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Research article

Comparative phytochemical and antioxidant characterization of propolis from different species of Malaysian stingless bees

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Abstract: Propolis derived from stingless bees is recognized for its rich bioactive composition and potential health-promoting properties. This study evaluated the phytochemical profile and antioxidant capacity of propolis extracts obtained from three Malaysian stingless bee species: *Geniotrigona thoracica, Heterotrigona itama,* and *Tetrigona binghami*. Gas chromatography–mass spectrometry (GC–MS) analysis identified diverse bioactive constituents and revealed marked compositional variations among the species examined. Antioxidant activities were assessed using DPPH, ABTS, and FRAP assays. All extracts exhibited strong antioxidant potential, as reflected by favorable IC_{50}/EC_{50} values in the DPPH assay and consistent performance in the ABTS and FRAP assays. Significant correlations between antioxidant capacity and total phenolic and flavonoid contents indicated that phenolic compounds are the principal contributors to the observed effects. Overall, these findings demonstrate that Malaysian stingless bee propolis represents a valuable source of natural antioxidants and bioactive phytochemicals, underscoring its potential as a health-promoting and nutraceutical agent.

Keywords: propolis; stingless bees; antioxidant activity; phytochemicals; nutraceutical potential

1. Introduction

Propolis, commonly known as "bee glue", is a resinous substance produced by bees from plant exudates such as buds, leaves, and tree bark, which are mixed with beeswax and salivary enzymes [1,2]. Within the hive, propolis functions as a protective sealant, safeguarding the colony against pests, pathogens, and microbial invasion. Its physicochemical characteristics, including texture, color, and chemical composition, vary considerably depending on the botanical sources and geographical origin of the raw materials [3].

Stingless bees (Hymenoptera: Apidae, tribe Meliponini) represent a diverse group of eusocial insects comprising approximately 60 genera and over 600 species, predominantly distributed across tropical and subtropical regions, including Malaysia [4]. These bees exhibit unique biological traits, such as specialized nectar foraging behavior, colony organization without conventional honeycombs, and distinct resin-collecting activities. Among the most prominent Malaysian stingless bee species are *Heterotrigona itama*, *Geniotrigona thoracica*, and *Tetrigona binghami* [5].

The therapeutic value of propolis is largely attributed to its complex chemical composition, which includes resins, waxes, essential oils, phenolic compounds, flavonoids, and various trace elements such as vitamins and minerals [6,7]. Major bioactive constituents—including carboxylic acids, terpenoids, steroids, hydrocarbons, sugars, alkaloids, flavonoids, phenols, ketones, and amino acids—collectively contribute to its broad pharmacological properties, including antioxidant, antimicrobial, anti-obesity, and anticancer effects. Propolis derived from stingless bees has been reported to exhibit strong free radical–scavenging capacity, inhibit lipid peroxidation, and enhance the activity of antioxidant enzymes such as catalase and superoxide dismutase [8,9]. Additionally, its metal ion–chelating activity further contributes to protection against oxidative damage. Collectively, these findings highlight stingless bee propolis as a rich natural source of bioactive compounds with promising potential for mitigating oxidative stress–related disorders [10].

2. Materials and methods

2.1. Propolis sample collection

Raw propolis samples were collected in November from the hives of three stingless bee species, namely *G. thoracica, H. itama,* and *T. binghami*, located in Kuala Kangsar, Perak (4°53′57.4″N, 100°53′46.9″E). One representative sample was obtained from each species by carefully scraping the inner surfaces of hive frames. Species identification was confirmed by specialists from the Malaysian Agricultural Research and Development Institute (MARDI), Serdang, Malaysia, for the corresponding stingless bee species (Figure 1). Following collection, the propolis samples were placed in sealed containers, transported under controlled conditions, and stored at −20 °C until further extraction and analysis.

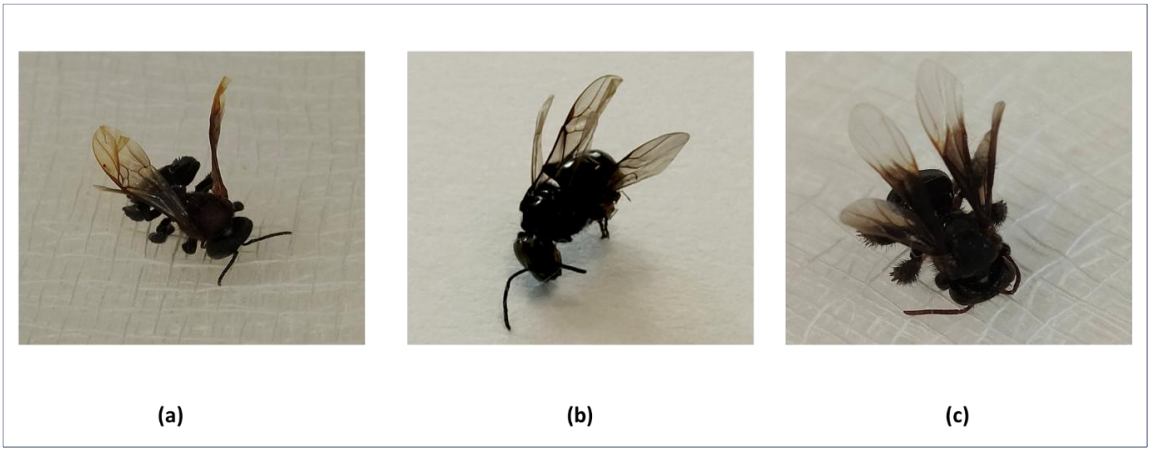


Figure 1. Stingless bees species: (a) *G. thoracica*, (b) *H. itama*, (c) *T. binghami*.

2.2. Propolis extracts preparation

Ethanolic extracts of propolis from *G. thoracica, H. itama,* and *T. binghami* were prepared following modified protocols described by Mihai et al. (2011) and Zin et al. (2018). Propolis samples were cryogenically stored at −20 °C and subsequently ground into a fine powder. A 10 g portion of powdered propolis was mixed with 100 mL of 80% ethanol (1:10 w/v) and subjected to magnetic stirring at 37 °C for 24 h at 400 rpm. After extraction, the mixture was centrifuged at 3000 rpm for 10 min, and the resulting supernatant was filtered through Whatman No. 4 filter paper. The extraction process was repeated twice to ensure maximum yield. The combined filtrates were collected in a volumetric flask and concentrated using a rotary evaporator (BUCHI, Switzerland) at 40 °C. The concentrated extract was subsequently freeze-dried (Alpha 1-4 LSC, Germany) to obtain a powdered form. All dried extracts were stored at −20 °C until further analysis [11].

2.3. Chemical profiling by GC–MS analysis

Gas chromatography–mass spectrometry (GC–MS) analysis of the propolis extracts was conducted according to the protocol described in [12]. The analysis was performed using an Agilent GC7890A system equipped with an Agilent mass-selective detector and a split injector. Compound separation was achieved using an Agilent HP-5MS capillary column (30.0 m × 0.25 mm, 0.25 μm film thickness; Agilent 190913-433). High-purity helium (99.99%) was employed as the carrier gas at a constant flow rate of 1 mL/min. A 1 μL aliquot of each extract was injected in split mode (10:1 ratio) at an injector temperature of 250 °C.

The oven temperature was initially set at 50 °C for 1 min and then increased at 8 °C/min to 120 °C (held for 1 min), followed by a ramp of 6 °C/min to 250 °C, and maintained at 250 °C for 15 min. The solvent delay was set from 0 to 3 min, resulting in a total runtime of 47 min. Compound identification was performed by matching the obtained mass spectra with entries in the National Institute of Standards and Technology (NIST) library, enabling the determination of compound names, molecular weights, and structural characteristics. The detected compounds were identified based on their retention times and comparison of their mass spectra with library data. All experiments were conducted in triplicate.

2.4. Determination of phytochemical content

2.4.1. Total phenol content

The total phenolic content of the propolis samples was determined using a modified Folin–Ciocalteu method. Propolis extracts were initially prepared in methanol, and 100 µL of each extract was mixed with 100 µL of Folin–Ciocalteu reagent and incubated at room temperature for 5 min. Subsequently, 300 µL of 7.5% Na_2CO_3 solution was added, and the reaction mixture was further incubated for 30 min under low-light conditions. Following incubation, 200 µL of the final reaction mixture was transferred into a 96-well microplate, and absorbance was measured at 760 nm using a microplate reader. Gallic acid standard solutions (50–400 µg/mL) were used to generate a calibration curve. All measurements were performed in triplicate (n = 3), and results were expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g) and reported as mean ± standard deviation.

2.4.2. Total flavonoid content

The total flavonoid content of the propolis extracts was determined using a modified aluminum chloride colorimetric assay adapted to a 96-well microplate format. Propolis extracts were first prepared in methanol to obtain stock solutions. Briefly, 100 µL of each sample was mixed with 0.3 mL of 5% $NaNO_2$ solution and allowed to react for 5 min at room temperature. Subsequently, 0.3 mL of 10% $AlCl_3$ solution was added, followed by a 6-min incubation. Thereafter, 0.2 mL of 1 M NaOH was introduced to complete the reaction, and the mixture was incubated for an additional 15 min. A 200 µL aliquot of the final reaction mixture was then transferred to a 96-well microplate, and absorbance was measured at 415 nm using a microplate reader. All measurements were performed in triplicate, and results were expressed as mean ± standard deviation. Quercetin standard solutions (10–100 µg/mL) were used to construct the calibration curve, and total flavonoid content was expressed as milligrams of quercetin equivalents per gram of dry extract (mg QE/g).

2.5. Propolis extracts antioxidant potency

The antioxidant capacity of propolis was investigated through various in vitro assays, grounded in the mechanism of free radical suppression.

2.5.1. Scavenging activity of DPPH (2,2-diphenyl-1-picrylhydrazyl) assay

Propolis extracts were initially dissolved in methanol to prepare stock solutions. Serial dilutions were subsequently performed to obtain solutions of varying concentrations. For the assay, 50 µL of each diluted extract was mixed with 200 µL of freshly prepared 0.2 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution in a 96-well microplate, ensuring complete homogenization.

The reaction mixtures were incubated in the dark at room temperature for 30 min. Following incubation, absorbance was measured at 517 nm using a microplate spectrophotometer [13]. All measurements were conducted in triplicate, and results were expressed as mean ± standard deviation. Trolox was used as the reference antioxidant standard. The percentage of radical scavenging activity was calculated using the following formula:

$$\text{Inhibition ratio } \% = \frac{OD_{control} - OD_{sample}}{OD_{control}} * 100 \quad (1)$$

Where $OD_{control}$ is the control absorbance, and OD_{sample} is the sample absorbance.

2.5.2. Scavenging activity of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^+$) assay

The $ABTS^+$ radical scavenging assay was performed to evaluate the antioxidant capacity of the propolis samples, following the method described in [14] with minor modifications. A stock solution of the $ABTS^+$ radical was prepared by mixing equal volumes of 7 mM 2,2′ -azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) solution and 2.5 mM potassium persulfate, followed by incubation in the dark at room temperature for 16 h to allow radical formation.

Subsequently, 20 µL of Trolox (positive control) or 20 µL of propolis extracted at various concentrations were added to the wells of a 96-well microplate, followed by the addition of 180 µL of the $ABTS^+$ working solution. The plate was incubated at room temperature in the dark, and absorbance was measured at 734 nm after 5 min of reaction.

The $ABTS^+$ radical scavenging activity was expressed as the percentage of decolorization, calculated using the following equation (Equation 2):

$$Inhibition\ \% = \frac{OD_{control} - OD_{sample}}{OD_{control}} * 100 \quad (2)$$

Where $OD_{control}$ is the control absorbance, and OD_{sample} is the sample absorbance.

2.5.3. Evaluation of ferric reducing antioxidant power (FRAP) assay

The FRAP assay is a widely used method that measures the reduction of ferric ions (Fe^{3+}) to the blue-colored ferrous form (Fe^{2+}) under acidic conditions in the presence of antioxidants. Following the procedure described in [3] with slight modifications, the FRAP reagent was prepared by mixing 10 mM 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), 20 mM $FeCl_3$, and 300 mM acetate buffer in a ratio of 1:1:10 (v/v/v). The reagent mixture was prewarmed at 37 °C for 10 min prior to use.

The reaction mixture consisted of 200 µL of sample and 1.5 mL of freshly prepared FRAP working solution. The mixture was incubated at room temperature in the dark for 30 min. Absorbance was measured at 593 nm against distilled water as a blank. All measurements were performed in triplicate. A standard curve was constructed using aqueous solutions of $FeSO_4 \cdot 7H_2O$ (100–1000 µmol), and FRAP values were expressed as µmol Fe^{2+} equivalents.

2.5.4. Statistical analysis

Statistical analyses were performed to evaluate total polyphenol and flavonoid contents, as well as the antioxidant activities of the propolis extracts determined by the DPPH, ABTS, and FRAP assays. All experiments were conducted in triplicate, and data are presented as mean ± standard deviation (SD).

Pearson's correlation coefficient was used to assess the relationships between phytochemical content and antioxidant capacity. Coefficients of determination (R^2) for the spectrophotometric assays were calculated using Microsoft Excel. One-way analysis of variance (ANOVA) was performed using GraphPad Prism (version 10) to determine significant differences among treatment groups. Post hoc

comparisons were conducted using Tukey's HSD test in IBM SPSS Statistics (version 27).

Statistical significance was defined as $p < 0.05$, $p < 0.01$, and $p < 0.001$, while values of $p \geq 0.05$ were considered not significant (ns). Additionally, IC_{50} and EC_{50} values were analyzed using the Kruskal–Walli's test in IBM SPSS Statistics (version 27).

3. Results

3.1. Physical characteristics of propolis

The physical and visual characteristics of propolis varied according to its botanical origin and bee species classification. The samples examined demonstrated distinct differences in color, ranging from dark golden in *T. binghami* to dark brown in *G. thoracica* and blackish brown in *H. itama*. Propolis from *T. binghami* was characterized by a smooth, lustrous, and brittle surface with a mild balsamic aroma. In contrast, *G. thoracica* propolis exhibited a coarse texture, high density resembling compressed layers, and a strong aromatic odor. The *H. itama* sample displayed a thick, branching structure attributed to its resin-rich composition and emitted a pronounced aromatic fragrance (Figure 2). All samples were solid at room temperature and upon cooling, but softened and melted when exposed to temperatures above 60 °C.

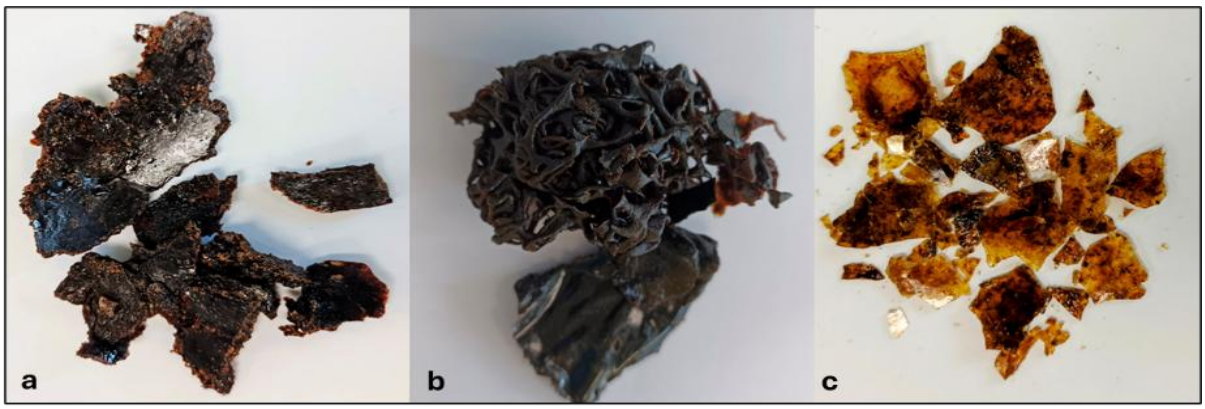


Figure 2. Morphology of raw propolis. (a) *G. thoracica*, (b) *H. itama*, (c) *T. binghami*.

3.2. Percentage yields

Propolis samples from the stingless bee species were individually weighed at 10 g and extracted using 100 mL of 80% ethanol. The percentage yield for extractions was calculated as follows:

$$\% \text{ Yield} = [100 * \frac{(\text{Weight of propolis extracts (g)})}{(\text{Weight of propolis raw powder (g)})}] \quad (3)$$

The extraction yielded 26.3% for *H. itama*, 31.2% for *G. thoracica,* and 24.19% for *T. binghami* (Table 1). The highest yield was obtained from *G. thoracica*, potentially due to differences in resin origin, wax content, or species-specific foraging behaviour. These variations suggest a distinct chemical profile across the samples.

Table 1. Percentage yields of propolis extracts.

Species name	Propolis raw powder weight (g)	Yield percentage (%)
H. itama	10	26.3
T. binghami	10	24.19
G. thoracica	10	31.2

3.3. Chemical profiling of propolis extracts

3.3.1. Gas chromatography–mass spectrometry (GC–MS) analysis

The GC–MS analysis of *G. thoracica* propolis revealed a chemically diverse profile. Compound identification was based on the total ion chromatogram (Figure 3), and only compounds with a spectral matching quality ≥80% against the NIST Mass Spectrometry Database were considered (Table 2).

Identified phenolic constituents included phenol, 2-methoxy- (92%), benzenecarboxylic acid (96%), 1,2-benzenediol (95%), phenol, 4-ethyl-2-methoxy- (91%), and vanillin (95%). The terpene fraction comprised both mono- and sesquiterpenes, including 1,3-cyclohexadiene, 1-methyl-4-(1-methylethyl)- (91%), α-cubebene (87%), copaene (93%), caryophyllene (99%), γ-elemene (95%), 1,4,7-cycloundecatriene, 1,5,9,9-tetramethyl-, (Z,Z,Z)- (98%), 1H-cyclopenta[1,3]cyclopropa[1,2]benzene, octahydro-7-methyl-3-methylene-4-(1-methylethyl)- (95%), naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)- (94%), (+)-epi-bicyclosesquiphellandrene (83%), caryophyllene oxide (93%), aromadendrene oxide-(1) (80%), and longifolenaldehyde (91%).

Fatty acid–related compounds included hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (98%) and 1,9-tetradecadiene (97%). Alkylated phenols such as 3-pentadecyl- (94%) were also detected. The sterol and triterpenoid fractions were represented by ergosta-8,24(28)-dien-3-ol (91%), 4,4,6a,6b,8a,11,11,14b-octamethyl-2H-picen-3-one (90%), α,β-amyrin (94%), and 9,19-cyclolanost-24-en-3-ol (98%).

This chemical profile highlights the compositional complexity of *G. thoracica* propolis, encompassing phenolics, terpenes, fatty acid derivatives, and sterol/triterpenoid constituents that collectively contribute to its bioactive potential.

The GC–MS analysis of *T. binghami* propolis (Table 3) revealed a chemically rich composition encompassing phenolic compounds, fatty acids, sesquiterpenes, and terpenoids. The phenolic fraction consisted of 2-methoxy-4-vinylphenol (80%) and phenol, 2,4-bis(1,1-dimethylethyl)- (97%), indicating the presence of alkylated phenolic antioxidants.

Fatty acid derivatives were also detected (Figure 4), most notably n-decanoic acid (98%) and hexadecanoic acid, ethyl ester (95%). Sesquiterpenes constituted a major component of the volatile fraction. Key representatives included cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)- (95%), caryophyllene (99%), and 1,4,7-cycloundecatriene, 1,5,9,9-tetramethyl- (98%). Oxygenated sesquiterpenes were also identified, including caryophyllene oxide (94%) and isoaromadendrene epoxide (90%), reflecting oxidative derivatives of terpene metabolism.

Additional terpenoid constituents comprised a monoterpenoid aldehyde (80%) and a terpenoid ketone (83%), further contributing to the aromatic and bioactive profile of the extract. High-molecular-weight terpenoids were well represented within the sterol and triterpenoid fractions. The sterols included lanosterol (86%) and 9,19-cyclolanost-24-en-3-ol (96%), while the triterpenes comprised olean-12-ene (83%), α,β-amyrin (90%), and the triterpenoid ketone 4,4,6a,6b,8a,11,12,14b-octamethyl-picen-3-one (86%).

Collectively, the chemical profile of *T. binghami* propolis demonstrates a complex mixture of phenolics, fatty acids, sesquiterpenes, sterols, and triterpenoids, underscoring its potential for diverse biological activities.

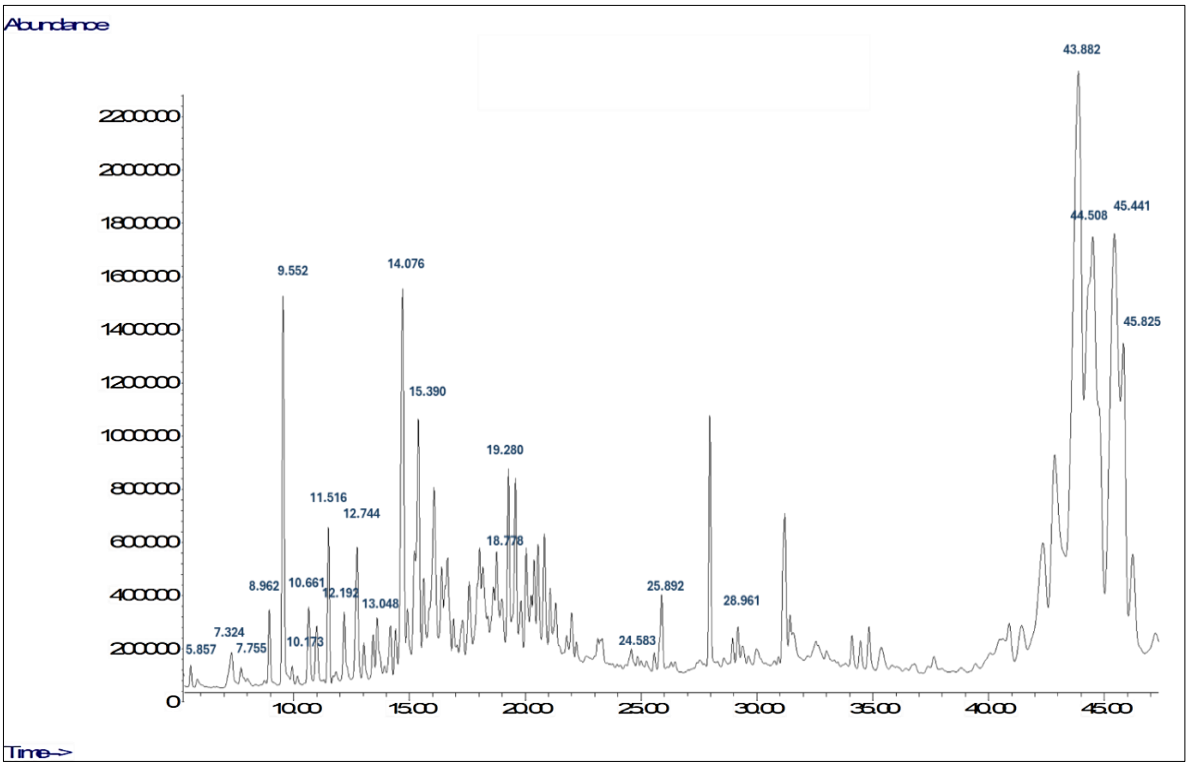


Figure 3. Total ion chromatogram (TIC) of stingless bee *G. thoracica* propolis ethanolic extract.

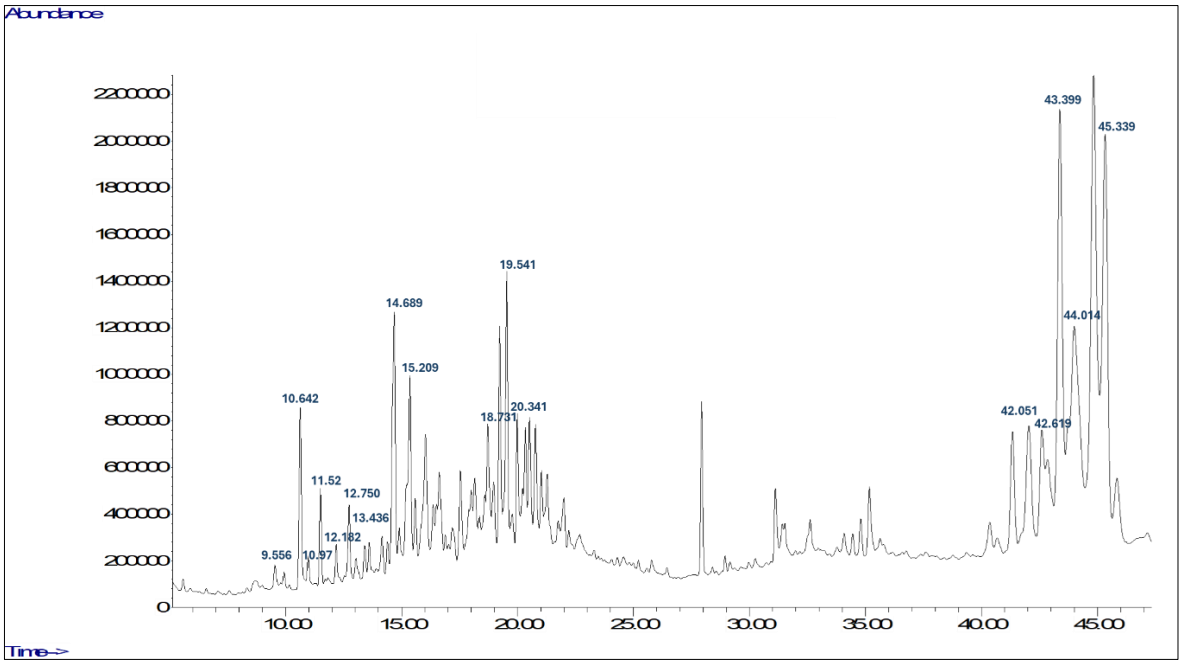
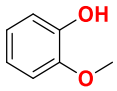
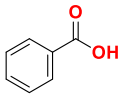
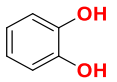
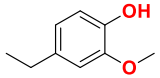
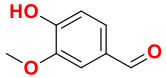
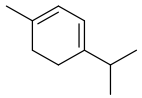
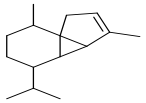
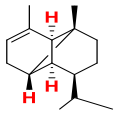
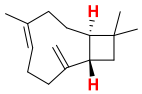


Figure 4. Total ion chromatogram (TIC) of stingless bee *T. binghami* propolis ethanolic extract.

Table 2. Compounds identified in *G. thoracica* propolis extract by GC–MS analysis.

Compound	Chemical formula	Structure	Molecular weight (g/mol)	Retention time (min)	Classification	Matching quality (%)
Phenol, 2-methoxy-	$C_7H_8O_2$		124.14	5.857	Methoxyphenol	92
Benzenecarboxylic acid	$C_7H_6O_2$		122.12	7.324	Benzoic acid derivative	96
1,2-benzenediol	$C_6H_6O_2$		110.11	7.743	Catechol (Polyphenol)	95
Phenol, 4-ethyl-2-methoxy-	$C_9H_{12}O$		136.19	8.962	Alkyl methoxyphenol	91
Vanillin	$C_8H_8O_3$		152.15	8.962	phenolic aldehyde	95
1,3-cyclohexadiene, 1-methyl-4-(1-methylethyl)	$C_{10}H_{16}$		136.23	9.552	Monoterpene (p-Cymene)	91
α-cubebene	$C_{15}H_{24}$		204.35	10.173	Sesquiterpene	87
Copaene	$C_{15}H_{24}$		204.35	10.661	Sesquiterpene	93
Caryophyllene	$C_{15}H_{24}$		204.35	11.516	Sesquiterpene	99

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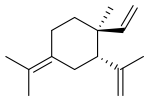
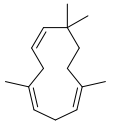
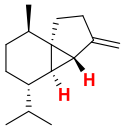
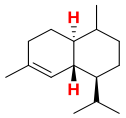
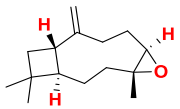
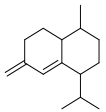
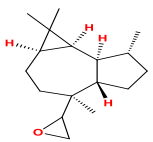
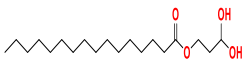
Compound	Chemical formula	Structure	Molecular weight (g/mol)	Retention time (min)	Classification	Matching quality (%)
γ-elemene	$C_{15}H_{24}$		204.35	11.516	Sesquiterpene	95
1,4,7-cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z-	$C_{15}H_{24}$		204.35	12.192	Sesquiterpene	98
1H-cyclopenta[1,3]cyclopropa[1,2]benzene, octahydro-7-methyl-3-methylene-4-(1-methylethyl)-, [3aS-(3a.alpha.,3b.beta.,4.beta.,7.alpha.,7aS*)]-	$C_{15}H_{24}$		204.35	12.744	Sesquiterpene	95
Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)- (1S-cis)	$C_{15}H_{24}$		204.35	13.048	Sesquiterpene	94
Caryophyllene oxide	$C_{15}H_{24}O$		220.35	14.076	Sesquiterpene oxide	93
(+)-epi-bicyclosesquiphellandrene	$C_{15}H_{24}$		204.35	15.390	Sesquiterpene	83
Aromadendrene oxide-(1)	$C_{15}H_{24}O$		220.35	18.778	Sesquiterpenoid	80
Hexadecanoic acid, 2-hydroxy-1(hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$		330.51	19.280	Fatty acid ester	98

Continued on the next page

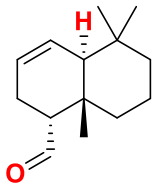
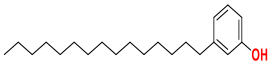
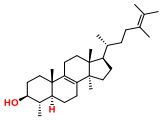
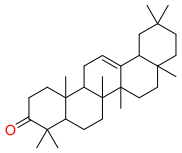
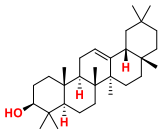
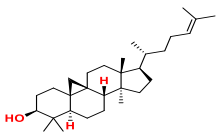
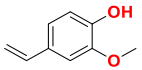
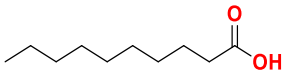
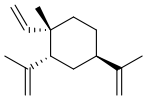
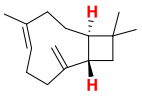
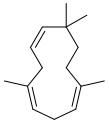
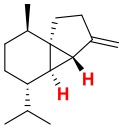
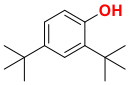
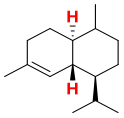
Compound	Chemical formula	Structure	Molecular weight (g/mol)	Retention time (min)	Classification	Matching quality (%)
Longifolenaldehyde	$C_{15}H_{24}O$		220.35	24.583	Sesquiterpene aldehyde	91
1,9-tetradecadiene	$C_{14}H_{26}$		194.36	25.892	Unsaturated hydrocarbon	97
Phenol, 3-pentadecyl-	$C_{21}H_{36}O$		304.50	28.961	Alkylphenol	94
Ergosta-8,24(28)-dien-3-ol	$C_{30}H_{50}O$		426.70	43.882	Sterol (ergosterol derivative)	91
4,4,6a,6b,8a,11,11,14b-octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-octadecahydro-2H-picen-3-one	$C_{30}H_{50}O$		424.70	44.508	Triterpenoid	90
α, β-amyrin	$C_{30}H_{50}O$		426.72	45.44	Pentacyclic triterpenoid	94
9,19-cyclolanost-24-en-3-ol, (3.beta.)-	$C_{30}H_{50}O$		426.70	45.82	Cycloartane-type triterpenoid (cycloartenol)	98

Table 3. Bioactive compounds identified in T. binghami propolis extract by GC-MS analysis.

Compound	Chemical formula	Structure	Molecular weight (g/mol)	Retention time (min)	Classification	Matching quality (%)
2-methoxy-4-vinylphenol	$C_9H_{10}O_2$		150.18	9.556	Phenolic compound	80
n-decanoic acid	$C_{10}H_{20}O_2$		172.26	10.642	Saturated fatty acid	98
Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1.alpha.,2.beta.,4.beta.)]-	$C_{15}H_{24}$		204.35	10.976	Sesquiterpene	95
Caryophyllene	$C_{15}H_{24}$		204.35	11.520	Sesquiterpene	99
1,4,7-cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z-	$C_{15}H_{24}$		204.35	12.182	Sesquiterpene	98
1H-cyclopenta[1,3]cyclopropa[1,2]benzene, octahydro-7-methyl-3-methylene-4-(1-methylethyl)-, [3aS-(3a.alpha.,3b.beta.,4.beta.,7.alpha.,7aS*)]-	$C_{15}H_{24}$		204.35	12.750	Sesquiterpene	92
Phenol, 2,4-bis(1,1-dimethylethyl)	$C_{14}H_{22}O$		206.32	13.436	Alkylphenol	97
Naphthalene, 1,2,3,5,6,8 a-hexahydro-4,7-dimethyl-1-(1-methylethyl)- (1S-cis)	$C_{15}H_{24}$		204.35	13.625	Sesquiterpene	97

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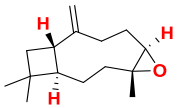
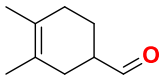
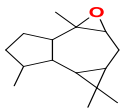
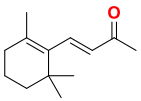
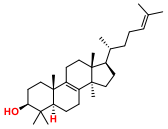
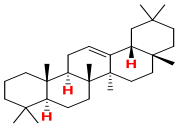
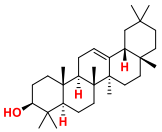
Compound	Chemical formula	Structure	Molecular weight (g/mol)	Retention time (min)	Classification	Matching quality (%)
Caryophyllene oxide	$C_{15}H_{24}O$		220.35	14.689	Sesquiterpene oxide	94
3-cyclohexenecarboxaldehyde -	$C_9H_{14}O$		138.21	15.209	Monoterpenoid aldehyde	80
Isoaromadendrene epoxide	C15H24O		220.35	18.731	Sesquiterpene epoxide	90
Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$		284.47	19.541	Fatty acid ethyl ester	95
3-buten-2-one, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)- (E)	$C_{13}H_{20}O$		192.30	20.341	Terpenoid ketone	83
Lanosterol	$C_{30}H_{50}O$		426.72	42.051	Triterpenoid sterol	86
Olean-12-ene	$C_{30}H_{50}$		426.72	42.619	Triterpene hydrocarbon	83
α,β-amyrin	$C_{30}H_{50}O$		426.72	43.399	Pentacyclic triterpenoid	90

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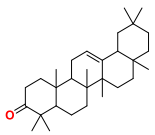
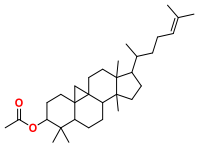
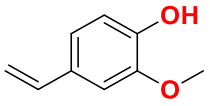
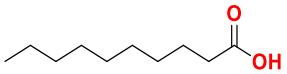
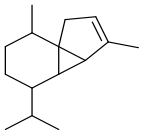
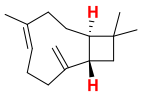
Compound	Chemical formula	Structure	Molecular weight (g/mol)	Retention time (min)	Classification	Matching quality (%)
4,4,6a,6b,8a,11,12,14b-Octamethyl-...picen-3-one	$C_{30}H_{50}O$		426.72	44.014	Triterpenoid ketone	86
9,19-cyclolanost-24-en-3-ol, (3β)	$C_{30}H_{50}O$		426.72	45.339	Cycloartane-type triterpenoid (Cycloartenol)	96

Table 4. Bioactive compounds identified in *H. itama* propolis extract by GC–MS analysis.

Compound	Chemical formula	Structure	Molecular weight (g/mol)	Retention time (min)	Classification	Matching quality (%)
2-methoxy-4-vinylphenol	$C_9H_{10}O_2$		150.17	9.527	Phenolic volatile	96
n-decanoic acid	$C_{10}H_{20}O_2$		172.26	9.782	Fatty acid	98
α-vubebene	$C_{15}H_{24}$		204.35	10.813	Sesquiterpene	96
Caryophyllene	$C_{15}H_{24}$		204.35	11.512	Sesquiterpene	99

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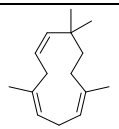
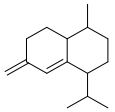
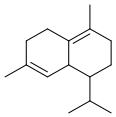
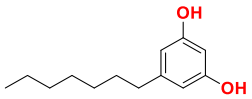
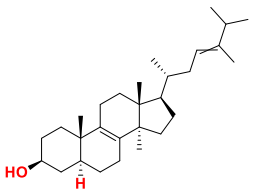
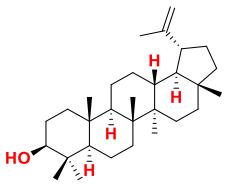
Compound	Chemical formula	Structure	Molecular weight (g/mol)	Retention time (min)	Classification	Matching quality (%)
1,4,7-cycloundecatriene,1,5,9,9-tetramethyl-, Z,Z,Z-	$C_{15}H_{24}$		204.35	12.192	Sesquiterpene	98
(+)-epi-bicyclosesquiphellandrene	$C_{15}H_{24}$		204.35	12.742	Sesquiterpene	90
Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)- (1S-cis)	$C_{15}H_{24}$		204.35	13.615	Sesquiterpene	85
Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$		284.477	22.209	Fatty acid ester	93
5-heptylresorcinol	$C_{13}H_{20}O_2$		208.30	35.198	Phenolic lipid	80
(E)-14. alpha. -methyl-5. alpha. -ergosta-8,23-dien-3. beta. -ol	$C_{29}H_{48}O$		412.70	40.375	Sterol	91
Lupeol	$C_{30}H_{50}O$		426.72	42.089	Triterpenoid	83

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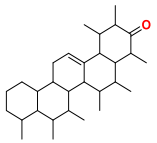
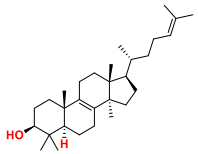
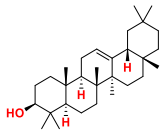
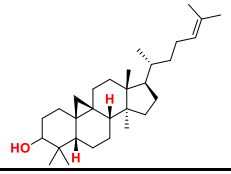
Compound	Chemical formula	Structure	Molecular weight (g/mol)	Retention time (min)	Classification	Matching quality (%)
4,4,6a,6b,8a,11,12,14b-octamethyl 1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12 ,12a,14,14 a,14b-octadecahydro 2H-p icen-3-one	$C_{30}H_{50}O$		426.72	42.649	Triterpenoid ketone	89
Lanosterol	$C_{30}H_{50}O$		426.72	44.204	Triterpenoid sterol	86
α,β-amyrin	$C_{30}H_{50}O$		426.72	44.961	Pentacyclic triterpenoid	91
9,19-cyclolanost-24-en-3-ol	$C_{30}H_{50}O$		426.72	45.517	Cycloartane triterpenoid	99

H. itama propolis revealed a chemically diverse mixture of bioactive constituents spanning several major classes (Table 4). The phenolic fraction was represented by 2-methoxy-4-vinylphenol (96%), a volatile phenolic compound commonly associated with antioxidant and antimicrobial activities. Another notable phenolic compound was 5-heptylresorcinol (80%), classified as a phenolic lipid.

Fatty acid derivatives were also prominent. The analysis identified n-decanoic acid (98%), a medium-chain fatty acid, along with hexadecanoic acid, ethyl ester (93%), a fatty acid ester frequently associated with lipid-rich natural extracts.

The sample exhibited a strong representation of sesquiterpenes (Figure 5), including α-cubebene (96%), caryophyllene (99%), 1,4,7-cycloundecatriene, 1,5,9,9-tetramethyl- (98%), (+)-epi-bicyclosesquiphellandrene (90%), and a naphthalene, hexahydro-dimethyl-isopropyl derivative (85%). These compounds indicate a substantial contribution of volatile terpenes to the overall chemical profile.

Higher-molecular-weight terpenoids were also abundant. The sterol fraction included (E)-14α-methyl-5α-ergosta-8,23-dien-3β-ol (91%), while the triterpenoid constituents comprised lupeol (83%), 4,4,6a,6b,8a,11,12,14b-octamethyl-picen-3-one (89%), lanosterol (86%), α,β-amyrin (91%), and 9,19-cyclolanost-24-en-3-ol (99%).

Overall, the GC–MS chromatographic profile demonstrates that *H. itama* propolis contains a complex assemblage of phenolics, fatty acids, sesquiterpenes, sterols, and triterpenoids, collectively contributing to its potential bioactivity.

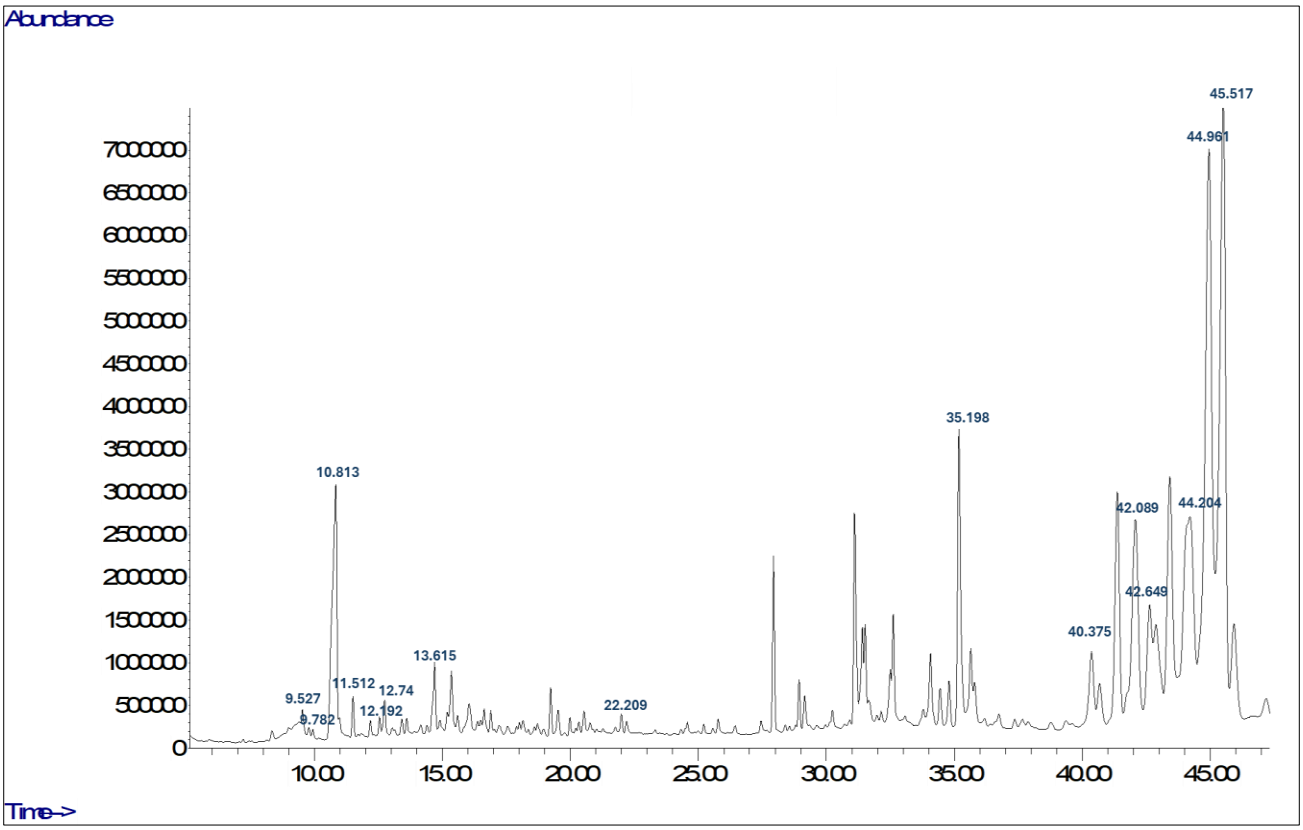


Figure 5. Total ion chromatogram (TIC) of stingless bee *H. itama* propolis ethanolic extract.

3.4. Phytochemical content

Phytochemical analysis revealed distinct compositional differences among the three stingless bee propolis samples (Table 5). The extract from *G. thoracica* contained markedly higher levels of total phenolic compounds (206.49 ± 6.19 mg GAE/g), approximately double those of *H. itama* (119.65 ± 3.59 mg GAE/g) and *T. binghami* (108.45 ± 3.25 mg GAE/g).

A similar trend was observed for total flavonoid content, with *G. thoracica* again exhibiting the highest value (88.18 ± 1.61 mg QE/g), followed by *H. itama* (83.91 ± 1.51 mg QE/g) and *T. binghami* (69.38 ± 1.14 mg QE/g). This significant interspecies variation is likely attributable to differences in foraging behavior, preferred botanical resin sources, and local environmental conditions.

Table 5. Phytochemical content of ethanolic propolis extracts.

Sample	Total phenol content (mg GAE/g)	Total flavonoid content (mg QE/g)
G. thoracica	206.49 ± 6.19	88.18 ± 1.61
H. itama	119.65 ± 3.59	83.91± 1.51
T. binghami	108.45 ± 3.25	69.38 ± 1.14

3.5. Propolis extracts antioxidant potency

3.5.1. Scavenging activity of DPPH (2,2-diphenyl-1-picrylhydrazyl) assay

The antioxidant activity of the propolis samples was evaluated using the DPPH radical scavenging assay, with results expressed as IC_{50} values (mg/mL) and Trolox equivalent antioxidant capacity (TEAC; mg Trolox/mg sample). Trolox was used as the reference standard. The IC_{50} represents the concentration required to scavenge 50% of DPPH radicals, whereas TEAC quantifies antioxidant capacity relative to Trolox, based on the calibration curve ($y = 1.2634x + 34.281$, $R^2 = 0.9888$) (Figure 6).

All extracts exhibited a clear concentration-dependent radical scavenging effect. *G. thoracica* demonstrated the highest antioxidant potency, as indicated by the lowest IC_{50} value (0.0524 ± 0.001 mg/mL) (Figure 8). *H. itama* showed moderate activity (IC_{50} = 0.0824 ± 0.003 mg/mL), whereas *T. binghami* exhibited the lowest potency (IC_{50} = 0.1030 ± 0.017 mg/mL). All dose–response curves fell within the linear range of the Trolox standard.

The TEAC values confirmed this ranking. *G. thoracica* exhibited the highest TEAC (0.2370 ± 0.022 mg Trolox/mg), followed by *H. itama* (0.1508 ± 0.019 mg Trolox/mg) and *T. binghami* (0.1207 ± 0.018 mg Trolox/mg). The consistent inverse relationship between IC_{50} and TEAC values (Table 6) enhances the internal consistency and reliability of antioxidant assessment across all samples.

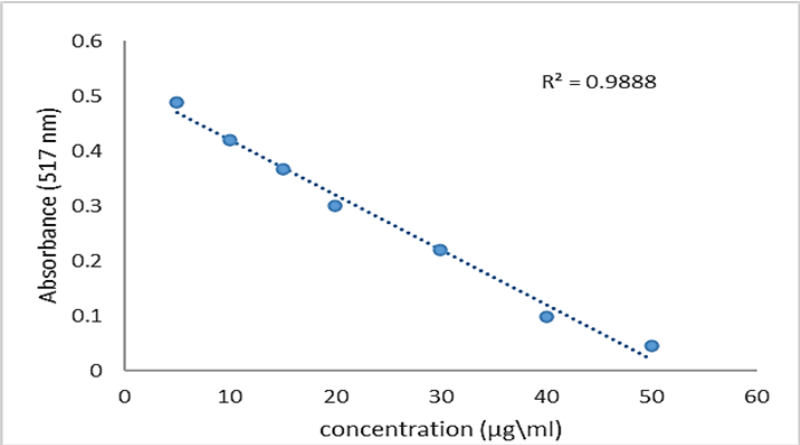


Figure 6. Trolox standard curve for the DPPH method. Data expressed by mean ± SD.

3.5.2. 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS+) assay

The antioxidant capacity of the propolis extracts was evaluated using the ABTS^{+} radical cation scavenging assay, a standard spectrophotometric method. All three extracts exhibited concentration-dependent scavenging activity, with 50% inhibition (IC_{50}) values of 0.0796 ± 0.0043 mg/mL for *G. thoracica*, 0.0961 ± 0.0039 mg/mL for *T. binghami*, and 0.0978 ± 0.0053 mg/mL for *H. itama* (Figure 7). Among the tested samples, *G. thoracica* demonstrated the highest radical scavenging potency, as indicated by its lowest IC_{50} value, reflecting superior antioxidant capacity.

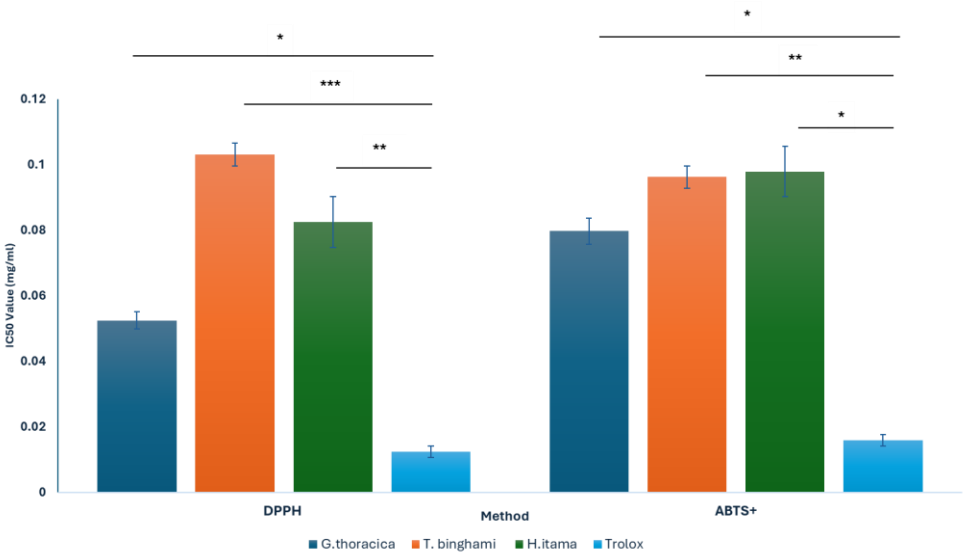


Figure 7. IC_{50} values of ethanolic propolis extracts from *G. thoracica, T. binghami,* and *H. itama* determined using DPPH and ABTS^{+} radical scavenging assays. Trolox was included as the reference antioxidant. Statistical differences between groups were assessed using the Kruskal–Wallis test, followed by appropriate post hoc comparisons. Significance levels are indicated as *$p \leq 0.05$, **$p < 0.01$, and ***$p < 0.001$.

The TEAC results further supported this ranking. *G. thoracica* exhibited the highest TEAC value (0.1994 ± 0.019 mg Trolox/mg dry weight), followed by *T. binghami* (0.1652 ± 0.015 mg Trolox/mg dry weight) and *H. itama* (0.1623 ± 0.021 mg Trolox/mg dry weight). Trolox served as the reference compound, and quantification was based on the calibration curve described by the equation $y = 1.1214x + 32.181$ ($R^2 = 0.9976$) (Figure 8).

A clear inverse relationship was observed between IC_{50} and TEAC values across all samples, as presented in Table 6. This consistent inverse association between IC_{50} (potency) and TEAC (capacity) confirms the internal consistency of the assay and underscores the significant antioxidant potential of Malaysian stingless bee propolis, particularly that derived from *G. thoracica*.

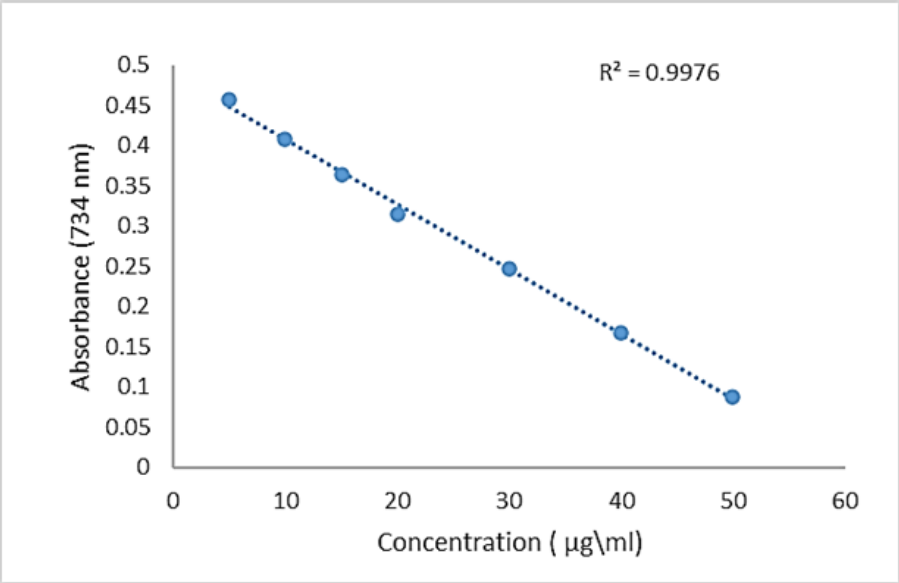


Figure 8. Trolox standard curve for the TEAC measurement of propolis ethanolic extracts in the $ABTS^+$ method. Data expressed by mean ± SD.

3.5.3. Ferric reducing antioxidant power (FRAP)

The FRAP assay, which assesses the ability of antioxidants to reduce ferric (Fe^{3+}) to ferrous (Fe^{2+}) ions, confirmed that all extracts exhibited concentration-dependent antioxidant activity. Among the three species, *G. thoracica* exhibited the most significant reducing power, achieving the highest FRAP value (555.8 µM Fe^{2+}/g) and the lowest EC_{50} (0.0295 ± 0.003 mg/mL). *H. itama* showed moderate activity (FRAP: 490.2 µM Fe^{2+}/g; EC_{50}: 0.0331 ± 0.007 mg/mL), while *T. binghami* exhibited the lowest efficacy (FRAP: 446.0 µM Fe^{2+}/g; EC_{50}: 0.0342 ± 0.004 mg/mL), as illustrated in Figure 9. The inverse relationship between EC_{50} (potency) and FRAP value (reducing capacity), detailed in Table 6, clearly confirms the sequence of antioxidants. Ferrous sulfate ($FeSO_4$) served as the reference standard, with the calibration curve ($y = 0.0008x + 0.1807$, $R^2 = 0.992$) shown in Figure 10.

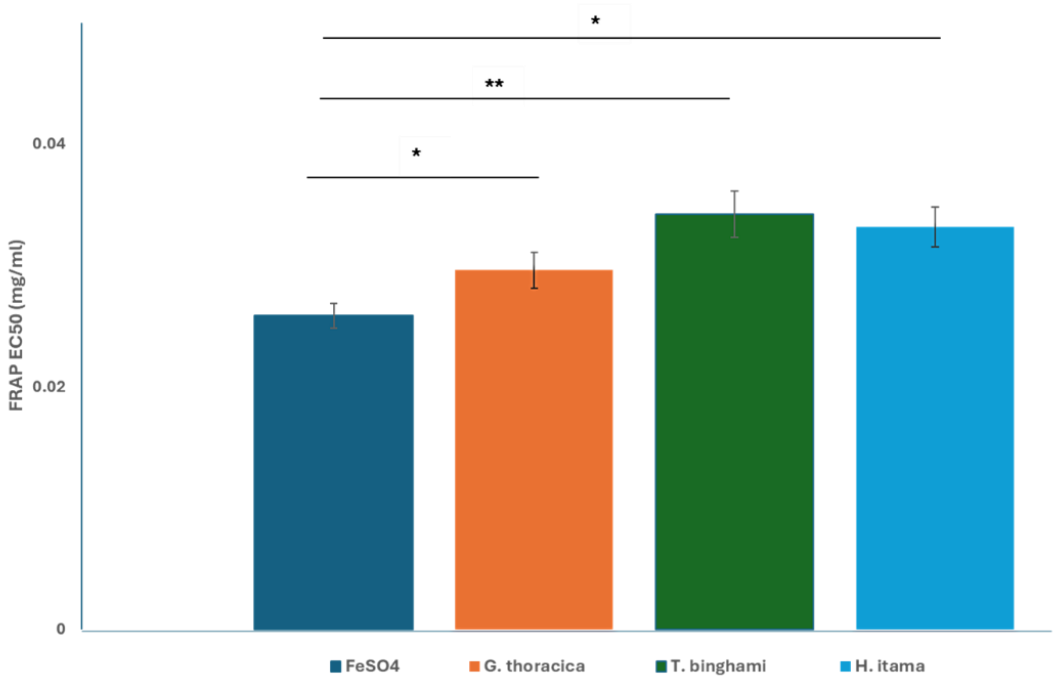


Figure 9. EC_{50} values of propolis extracts derived from *G. thoracica, T. binghami, H. itama*, and $FeSO_4$ standard compound, on FRAP.

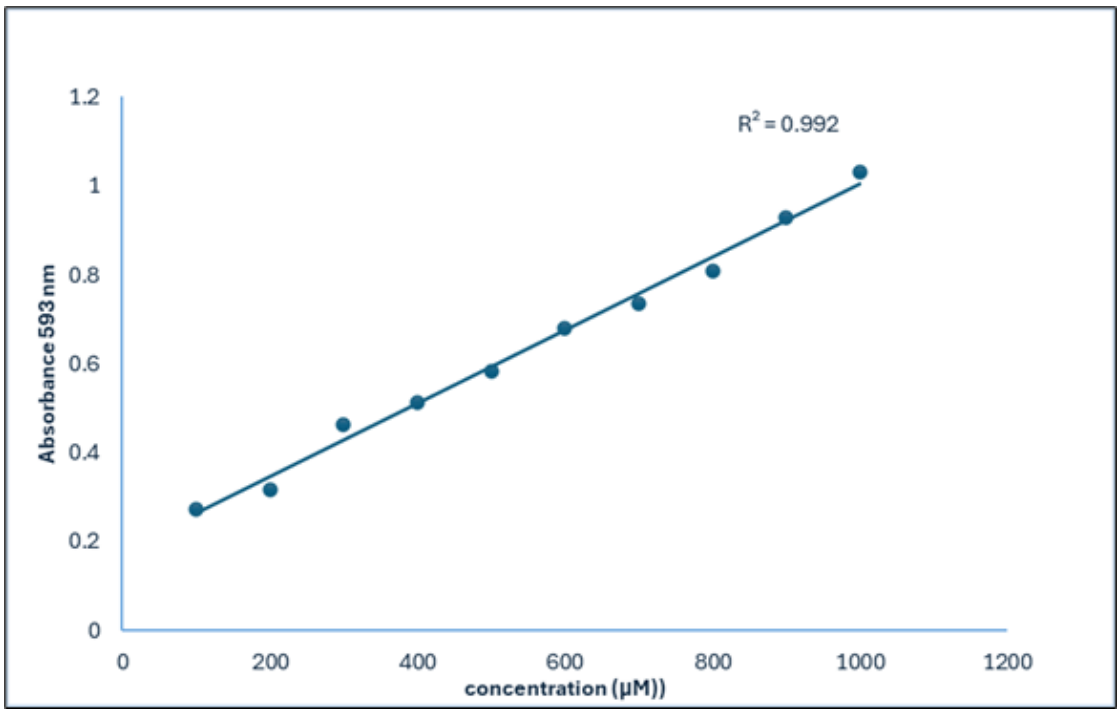


Figure 10. $FeSO_4$ standard curve for the measurement of propolis ethanolic extracts (FRAP value method). Data expressed by mean ± SD.

Table 6. Comparing the antioxidant activity of propolis extracts by DPPH, ABTS+, and FRAP assays.

Sample source	DPPH IC_{50} (mg/mL ± SD)	TEAC (mg Trolox/mg sample ± SD)	$ABTS^+$ IC_{50} (mg/mL ± SD)	TEAC (mg Trolox/mg sample ± SD)	FRAP EC_{50} (mg/mL ± SD)	FRAP value (μM Fe^{+2} g^{-1})
G. thoracica	0.0524 ± 0.001	0.2370 ± 0.021	0.0796 ± 0.004	0.1994 ± 0.019	0.0295 ± 0.003	555.800 ± 29.235
T. binghami	0.1030 ± 0.017	0.1207 ± 0.017	0.0961 ± 0.003	0.1652 ± 0.015	0.0342 ± 0.004	446.000 ± 31.292
H. itama	0.0824 ± 0.002	0.1508 ± 0.019	0.0978 ± 0.005	0.1623 ± 0.021	0.0331 ±0.007	490.200 ± 33.000

3.6. Correlation between total phenolic and flavonoid contents and antioxidant activities

Pearson correlation analysis was conducted to examine the relationships between total phenolic content (TPC), total flavonoid content (TFC), and antioxidant capacities as determined by the DPPH, $ABTS^+$, and FRAP assays. This analysis aimed to elucidate the contribution of phenolic and flavonoid constituents to the antioxidant potential of the propolis extracts and to assess the interrelationships among the different antioxidant assays (Table 7).

The results demonstrated strong positive correlations between TPC and all antioxidant assays, underscoring the central role of phenolic compounds in free radical scavenging and redox activity. Specifically, TPC exhibited high correlation coefficients with DPPH (R = 0.9890), $ABTS^+$ (R = 0.9848), and FRAP (R = 0.9530), indicating that phenolics substantially contribute to both radical scavenging capacity and reducing power.

TFC also showed significant positive correlations with antioxidant activities, particularly with FRAP (R = 0.9144), highlighting the contribution of flavonoids to electron transfer–based reducing mechanisms. Moderate to strong correlations were observed between TFC and DPPH (R = 0.8383), as well as between TFC and $ABTS^+$ (R = 0.6226). Moreover, a substantial correlation between TFC and TPC (R = 0.7488) suggests partial overlap in phytochemical composition and indicates that flavonoids represent a major subclass within the total phenolic fraction.

Regarding assay interrelationships, DPPH exhibited strong correlations with both $ABTS^+$ (R = 0.9485) and FRAP (R = 0.9872), suggesting a shared underlying mechanism predominantly based on electron transfer. $ABTS^+$ also correlated positively with FRAP (R = 0.8860), albeit to a slightly lesser extent.

Collectively, these strong inter-assay correlations demonstrate methodological consistency and confirm that the applied assays consistently capture the broad-spectrum antioxidant capacity of stingless bee propolis, primarily driven by its phenolic and flavonoid constituents.

Table 7. Pearson's correlation coefficient of antioxidant activities, total phenolic, and flavonoid content of propolis extracts derived from *G. thoracica, T. binghami,* and *H. itama.*

Assay	TPC	TFC	DPPH	ABTS	FRAP
TPC	1	0.748826791	0.989082567	0.984875571	0.953003151
TFC	0.748826791	1	0.838318271	0.622668349	0.914426057
DPPH	0.989082567	0.838318271	1	0.948590790	0.987243770
ABTS	0.984875571	0.622668349	0.948590790	1	0.886097659
FRAP	0.953003151	0.914426057	0.987243770	0.886097659	1

4. Discussion and conclusions

The GC–MS analysis revealed that propolis derived from *G. thoracica*, *T. binghami*, and *H. itama* is characterized by a diverse array of phenolic compounds, terpenes, fatty acid derivatives, sterols, and triterpenoids, reflecting both botanical origin and species-specific metabolic contributions.

In *G. thoracica* propolis, the predominance of simple phenolic compounds, including phenol, 2-methoxy-, benzenecarboxylic acid, 1,2-benzenediol, phenol, 4-ethyl-2-methoxy-, and vanillin, indicates a pronounced phenolic profile. Phenolic acids and simple phenols are widely recognized for their antioxidant and free radical–scavenging activities, contributing to protection against oxidative stress [15]. Notably, vanillin has been reported to exhibit antioxidant, anti-inflammatory, and analgesic properties, as well as protective effects against oxidative tissue damage [16]. Furthermore, the presence of alkylated phenols such as phenol, 3-pentadecyl-, suggests increased lipophilicity, which may enhance membrane interaction and antimicrobial activity [17].

Furthermore, the terpene-rich fraction of *G. thoracica*, including α-cubebene, copaene, caryophyllene, γ-elemene, 1,4,7-cycloundecatriene, 1,5,9,9-tetramethyl-, and (+)-epi-bicyclosesquiphellandrene, is consistent with the well-documented antimicrobial and anti-inflammatory properties of sesquiterpenes [18]. Caryophyllene and its oxygenated derivative, caryophyllene oxide, have been extensively associated with anti-inflammatory, analgesic, and antimicrobial activities [19]. Oxygenated sesquiterpenes such as aromadendrene oxide-(1) and aldehydes like longifolenaldehyde are reported to exhibit enhanced bioactivity compared with their hydrocarbon counterparts, particularly against microbial pathogens [20].

Fatty acid–related compounds, including hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester, and 1,9-tetradecadiene, are commonly associated with antibacterial and anti-inflammatory effects in natural extracts [21,22]. The sterol and triterpenoid constituents [ergosta-8,24(28)-dien-3-ol, α,β-amyrin, 9,19-cyclolanost-24-en-3-ol, and 4,4,6a,6b,8a,11,11,14b-octamethyl-2H-picen-3-one] are particularly noteworthy, as plant sterols and pentacyclic triterpenes are widely reported to exert anti-inflammatory, hepatoprotective, and cardioprotective effects [23,24].

Similarly, in *T. binghami* propolis, the presence of alkylated phenolic compounds such as 2-methoxy-4-vinylphenol and phenol, 2,4-bis(1,1-dimethylethyl)- supports a strong antioxidant potential, as alkyl substitution enhances phenolic stability and radical-scavenging efficiency [25,26]. Fatty acids and esters, including n-decanoic acid and hexadecanoic acid, ethyl ester, are frequently associated with antimicrobial and membrane-disruptive activities [21,22]. Sesquiterpenes and their oxygenated derivatives (caryophyllene, caryophyllene oxide, and isoaromadendrene epoxide) further contribute anti-inflammatory and antimicrobial properties [18,19]. The detection of sterols (lanosterol and 9,19-cyclolanost-24-en-3-ol) and triterpenes (olean-12-ene, α,β-amyrin, *and* 4,4,6a,6b,8a,11,12,14b-octamethyl-picen-3-one) reinforces the therapeutic relevance of this propolis, as these compounds are associated with lipid-lowering, anti-inflammatory, and anticancer activities [23,24].

In addition, *H. itama* propolis contained phenolic constituents such as 2-methoxy-4-vinylphenol and 5-heptylresorcinol, both reported to possess antioxidant and antimicrobial properties [25,26]. The sesquiterpene profile, dominated by α-cubebene, caryophyllene, (+)-epi-bicyclosesquiphellandrene, and related hydrocarbons, is consistent with the documented bioactivities of volatile terpenes, particularly their antimicrobial and anti-inflammatory effects [18]. The sterol and triterpenoid fraction (lupeol, lanosterol, α,β-amyrin, and 9,19-cyclolanost-24-en-3-ol) is particularly noteworthy, as these compounds are well recognized for their anti-inflammatory, cardioprotective, and immunomodulatory activities [23,24].

In summary, the biological activities attributed to the identified compounds, including antioxidant, antimicrobial, anti-inflammatory, and cardioprotective effects, are consistent with the chemical classes detected across all three propolis types. Therefore, these GC–MS findings provide strong chemical evidence supporting the biological potential traditionally associated with stingless bee propolis.

Building upon the chemical characterization, ethanolic extracts of Malaysian stingless bee propolis exhibited high total phenolic and flavonoid contents, with significant interspecies variation influencing their bioactivity. Polyphenols play a central role in neutralizing free radicals and mitigating oxidative damage. Consistent with previous studies [27], *G. thoracica* demonstrated the highest total phenolic content (TPC), followed by *H. itama* and *T. binghami*. Ethanol extraction yielded higher TPC values than aqueous extraction methods [28,29], likely due to its greater efficiency in solubilizing a broader spectrum of phenolic compounds. Nevertheless, regional and botanical variability can substantially influence resin composition and may account for differences reported among studies [30,31]. These findings highlight the critical roles of bee species and environmental factors in determining the chemical composition and therapeutic quality of propolis, underscoring the importance of standardized extraction protocols and quality control measures.

Similarly, total flavonoid content (TFC), determined using the aluminum nitrate colorimetric method, followed the same trend, with *G. thoracica* exhibiting the highest concentration, followed by *H. itama* and *T. binghami*. These species-dependent differences are consistent with previous reports [30,31] and are likely attributable to variations in floral sources and foraging behavior. Given the well-established role of flavonoids in antioxidant defense, such compositional differences directly influence the biological activities observed among stingless bee species.

To assess the functional implications of these phytochemicals, antioxidant assays confirmed strong radical-scavenging and reducing capacities across all extracts. *G. thoracica* exhibited the most pronounced activity, with a DPPH IC_{50} value of 0.0524 mg/mL and the highest TEAC and ABTS values, surpassing several internationally reported propolis samples [32,33]. These findings are consistent with previous studies on Malaysian propolis [34] and further emphasize the pivotal contribution of phenolic constituents to antioxidant efficacy [35,36].

Further corroborating these findings, FRAP analysis demonstrated strong ferric-reducing capacity (446.0–555.8 μM Fe^{2+}), comparable to values reported in previous studies [37,38]. Variations in FRAP performance likely reflect differences in phenolic and flavonoid composition arising from species-specific foraging behavior and botanical diversity [39,40]. Pearson correlation analysis revealed strong positive correlations between TPC, TFC, and antioxidant activities, with TPC exhibiting the highest correlation across all assays (R^2 = 0.953–0.989). These robust inter-assay correlations confirm methodological consistency and highlight the synergistic contribution of phenolic and flavonoid compounds to the antioxidant potential of stingless bee propolis.

In comparison with honeybee (*Apis mellifera*) propolis, stingless bee propolis exhibits notable differences in chemical composition and biological activity. Honeybee propolis, particularly from temperate regions, is often dominated by flavonoid aglycones, such as pinocembrin, galangin, and chrysin, and phenolic acids and their esters, which contribute significantly to its antioxidant and antimicrobial properties [41,42]. In contrast, Malaysian stingless bee propolis, as demonstrated in the present study, contains a broader array of terpenoids, sesquiterpenes, sterols, and triterpenoids in addition to phenolics, reflecting both botanical diversity and species-specific foraging behavior. Functionally, while both types of propolis exhibit antioxidant and anti-inflammatory activities, several studies report that stingless bee propolis often displays higher total phenolic content and stronger

radical-scavenging activity than honeybee propolis from similar tropical environments [43,44]. These interspecific differences likely stem from variations in resin sources and bee ecology, suggesting that the superior antioxidant performance observed in *G. thoracica* extracts is attributable not only to elevated phenolic and flavonoid concentrations but also to the synergistic effects of terpenoid and triterpenoid constituents that are less prominent in many honeybee propolis profiles.

In conclusion, the present study demonstrates that Malaysian stingless bee propolis, particularly from *G. thoracica*, exhibits superior antioxidant capacity attributable to its elevated phenolic and flavonoid contents. The strong correlations between total phenolic content, total flavonoid content, and multiple antioxidant assays confirm that these phytochemicals are the primary contributors to its bioactivity. Species-specific differences, influenced by foraging patterns and botanical origin, significantly affect the chemical composition and functional efficacy of propolis. These findings underscore the therapeutic potential of stingless bee propolis as a natural antioxidant source and emphasize the importance of species selection and standardized extraction protocols for its advancement in pharmaceutical and nutraceutical applications.

Author contributions

Nosiba A. Alsarayrah, Rafeezul Mohamed, Mohd Azwan Jaafar, and Eshaifol A. Omar: Concept and design; analysis and interpretation of data; data collection; statistical analysis; writing – original draft; writing – review and editing; critical revision of the manuscript; funding acquisition; overall supervision. All authors have read and agreed to the published version of the manuscript.

Use of Generative-AI tools declaration

The authors declare that they have not used artificial intelligence tools in the creation of this article.

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Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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