

# Phytochemical Screening and Antioxidant Activities of *Geniotrigona thoracica* Propolis Extracts Derived from Different Locations in Malaysia

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**Abstract** Propolis, a natural resinous substance secreted by bees, has garnered considerable interest due to its diverse bioactive compounds and potential health benefits. Nevertheless, the phytochemical composition of propolis exhibits significant variation, influenced by multiple factors including geographical region, and botanical origin. These determinants exert profound effects on the distinctive properties and biological diversities of propolis. This study aimed to investigate the phytochemical composition and antioxidant activities of *Geniotrigona thoracica* propolis extracts collected from three apiary sites, designated as apiary A, apiary B, and apiary C, located in different regions within Selangor. The ethanolic extracts of propolis were prepared using 70% of ethanol and subjected to phytochemical screening to identify the presence of flavonoids, terpenoids, alkaloids, saponins, tannins, steroids, and cardiac glycosides, whilst the total phenolic content (TPC) and total flavonoid content (TFC) were measured using the Folin-Ciocalteu colorimetric and aluminium chloride methods, respectively. Additionally, the antioxidant activities of the propolis extracts were evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and ferric-reducing antioxidant power (FRAP) assays. The phytochemical screening revealed the presence of flavonoids, terpenoids, alkaloids, saponins, tannins, steroids, and cardiac glycosides in all propolis extracts. The propolis from apiary A exhibited significantly higher TPC ( $302.21 \pm 0.11$  mg/mL GAE) and TFC values ( $99.08 \pm 0.03$  mg/mL QE) compared to apiary B and C. The results also indicated that propolis from apiary A possessed significantly higher antioxidant activities, with  $IC_{50}$  value of DPPH at  $25.27 \mu\text{g/mL}$  and FRAP value of  $727.53 \pm 0.09 \mu\text{M Fe}^{2+}$ , in comparison to apiary B and C. A strong correlation was observed between TPC, TFC, and  $IC_{50}$  of DPPH. This study highlights significant variations in the phytochemical compositions and antioxidant activities of propolis samples collected from different geographical and botanical sources. Further investigation is in progress to identify the specific phytochemical constituents responsible for these variations.

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## Introduction

Stingless bee is the largest group of eusocial bees, with more than 500 documented species found in tropical, and subtropical regions of the world [1]. It belongs to the family Apidae and is known for its abilities to produce propolis, aside from honey and bee pollen [2]. Stingless bees produce propolis to repair beehives that acts as a natural defense mechanism against predators and microbial infections [3].

Due to its diverse and complex chemical composition, propolis exhibits various pharmacological activities, such as antioxidant, antibacterial, anticancer, antifungal, anti-inflammatory, antiviral, and antidiabetic effects [4]. *Geniotrigona thoracica* is one of the commonly cultivated stingless bee species in the Malaysian meliponiculture industry [5]. It has distinct physical characteristics with brown in colour and larger body size, setting it apart from other stingless bee species [6]. *G. thoracica* is endemic to Malaysia and has been reported to produce a large amount of propolis with complex chemical constituents, in comparison to other stingless bee species [7, 8]. Additionally, a study by Adli *et al.* [9] demonstrated that ethanolic extract derived from *G. thoracica* exhibited the highest antioxidant activity compared to propolis produced by *Heterotrigona itama*, *Tetrigona apicalis*, *Trigona binghami*, *Tetragonula laeviceps*, and *Lepidotrigona terminata*. Stingless bee propolis exhibits antioxidant activity that can counteract, and repair cellular damage induced by free radicals [10]. These free radicals are constantly generated within human bodies during regular metabolic processes and can impair cellular function, potentially initiating the development of pathological conditions such as cardiovascular disease, cancer, and neurological disorders [11].

Several antioxidant assays are widely utilized to assess antioxidant capacities of various extracts, including two well-established methods such as 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric ion reducing antioxidant potential (FRAP) [12]. The DPPH assay assesses the capacity of antioxidants to scavenge free radicals, while the FRAP assay measures the ability of antioxidants to reduce ferric ions [12]. Both methods offer a comprehensive assessment of the antioxidant activity of a sample through distinct mechanisms [13]. As different antioxidants can act through a range of mechanisms of action to counteract oxidative stress and protect cells and tissues, utilizing both the DPPH and FRAP assays can provide a more comprehensive understanding of the antioxidant activity of a sample, as these assays capture various aspects of antioxidant functionality [14].

Higher antioxidant activity in propolis is attributed to its complex chemical compounds, including polyphenols, terpenoids, amino acids, aldehyde, and steroids [15]. Polyphenol compounds are considered as the main biologically active substances in propolis, contributing to its various pharmacological activities, such as antioxidant, anti-inflammatory, antimicrobial, and anticancer properties [19]. Polyphenols are a diverse group of phytochemical compounds that contain multiple phenolic groups such as phenolic acids and flavonoids [17]. Phenolic acids contain one or more hydroxyl groups attached to an aromatic ring, whereas flavonoids comprise of 15 carbons atoms, organized in two aromatic rings connected by a three-carbon bridge [18]. These molecules are important antioxidant compounds based on their ability to donate hydrogen atoms to free radicals and directly scavenge reactive oxygen free radicals, as well as chelating metal ions [19].

The chemical composition of propolis is greatly impacted by factors such as geographical location, climate, and floral sources, which exert significant effects on its biological diversities [20]. Moreover, native vegetation surrounding the beehives have been reported to influence the composition and chemical content of propolis, as specific flowers in the immediate vicinity of the beehives can contribute to the phytochemicals and bioactive compounds of the stingless bee propolis [4]. Hence, the aim of this study was to determine the phytochemical composition and antioxidant activities of *G. thoracica* propolis extracts obtained from three different locations. Additionally, the correlation between the phenolic and flavonoid contents with the antioxidant property was also evaluated.

## Materials and Methods

### Propolis Collection

Raw propolis produced by *G. thoracica* were collected in July 2022 from three different apiaries with varying botanical sources and assigned as apiary A, B, and C. Apiary A is located in Serdang, Selangor (N 2° 58' 45.84" E 101° 41' 51.72"), predominantly surrounded by medicinal plants from the *Simaroubaceae*, *Myrsinaceae*, *Primulaceae*, *Zingiberaceae*, *Acanthaceae* and *Lamiaceae* families. Apiary B, located in Shah Alam, Selangor (N 3° 3' 46.08" E 101° 32' 2.4"), features a landscape dominated by ornamental trees specifically *Malvaceae*, *Fabaceae*, and *Combretaceae*, along with fruit trees from the *Rutaceae* and *Sapindaceae* families. Meanwhile, apiary C, situated in Hulu Bernam, Selangor (N 3° 40' 42.1818" E 10° 31' 14.5416"), is distinctly characterized by an abundance presence of ornamental trees, notably from the *Myrtaceae*, *Fabaceae*, *Scrophulariaceae*, *Lythraceae*, *Elaeocarpaceae* families, in addition to fruit trees including *Moraceae*, *Anacardiaceae*, *Sapindaceae*. Notably, the propolis samples from apiary C were collected from the same location as reported by Adli *et al.* [9]. The propolis samples were cleaned, placed in labelled plastic bags, and stored at -20 °C until further analysis.

## Preparation of Ethanolic Extract Propolis

The ethanolic extract of propolis was prepared according to the method described by Pobiega *et al.* [21] with minor modifications. Briefly, 10 g of each propolis sample was ground into powder and dissolved in 100 mL of 70% ethanol at a 1:10 (w/v) ratio. The samples were then incubated at 25 °C and shaken at 250 rpm for 48 hours. The suspensions were filtered through Whatman No. 1 filter paper (Millipore, USA). The filtrate was evaporated under vacuum pressure (Rotavapor R-215, Büchi, Switzerland) at 995 hPa and 30 °C. The concentrated extracts were kept in a freezer at -18 °C overnight. The extract was centrifuged at 5000 rpm (10 °C) for 11 minutes to eliminate wax. The propolis extract was then freeze-dried at -110 °C and stored at -20 °C.

## Phytochemical Screening of Propolis Extracts

Phytochemical screening was performed to identify the presence of diverse phytoconstituents in the ethanolic extract of propolis. Specific chemical reagents were prepared for individual screening tests, qualitatively assessing the existence of secondary metabolites such as flavonoids, terpenoids, alkaloids, saponins, tannins, steroids, and cardiac glycosides. The screening procedures followed the method outlined by Sharma *et al.* [22] with minor modifications. The respective methods for each screening test were as follows:

### Test for Flavonoids

1 mg/mL of ethanol extract of propolis was transferred to each test tube. Subsequently, few drops of 1% sodium hydroxide (NaOH) were added, followed by the addition of 1% hydrochloric acid (HCl). In the presence of flavonoids, the intense yellow colour changes to colourless.

### Test for Terpenoids

1 mg/mL of the ethanol extract of propolis was transferred to each test tube. Then, 1 mL of 2% HCl was added, followed by 1 mL of Dragendorff's reagent. The reddish-brown colour of the solution indicated the presence of terpenoids.

### Test for Alkaloids

1 mg/mL of the ethanol extract of propolis was transferred to each test tube. Then, 0.5 mL of chloroform was added, followed by few drops of concentrated sulfuric acid (H<sub>2</sub>SO<sub>3</sub>). The reddish-brown colour of the solution indicated the presence of terpenoids.

### Test for Saponins

1 mg/mL of the ethanol extract of propolis was added into 5 ml of distilled water (dH<sub>2</sub>O), then heated in water bath for two minutes. The mixture was vigorously shaken, and the formation of frothing indicated the presence of saponins.

### Test for Tannins

1 mg/mL of the ethanol extract of propolis was added to 5 ml of dH<sub>2</sub>O and heated in water bath with for two minutes. Subsequently, 1% ferric chloride (FeCl<sub>3</sub>) was added dropwise. The change in colour to green indicated the presence of tannins.

### Test for Steroids

1 mg/mL of the ethanol extract of propolis was transferred into each test tube. Subsequently, a few drops of concentrated acetic acid were added, followed by the addition of a drop of concentrated sulfuric acid (H<sub>2</sub>SO<sub>3</sub>). The upper layer turned reddish-brown, indicating that the extract contained steroids.

### Test for Cardiac Glycosides

1 mg/mL of the ethanol extract of propolis was transferred into each test tube. Subsequently, 2 ml of glacial acetic acid was added, along with one drop of 1% FeCl<sub>3</sub>. The formation of a violet or greenish colour indicated the presence of cardiac glycosides.

### Determination of Total Phenolic Content (TPC)

The total phenolic content (TPC) of propolis extracts was determined using Folin-Ciocalteu method, with gallic acid serving as the standard solution [23]. Briefly, 1 mL of gallic acid was prepared at eight different concentrations (5, 50, 75, 100, 250, 500, 750, and 1000 µg/mL). Then, 25 µL of ethanol extract of propolis

and the standard solutions were mixed with 100  $\mu\text{L}$  of 25% Folin reagent in a 96-well plate, incubated, and shaken for four minutes at room temperature. Subsequently, 75  $\mu\text{L}$  of 7.5% sodium carbonate was added to the reaction mixture, shaken for 60 s, and incubated at room temperature for two hours. The absorbance was measured at 765 nm using a microplate reader. The results of the regression line obtained from the standard curve were used to calculate the TPC value and were expressed as mg/mL Gallic Acid Equivalent (GAE). The test was performed in triplicate.

### Determination of Total Flavonoid Content (TFC)

The total flavonoid content (TFC) of propolis extracts was determined using the aluminium chloride colorimetric method, with quercetin used as the standard solution [24]. Briefly, 1 mL of quercetin at eight different concentrations (5, 50, 75, 100, 250, 500, 750, and 1000  $\mu\text{g}/\text{mL}$ ) were prepared to obtain the standard curve. Then, 20  $\mu\text{L}$  of prepared standard solution and 1 mg/mL of propolis extracts will be added into a 96-well plate, mixed with 20  $\mu\text{L}$  of 10% aluminium chloride solution, 20  $\mu\text{L}$  of 1M potassium acetate, and 140  $\mu\text{L}$  distilled water. The plate was shaken continuously for 1 minute before incubation in the dark for 30 minutes at room temperature. The absorbance was measured at 415 nm using a microplate reader. The TFC value was calculated using a linear regression line plotted on the standard curve and expressed as mg/mL Quercetin Equivalent (QE). The test was performed in triplicate.

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

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical-scavenging activity of propolis extracts was determined following the method outlined by Nafi *et al.* [25]. Briefly, a 1 mM DPPH solution was prepared by diluting 5 mg of DPPH solution in 100 mL of methanol. Quercetin was used as reference standard. Different concentrations of propolis extract and standard quercetin were prepared (7.8125, 15.625, 31.25, 62.5, 125, 250, and 500  $\mu\text{g}/\text{mL}$ ) using a two-fold serial dilution method with dimethyl sulfoxide (DMSO) as the solvent. Approximately 25  $\mu\text{L}$  of standard and sample solutions were added to a 96-well plate. Then, DPPH solution (200  $\mu\text{M}$ ) was added to each well and mixed. A negative blank solution was prepared by mixing 200  $\mu\text{L}$  of 1 mM DPPH with 25  $\mu\text{L}$  DMSO. The plate was then incubated at room temperature in the dark for 30 minutes. After incubation, the absorbance was measured at 517 nm using a microplate reader, and the experiment was repeated in triplicate. DPPH radical scavenging activity was calculated using equation (1). The radical scavenging activities of propolis extracts were interpreted using  $\text{IC}_{50}$  values. The  $\text{IC}_{50}$  is a concentration that has the ability to scavenge the 50% of DPPH free radical.

$$\text{Inhibition (\%)} = \frac{(\text{Abs of blank} - \text{Abs of sample})}{\text{Abs of blank}} \times 100\% \quad (1)$$

### Ferric Reducing Antioxidant Power (FRAP) Assay

The ferric reducing ability of the propolis extracts was determined following the method described by Zarate *et al.* [26] with slight modifications. The FRAP reagent, consisting of three solutions, was prepared as follows: solution 1 (300  $\mu\text{M}$  pH 3.8 acetate buffer) was prepared by diluting 3.12 g of sodium acetate anhydrous ( $\text{C}_2\text{H}_3\text{NaO}_2$ ) and 1.6 mL of glacial acetic acid ( $\text{CH}_3\text{COOH}$ ) in 1 L of distilled  $\text{H}_2\text{O}$ . For solution 2, 0.031 g of 2,4,6-Tripyridyl-S-triazine (TPTZ) solution was dissolved in 10 mL of 37% HCl, and 1.46 mL of HCl (36.46 mol) was added to 1 L of distilled  $\text{H}_2\text{O}$ . Solution 3 (20mM of Iron (III) chloride hexahydrate ( $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ ), was prepared by dissolving 0.054 g of  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  in 10 mL of distilled  $\text{H}_2\text{O}$ . The working FRAP reagent was prepared by mixing all 3 solutions, heated at 37  $^\circ\text{C}$  for 10 minutes before use. Then, 30  $\mu\text{L}$  of ethanol extract of propolis was added successively. The resulting mixture was allowed to rest for 30 minutes at 20  $^\circ\text{C}$  and was protected from light. The absorbance was read at 593 nm using a microplate reader spectrophotometer. All measurements were performed in triplicate. The results were calculated using a calibration curve of ferrous sulphate as the standard.

### Statistical Analysis

The results were expressed as mean  $\pm$  standard deviation (SD). The data were analysed using one-way Analysis of Variance (ANOVA), followed by Tukey test and Pearson's correlation coefficient test using GraphPad Prism version 7.0 Software (CA, USA). Differences between the experimental groups were considered significant if  $P < 0.05$ .

## Results and Discussion

### Phytochemical Screening of Propolis Extracts

The results of the phytochemical screening obtained during the experiment are shown in Table 1.

**Table 1.** Qualitative analysis of phytochemical screening of ethanol extracts of propolis from three different apiary sites

Phytochemical compounds	<i>G. thoracica</i>		
	Apiary A	Apiary B	Apiary C
Flavonoids	+++	+++	+++
Terpenoids	+++	+++	+++
Alkaloids	+++	+++	+++
Saponins	+++	+++	+++
Tannins	++	++	+++
Steroids	+++	+++	+++
Cardiac Glycosides	+++	++	++

Key: "+": present; "++": moderately present; "+++": highly present; "-": absent.

Phytochemical screening serves as a straightforward analytical approach to classify the diversity of secondary metabolites found in plants and natural products, with the potential to unveil compounds valuable for drug discovery [22]. This method offers a preliminary information into the biological active compounds within the tested sample [7]. In this study, the results indicated the presence of seven metabolic groups consisting of flavonoids, terpenoids, alkaloids, saponins, tannins, steroids, and cardiac glycosides, in all ethanolic propolis extracts (Table 1). These findings are in accordance with a study reported by Usman *et al.* [27] who reported the presence flavonoids, terpenoids, alkaloids, saponins, tannins, and cardiac glycosides in ethanolic extract of Malaysian propolis. However, Ibrahim *et al.* [7] noted the presence of flavonoids and terpenoids in the methanolic extract of *G. thoracica* propolis, with the absence of saponins and steroids. In contrast, Badiazaman *et al.* [28] found that the methanolic extract of *G. thoracica* propolis contained terpenoids, flavonoids, coumarins, essential oils, unsaturated and aromatic compounds. These phytochemical constituents play specific roles in enhancing therapeutic activities, such as antioxidant, anti-inflammatory, and antimutagenic effects [29]. However, it is noteworthy that differences in the phytochemical composition of propolis are significantly influenced by factors such geographical origin, botanical sources, and the solvent used for sample extraction [30].

### Total Phenolic and Flavonoid Content

The TPC and TFC of ethanolic propolis extracts are presented in Table 2. The gallic acid standard curve was employed to calculate the TPC values with a regression line of  $y = 0.0024x + 0.0477$ , ( $R^2 = 0.993$ ). The highest TPC value is observed in ethanolic extracts of propolis from apiary A ( $302.21 \pm 0.11$  mg/mL GAE), which is significantly different from apiary B ( $156.79 \pm 0.06$  mg/mL GAE), and apiary C ( $111.38 \pm 0.06$  mg/mL GAE). In contrast, Asem *et al.* [31] demonstrated a lower TPC value of  $55.16 \pm 7.52$  mg/mL GAE for Malaysian ethanolic *G. thoracica* propolis extracts. However, Adli *et al.* [9] demonstrated that the ethanolic extracts of *G. thoracica* propolis exhibited a TPC value of  $259.84 \pm 4.97$  mg/mL GAE, which was consistent with the current results.

The TFC values of ethanolic propolis extracts were obtained from the quercetin standard curve with the regression line equation of  $y = 0.0025x + 0.0767$ , ( $R^2 = 0.994$ ). The results indicated that the ethanolic extracts of propolis from apiary A exhibited the highest TFC value ( $99.08 \pm 0.03$  mg/mL QE), significantly different from apiary B ( $64.68 \pm 0.02$  mg/mL QE) and apiary C ( $73.08 \pm 0.01$  mg/mL QE). Notably, the TFC in this study is lower compared to the findings of Adli *et al.* [9], who reported that ethanolic extracts of *G. thoracica* propolis exhibited a TFC value of  $435.00 \pm 6.57$  mg/mL QE. Furthermore, the current results are in contrast to those reported by Usman *et al.* [27], who documented higher TFC values of 209.83 mg/mL QE for Malaysian ethanolic propolis extracts.



Numerous studies have consistently highlighted the substantial variations in TPC and TFC observed in propolis, a pattern strongly influenced by both botanical sources and geographical locations [2,8,32]. The diverse array of medicinal plants in apiary A, encompassing families such as *Simaroubaceae*, *Myrsinaceae*, *Primulaceae*, *Zingiberaceae*, *Acanthaceae*, and *Lamiaceae*, may contribute significantly to the intricate phytochemical composition of propolis. Each plant family contributes distinct phytochemicals, and the combined effect of these diverse compounds could potentially account for the higher TPC and TFC observed in the propolis samples [33]. Importantly, these medicinal plants, known for their richness in phenolic and flavonoid compounds, contribute significantly to the overall phytochemical profile, and antioxidant properties [33,34,35].

Additionally, the chemical composition of propolis is intricately linked to the time of harvesting and the specific colony of bees involved [36, 37]. Despite the consistent sampling site for apiary C with Adli *et al.* [9], slight variations in TPC and TFC were observed, possibly due to differences in the time of harvesting and the unique characteristics of the bee colony. It is important to note that the amount and quality of propolis collected will vary greatly across colonies based on genetics, environment, and colony strength [37]. Moreover, the chemical makeup of propolis extracts is highly sensitive to factors such as the time of harvesting, a critical element influencing the foraging behaviour of *Melipona* species [36]. Harvesting time significantly impacts foraging activities, dictating the selective floral preferences of the bees and, consequently, the availability of floral resources [38]. Teixeira *et al.* [39], demonstrated that the TPC and TFC in propolis are significantly influenced by the timing of harvesting at the same apiary over various intervals throughout the year.

**Table 2.** The total phenolic and flavonoid contents of ethanolic extract of propolis from three different apiary sites

Apiary sites	Total Phenolic Content (mg/mL GAE)	Total Flavonoids Content (mg/mL QE)
Apiary A	302.21 ± 0.11 <sup>c</sup>	99.08 ± 0.03 <sup>b</sup>
Apiary B	156.79 ± 0.06 <sup>b</sup>	64.68 ± 0.02 <sup>a</sup>
Apiary C	111.38 ± 0.06 <sup>a</sup>	73.08 ± 0.01 <sup>a</sup>

The results are presented as the mean ± standard deviation. Different letters in the same column indicate statistical significance at  $p < 0.05$ . "GAE": Gallic acid equivalent; "QE": Quercetin equivalent. Both assays were conducted in triplicate.

### DPPH Free Radical Scavenging Activity

Table 3 shows the percentage inhibition of DPPH and the  $IC_{50}$  values of ethanolic extract of propolis at concentrations ranging from 7.813  $\mu\text{g/mL}$  to 500  $\mu\text{g/mL}$ , using quercetin as the reference standard. In general, all propolis extracts exhibited a dose-dependent free radical scavenging activity in the DPPH assay. Among the samples tested, propolis from apiary C exhibited the highest DPPH inhibition (84.82% ± 18.7), followed by propolis from apiary A (82.51% ± 1.27) and apiary B (81.38% ± 30.1). Notably, propolis from apiary A displayed a significantly lower  $IC_{50}$  value (25.27  $\mu\text{g/mL}$ ) compared to apiary B (65.37  $\mu\text{g/mL}$ ) and apiary C (122.7  $\mu\text{g/mL}$ ). A lower  $IC_{50}$  value indicates stronger antioxidant activities [40]. Quercetin showed the best DPPH inhibitory effect at 90.08% ± 9.76 with an  $IC_{50}$  value of 18.93  $\mu\text{g/mL}$ . Badiazaman *et al.* [28] reported that the methanolic extract of *G. thoracica* propolis collected from various locations in Malaysian exhibited  $IC_{50}$  values ranging between 53 to 190  $\mu\text{g/mL}$ . In the current study, a similar trend was observed, with  $IC_{50}$  values ranging from 25.27 to 122.7  $\mu\text{g/mL}$ . In contrast, Adli *et al.* [9] demonstrated higher  $IC_{50}$  values at 104.20  $\mu\text{g/mL}$  for ethanolic extract of *G. thoracica* propolis. The mechanisms of antioxidant action in the DPPH assay rely on the electron transfer scavenging potential of substances capable on donating hydrogen atoms [41]. This process results in the loss of an intense violet-colour solution, causing decolorization to a colourless or light-yellow solution as the number of electrons increases [42].

**Table 3.** Percentage inhibition of DPPH and IC<sub>50</sub> value of ethanolic extract of propolis from three different apiary sites

Apiary sites	DPPH Inhibition (%)	IC <sub>50</sub> DPPH (µg/mL)
Apiary A	82.51 ± 1.27	25.27 <sup>a</sup>
Apiary B	81.38 ± 30.1	65.37 <sup>b</sup>
Apiary C	84.82 ± 18.7	122.7 <sup>c</sup>
Quercetin	90.08 ± 9.76	18.93 <sup>a</sup>

### Ferric Reducing Antioxidant Power (FRAP) Assay

The results presented in Table 4 outline the FRAP values of the ethanolic extract of propolis at concentrations ranging between 0 to 1000 µg/mL. The FRAP values were determined by constructing a standard curve using iron (II) sulphate heptahydrate (FeSO<sub>4</sub>·7H<sub>2</sub>O). These values were calculated by comparing the absorbance of the ethanolic extract of propolis with the standard curve. The obtained results indicated that the FRAP values of the ethanolic extract of propolis ranged from 308.20 ± 0.02 µM Fe<sup>2+</sup> to 727.53 ± 0.09 µM Fe<sup>2+</sup>. Notably, propolis from apiary A exhibited significantly higher FRAP value (727.53 ± 0.09 µM Fe<sup>2+</sup>) compared to apiary C (308.20 ± 0.02 µM Fe<sup>2+</sup>). However, there were no significant difference in FRAP value between apiary A and apiary B (692.73 ± 0.05). Gallic acid, serving as the standard, displayed the highest FRAP value (1708.20 ± 0.16 µM Fe<sup>2+</sup>). This obtained result was consistent with a study reported by Asem *et al.* [31], where the ethanolic extract of *G. thoracica* propolis exhibited a FRAP value of 587.044 mM Trolox/g. The mechanisms of antioxidant action in the FRAP assay involve the ability to donate electrons, leading to the reduction of the Fe<sup>3+</sup> TPTZ complex to a blue-coloured Fe<sup>2+</sup> TPTZ through the action of an antioxidant agent [40]. A higher FRAP value indicates a greater antioxidant capacity [43].

**Table 4.** FRAP values by *G. thoracica* propolis extracts from three different apiary sites

Apiary sites	FRAP values (µM Fe <sup>2+</sup> )
Apiary A	727.53 ± 0.09 <sup>b</sup>
Apiary B	692.73 ± 0.05 <sup>b</sup>
Apiary C	308.20 ± 0.02 <sup>a</sup>
Gallic Acid	1708.20 ± 0.16 <sup>c</sup>

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

Table 5 shows the correlation coefficients between TPC, TFC, IC<sub>50</sub> of DPPH, and FRAP values for propolis extracts. Notably, a strong positive correlation exists between TPC and TFC, (R<sup>2</sup> = 0.868, p < 0.05). Similarly, a strong negative correlation is observed between TPC and IC<sub>50</sub> of DPPH, (R<sup>2</sup> = -0.846, p < 0.05). Additionally, a moderate positive correlation is observed between TPC and FRAP values, (R<sup>2</sup> = 0.673, p < 0.05). However, there is a weak positive correlation between TFC and FRAP values (R<sup>2</sup> = 0.352, p < 0.05), whereas a strong negative correlation is observed between TFC and IC<sub>50</sub> of DPPH (R<sup>2</sup> = -0.784, p < 0.05). Furthermore, a strong negative correlation is observed between IC<sub>50</sub> of DPPH and FRAP values (R<sup>2</sup> = -0.846, p < 0.05). The observed strong correlation between TPC, TFC, and IC<sub>50</sub> of DPPH in this study could indicate that radical scavenging activity of propolis extract is influenced by the phenolic and flavonoid contents. The obtained results are in line with previous studies, indicating a correlation between the antioxidant activity of the propolis extracts and their TPC and TFC [33, 34]. This is primarily attributed to the presence of aromatic hydroxyl groups, which are known for their effective electron accepting abilities [46].

**Table 5.** Pearson's correlation coefficient ( $R^2$ ) of TPC, TFC,  $IC_{50}$  of DPPH and FRAP values of propolis extracts from three different apiary sites

Assays	Correlation ( $R^2$ )			
	TPC	TFC	$IC_{50}$ DPPH	FRAP values
TPC	1	0.868*	-0.846*	0.673*
TFC	0.868*	1	-0.784*	0.352
$IC_{50}$ DPPH	-0.846*	-0.784*	1	-0.846*
FRAP values	0.673*	0.352	-0.846*	1

\*Correlation is significant at  $p < 0.05$

## Conclusions

This study highlighted the significant variations in the phytochemical compositions and antioxidant activities of ethanolic extracts of *G. thoracica* propolis collected from different geographical and botanical sources. The findings suggested that the ethanolic extracts of *G. thoracica* propolis from apiary A is a rich source of phenolic and flavonoid compounds, which exhibited robust antioxidant properties through radical scavenging and ferric reducing power activities. Further research is underway to identify the specific phytochemical constituents responsible for these observed variations, which may offer valuable insights into the therapeutic potential and applications of propolis.

## Conflicts of Interest

The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper.

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