G. thoracica* exhibited the highest DPPH radical activity compared to *T. apicalis* and *H. itama*.

In general, higher TPC and TFC levels indicate higher antioxidant activity levels (Pratami *et al*., 2018). Rosli *et al*. (2016) demonstrated a strong association between TFC and TPC against DPPH scavenging activities, indicating the antioxidant activity of the ethanolic extract of the propolis was influenced by its phenolic and flavonoid contents. These findings were supported by Ibrahim *et al*. (2016) and Fikri *et al*. (2019) who demonstrated that higher TPC and TFC levels contributed to the higher DPPH free radical scavenging properties of the propolis. However, in the present study, only TPC was significantly negatively correlated to the DPPH assay as expressed in IC_{50} value (R^2 value of -0.785, $p < 0.05$). Therefore, it can be said high levels of phenolic contents in the propolis contribute significantly to its antioxidant property. Phenolic compounds are excellent antioxidants owing to the vast conjugated π-electron systems that aid the donation of electrons from the hydroxyl moieties to oxidising radical species (Bittencourt *et al*., 2015).

Conclusion

In conclusion, ethanolic extract of *G. thoracica* propolis possessed more phenolic and flavonoid contents and exhibited the highest antioxidant activity as compared to other propolis extracts, suggesting its potential use as a natural source of antioxidants. However, further studies into the isolation, purification, and identification of the active phytochemical compounds responsible for its antioxidant activity are warranted.

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References

Abdullah, N. A., Zullkiflee, N., Zaini, S. N. Z., Taha, H., Hashim, F., & Usman, A. (2020). Phytochemicals, mineral contents, antioxidants, and antimicrobial activities of propolis produced by Brunei stingless bees *Geniotrigona thoracica, Heterotrigona itama*, and *Tetrigona binghami*. *Saudi Journal of Biological Sciences*, *27*(11), 2902-2911. https://doi.org/10.1016/j.sjbs.2020.09.014

Ahangari, Z., Naseri, M., & Vatandoost, F. (2018). Propolis: Chemical composition and its applications in endodontics. *Iranian Endodontic Journal*, *13*(3), 285-292. https://doi.org/10.22037/iej.v13i3.20994

Al-Hatamleh, M. A. I., Boer, J. C., Wilson, K. L., Plebanski, M., Mohamud, R., & Mustafa, M. Z. (2020). Antioxidant-based medicinal properties of stingless bee products: Recent progress and future directions. *Biomolecules*, *10*(6), 1-28. https://doi.org/10.3390/biom10060923

Asem, N., Abdul Gapar, N. A., Abd Hapit, N. H., & Omar, E. A. (2019). Correlation between total phenolic and flavonoid contents with antioxidant activity of Malaysian stingless bee propolis extract. *Journal of Apicultural*

Research, *59*(4), 437-442. https://doi.org/10.1080/00218839.2019.1684050

Bittencourt, M. L. F., Ribeiro, P. R., Franco, R. L. P., Hilhorst, H. W. M., Castro, R. D. de, & Fernandez, L. G. (2015). Metabolite profiling, antioxidant and antibacterial activities of Brazilian propolis: Use of correlation and multivariate analyses to identify potential bioactive compounds. *Food Research International*, *76*, 449-457. https://doi.org/10.1016/j.foodres.2015.07.008

Devequi-Nunes, D., Machado, B. A. S., Barreto, G. de A., Silva, J. R., Silva, D. F. da, Rocha, J. L. C. da, Brandao, H. N., Borges, V. M., & Umsza-Guez, M. A. (2018). Chemical characterization and biological activity of six different extracts of propolis through conventional methods and supercritical extraction. *PLOS ONE*, *13*(12), 1-20. https://doi.org/10.1371/journal.pone.0207676 December

Farasat, M., Khavari-Nejad, R.-A., Mohammad, S., Nabavi, B., & Namjooyan, F. (2014). Antioxidant activity, total phenolics and flavonoid contents of some edible green seaweeds from northern coasts of the Persian Gulf. In *Iranian Journal of Pharmaceutical Research*, *13*(1), 163-170.

Fikri, A. M., Sulaeman, A., Marliyati, S. A., & Fahrudin, M. (2019). Antioxidant activity and total phenolic content of stingless bee propolis from Indonesia. *Journal of Apicultural Science*, *63*(1), 139-147. https://doi.org/10.2478/JAS-2019-0012

Galeotti, F., Maccari, F., Fachini, A., & Volpi, N. (2018). Chemical composition and antioxidant activity of propolis prepared in different forms and in different solvents useful for finished products. *Foods*, *7*(3). https://doi.org/10.3390/foods7030041

Gonçalves, A. C., Rodrigues, M., Santos, A. O., Alves, G., & Silva, L. R. (2018). Antioxidant status, antidiabetic properties and effects on Caco-2 cells of colored and non-colored enriched extracts of sweet cherry fruits. *Nutrients*, *10*(11). https://doi.org/10.3390/nu10111688

Huang, S., Zhang, C., Wang, K., Li, G. Q., & Hu, F. (2014). The effects of wine-processing on ascending and descending: The distribution of flavonoids in rat tissues after oral administration of crude and wine-processed. *Radix scutellariae*. *Journal of Ethnopharmacology, 155*(1), 649-664. https://doi.org/10.1016/j.jep.2014.05.063

Ibrahim, N., Zakaria, A. J., Ismail, Z., & Mohd, K. S. (2016). Antibacterial and phenolic content of propolis produced by two Malaysian stingless bees, *Heterotrigona itama* and *Geniotrigona thoracica*. *International Journal of Pharmacognosy and Phytochemical Research*, *8*(1), 156-161.

Kedare, S. B., & Singh, R. P. (2011). Genesis and development of DPPH method of antioxidant assay. *Journal of Food Science and Technology*, *48*(4), 412-422. https://doi.org/10.1007/s13197-011-0251-1

Kocot, J., Kiełczykowska, M., Luchowska-Kocot, D., Kurzepa, J., & Musik, I. (2018). Antioxidant potential of propolis bee pollen, and royal jelly: Possible medical application. *Oxidative Medicine and Cellular Longevity*, *2018*, 1-29. https://doi.org/10.1155/2018/7074209

Kubiliene, L., Jekabsone, A., Zilius, M., Trumbeckaite, S., Simanaviciute, D., Gerbutaviciene, R., & Majiene, D. (2018). Comparison of aqueous, polyethylene glycol-aqueous and ethanolic propolis extracts: Antioxidant and mitochondria modulating properties. *BMC Complementary and Alternative Medicine*, *18*(165), 1-10. https://doi.org/10.1186/s12906-018-2234-5

Kubiliene, L., Laugaliene, V., Pavilonis, A., Maruska, A., Majiene, D., Barcauskaite, K., Kubilius, R., Kasparaviciene, G., & Savickas, A. (2015). Alternative preparation of propolis extracts: Comparison of their composition and biological activities. *BMC*

Complementary and Alternative Medicine, *15*(156), 1-7. https://doi.org/10.1186/s12906-015-0677-5

Liang, N., & Kitts, D. D. (2014). Antioxidant property of coffee components: Assessment of methods that define mechanism of action. *Molecules*, *19*(11), 19180-19208. https://doi.org/10.3390/molecules191119180

Lobo, V., Patil, A., Phatak, A., & Chandra, N. (2010). Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacognosy Reviews*, *4*(8), 118-126. https://doi.org/10.4103/0973-7847.70902

Mokhtar, S. U., Hooi, ng S., T Lene, D. T., & Jayaraman, S. (2019). Comparison of total phenolic and flavonoids contents in Malaysian propolis extract with two different extraction solvents. *International Journal of Engineering Technology and Sciences*, *6*(2), 1-11. https://doi.org/10.15282/IJETS.V6I2.2577

Nafi, N. E. M., Zin, N. B. M., Pauzi, N., Khadar, A. S. A., Anisava, A. R., Badiazaman, A. A. M., & Mohd, K. S. (2019). Cytotoxicity, antioxidant and phytochemical screening of propolis extracts from four different Malaysian stingless bee species. *Malaysian Journal of Fundamental and Applied Sciences*, *15*(2-1), 307-312. https://doi.org/10.11113/mjfas.v15n2-1.1542

Pasupuleti, V. R., Sammugam, L., Ramesh, N., & Gan, S. H. (2017). Honey, propolis, and royal jelly: A comprehensive review of their biological actions and health benefits. *Oxidative Medicine and Cellular Longevity*, *2017*, 1-22. https://doi.org/10.1155/2017/1259510

Pobiega, K., Kraśniewska, K., Derewiaka, D., & Gniewosz, M. (2019). Comparison of the antimicrobial activity of propolis extracts obtained by means of various extraction methods. *Journal of Food Science and Technology*, *56*(12), 5386-5395. https://doi.org/10.1007/S13197-019-04009-9/TABLES/3

Pratami, D. K., Mun'im, A., Sundowo, A., & Sahlan, M. (2018). Phytochemical profile and antioxidant activity of propolis ethanolic extract from Tetragonula bee. *Pharmacognosy Journal*, *10*(1), 128-135. https://doi.org/10.5530/pj.2018.1.23

Prior, R. L., Wu, X., & Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry*, *53*(10), 4290-4302. https://doi.org/10.1021/jf0502698

Rosli, N. L., Roslan, H., & Omar, A. (2016). Phytochemical analysis and antioxidant activities of *Trigona apicalis* propolis extract. *AIP Conference Proceedings*, *1791*, 20018. https://doi.org/10.1063/1.4968873

Sahlan, M., Mahira, K. F., Wiratama, I., Mahadewi, A. G., Yohda, M., Hermansyah, H., & Noguchi, K. (2019). Purification and characterization of proteins in multifloral honey from kelulut bee (stingless bee). *Heliyon*, *5*(11). https://doi.org/10.1016/j.heliyon.2019.e02835

Salleh, S. N. A. S., Mohd Hanapiah, N. A., Ahmad, H., Wan Johari, W. L., Osman, N. H., & Mamat, M. R. (2021). Determination of total phenolics, flavonoids, and antioxidant activity and GC-MS analysis of Malaysian stingless bee propolis water extracts. *Scientifica*, *2021*, 1-11. https://doi.org/10.1155/2021/3789351

Samsudin, S. F., Mamat, M. R., & Hazmi, I. R. (2018). Taxonomic study on selected species of stingless bee (Hymenoptera: Apidae: Meliponini) in Peninsular Malaysia. *Serangga*, *23*(2), 203-258.

Seyidoglu, N., & Aydin, C. (2020). Stress, natural antioxidants and future perspectives. In *The Health Benefits of Foods - Current Knowledge and Further Development* (pp. 1-17). https://doi.org/10.5772/intechopen.91167

Siripatrawan, U., Vitchayakitti, W., & Sanguandeekul, R. (2013). Antioxidant and antimicrobial properties of Thai propolis extracted using ethanol aqueous solution. *International Journal of Food Science and Technology*, *48*(1), 22-27. https://doi.org/10.1111/j.1365-2621.2012.03152.x

Vendemiale, G., Grattagliano, I., & Altomare, E. (1999). An update on the role of free radicals and antioxidant defense in human disease. *International Journal of Clinical and Laboratory Research*, *29*(2), 49-55. https://doi.org/10.1007/s005990050063

Yıldırım, H. K. (2022). Assessment of propolis treated by different extraction methods. *Brazilian Archives of Biology and Technology*, *65*, 1-11. https://doi.org/10.1590/1678-4324-2022210251

Yusop, S. A. T. W., Sukairi, A. H., Sabri, W. M. A. W., & Asaruddin, M. R. (2019). Antioxidant, antimicrobial and cytotoxicity activities of propolis from Beladin, Sarawak stingless bees *Trigona itama* extract. *Materials Today: Proceedings*, *19*, 1752-1760. https://doi.org/10.1016/j.matpr.2019.11.213

Zheng, Y., Deng, G., Liang, Q., Chen, D., Guo, R., & Lai, R. (2017). Antioxidant activity of quercetin and its glucosides from propolis: A theoretical study. *Scientific Reports*, *7*, 1-11. https://doi.org/10.1038/s41598-017-08024-8

Zulhendri, F., Felitti, R., Fearnley, J., & Ravalia, M. (2021). The use of propolis in dentistry, oral health, and medicine: A review. *Journal of Oral Biosciences*, *63*(1), 23-34. https://doi.org/10.1016/j.job.2021.01.001