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Research Article

Antioxidant Activity of Stingless Bee Propolis (*Tetrigona apicalis*) Extracts from Dammar Forest Vegetation

Angga Yasir[1], Andi Adjeng[2], Sinta Firgianti[3], Aida Solehah[1], Veni Nurhayati[1], Untia Sari[3]

[1]Department of Cosmetic Engineering, Institut Teknologi Sumatera, Lampung, Indonesia, 35365.
[2]Department of Pharmacy, Universitas Lampung, Lampung, Indonesia, 35141.
[3]Department of Pharmacy, Institut Teknologi Sumatera, Lampung, Indonesia, 35365.

angga.yasir@km.itera.ac.id

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ABSTRACT

Propolis, a resinous substance produced by bees, is renowned for its diverse biological activities, including antioxidant properties. The antioxidant capacity of propolis is significantly shaped by the plant species from which bees collect resin. These botanical sources play a crucial role in determining the concentrations of phenolic compounds and flavonoids present in propolis, which in turn greatly impact its antioxidant properties. This study aimed to evaluate the antioxidant activity and chemical composition of stingless bee propolis from *Tetrigona apicalis*, sourced from dammar forest vegetation in Lampung Province. Ultrasonic-Assisted Extraction (UAE) was performed using 96% and 70% ethanol to assess the impact of solvent concentration on extraction efficiency and bioactive compound yield. The total flavonoid content was significantly higher in the 96% ethanol extract (0.147 mg QE/g dry propolis) compared to the 70% ethanol extract (0.015 mg QE/g dry propolis), indicating the superior efficacy of higher ethanol concentration in extracting non-polar flavonoids. Despite this, the antioxidant activity, measured by DPPH and FRAP assays, was relatively low, with an IC-50 value of 6001.33 µg/mL for DPPH and 0.067 mg AAE/g extract for FRAP. FTIR analysis confirmed the presence of functional groups associated with flavonoids, phenolic acids, and aliphatic hydrocarbons. The results suggest that the high resin content from dammar trees may dilute the concentration of potent antioxidant compounds, leading to weaker antioxidant activity. This study highlights the need for further optimization of extraction methods and comprehensive comparative studies to fully understand the bioactive potential of propolis from unique ecological settings like dammar forests. The findings contribute to the broader field of natural product research, with potential applications in cosmetics and pharmaceuticals.

Keywords: Antioxidant activity; dammar forest; flavonoids; propolis; ultrasonic-assisted extraction

INTRODUCTION

Bees create a remarkable natural compound known as propolis, a resinous material that has captured scientific interest due to its diverse biological effects. This bee-made substance has garnered attention for its impressive array of health-promoting properties, notably its capacity to combat microbes, reduce inflammation, and neutralize harmful free radicals through its potent antioxidant action (Anindya et al., 2023; Kocot et al., 2018; Przybyłek & Karpiński, 2019). Among the various types of propolis, stingless bee propolis has garnered interest due to its unique chemical composition, influenced by the specific vegetation where the bees source their resin (Popova et

al., 2021). The antioxidant activity of propolis is highly dependent on its botanical origin, which influences its phenolic and flavonoid content (Goh et al., 2023; Mahani et al., 2021).

The type and origin of propolis can significantly influence its characteristics and bioactivity. Previous research on propolis from *Tetragonula biroi* has exhibited strong antioxidant activity, as demonstrated by its 2,2-diphenyl-1-picrylhydrazyl DPPH radical scavenging capacity, reaching 82.31% at a concentration of 6.25 µg/mL (Arung et al., 2023). Similarly, propolis from *Heterotrigona item* has shown that ethanolic extracts yield significantly higher bioactive compounds than aqueous extracts, with phenolic content at 17.043 mg Gallic Acid Equivalent (GAE)/g, tannins at 5.411 mg GAE/g, and flavonoids at 0.83 mg QE/g, approximately two to four times greater than those in aqueous extracts and correlates with antioxidant activity (Lim, Chua, & Dawood, 2023). However, not all stingless bee propolis demonstrate high antioxidant potential. For instance, propolis from Brazilian stingless bees *Melipona quadrifasciata* and *Tetragonisca angustula* exhibited low antioxidant activity, with DPPH IC-50 values exceeding 1000 µg/mL (Santos et al., 2017). These findings indicate that the antioxidant properties of stingless bee propolis are highly variable, contingent upon both botanical origin and extraction processes, thus necessitating further investigation into propolis from diverse environmental and botanical contexts.

Antioxidant properties of *Tetrigona apicalis* propolis include studies from Peninsular Malaysia, where the ethanolic extract of propolis (EEP) demonstrated 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic (ABTS+) scavenging activity with an inhibition of 9.5% and an IC-50 value of 1.68 mg/mL (Mohamed et al., 2020). Further research by Mohamed (2022) revealed that the EEP exhibited IC-50 values of 1.78 mg/mL for DPPH and 1.68 mg/mL for ABTS+, along with total phenolic content (TPC) and total flavonoid content (TFC) of 31.99 mgGAE/g and 66.4 mg quercetin equivalent (QCE)/g, respectively, with strong correlations between these parameters (Mohamed et al., 2022). Despite these findings, a comprehensive assessment of the antioxidant potential of *T. apicalis* propolis from dammar forest vegetation is still necessary to expand the understanding of its bioactive properties within this unique ecological context.

Initial studies have indicated that the antioxidant activity of propolis from some tropical regions may be moderate and not necessarily superior to other sources (Huang et al., 2014). In tropical regions like Southeast Asia, stingless bee species such as *T. apicalis* are known to collect resin from diverse plant species found in dammar forests, a type of forest ecosystem characterized by high biodiversity (Popova et al., 2022). However, while propolis from other bee species and regions has been extensively studied, limited research exists on the antioxidant potential of *T. apicalis* propolis derived from dammar forest vegetation. Considering the distinctive flora within dammar forests, it is plausible that the propolis produced in these environments may possess unique antioxidant properties. However, preliminary studies suggest that the antioxidant potential of propolis from certain tropical regions may be moderate and not necessarily superior to those from other sources (Huang et al., 2014). Consequently, a targeted investigation into the antioxidant activity of *T. apicalis* propolis from dammar forest vegetation is essential to elucidate its potential and position it within the broader context of propolis research.

This research primarily aims to assess the antioxidant activity of propolis derived from *T. apicalis* in dammar forest vegetation from Lampung Province. The study not only seeks to determine the antioxidant capacity of this stingless bee propolis using various assays, such as DPPH and FRAP but also includes a comprehensive characterization process. Ethanol 70% was used as an initial screening solvent to determine the most suitable solvent for extracting propolis. This characterization involves phytochemical screening, determination of total flavonoid content, and Fourier-transform infrared spectroscopy (FTIR) analysis to identify the chemical bonds present in the propolis. Unlike previous studies that focused solely on identifying propolis with exceptionally high antioxidant activity, this research is exploratory, providing a baseline understanding of the antioxidant properties and chemical composition of this specific type of propolis. By doing so, the study contributes to the broader field of natural product research and offers insights into the potential applications of propolis from unique ecological settings.

METHODS

Extraction

The extraction process began with weighing 100 grams of propolis, which was obtained from a local

Micro, Small, and Medium Enterprise- MSME (PT Madu Suhita), located in Pesisir Barat, Lampung province, followed by the addition of 1 liter of 96% or 70% ethanol as a solvent (technical grade ethanol was obtained from PT Brataco), maintaining a ratio of 1:10. The mixture underwent Ultrasonic-Assisted Extraction (UAE) at 60 °C for 30 minutes. After extraction, the mixture was filtered using a Buchner funnel to separate the extract from the filtrate. The extract was then subjected to solvent removal using a rotary evaporator (IKA RV10, Germany) at 40 °C under 650 mmHg pressure. Finally, any residual solvent in the extract was evaporated in an oven set at 45 °C, ensuring thorough solvent removal and extract preparation for further analysis. The extraction efficiency is quantified by calculating the yield percentage. This is accomplished by first determining the ratio of the extracted material's mass to the initial propolis sample mass. This ratio is then converted to a percentage by multiplying it by 100, which measures how much propolis was successfully extracted.

Phytochemical Screening

Flavonoid

The process to detect flavonoids in the propolis extract begins by combining 1 gram of the ethanol-derived extract with 1 ml of methanol in a test tube. To this mixture, two drops of a 10% sodium hydroxide (NaOH) solution are introduced. The contents are then thoroughly agitated. The crucial indicator of flavonoid presence lies in the subsequent color transformation. A positive result is signaled by a notable shift in hue, with the initial light green color potentially changing to yellow, red, brown, or a deeper green shade. This distinctive chromatic alteration serves as a definitive marker, confirming the existence of flavonoid compounds within the propolis extract under examination (Kazia et al., 2017).

Tannin

The procedure for identifying tannins in the propolis extract commences with the preparation of a solution. This involves combining equal parts of the ethanol-based propolis extract and methanol, using 1 mL of each. The resulting mixture serves as the base for the subsequent chemical reaction. To this prepared solution, a small amount of ferric chloride is introduced. Specifically, 2-3 drops of a 5% ferric chloride solution are added to the extract-methanol mixture. Following the addition, the sample is agitated to ensure thorough mixing. The critical indicator for the presence of tannins lies in the color change that occurs after mixing. A positive result is characterized by the development of either a deep blue or a greenish-black hue. This distinctive chromatic shift serves as a definitive marker, confirming the existence of tannin compounds within the propolis extract under examination. (Mulyani, 2011).

Alkaloid

To detect the presence of alkaloids in the propolis extract, a specific chemical assay was employed. The process began with the preparation of a sample solution by dissolving a precise amount of the ethanol-based propolis extract gram- in an equal volume (1 mL) of methanol. The critical step in this test involved the introduction of Dragendorff's reagent to the prepared solution. Specifically, five drops of this reagent were carefully added to the propolis-methanol mixture. The definitive indicator for the presence of alkaloids in this test is a distinct visual change. A positive result is characterized by the formation of a red precipitate within the test tube. This color-specific precipitation serves as a clear marker, confirming the existence of alkaloid compounds in the propolis extract under examination. (Ergina, 2014).

Saponin

The procedure to identify saponins in the propolis extract involves a specific sequence of steps designed to exploit the characteristic properties of these compounds. Initially, a sample is prepared by combining 1 gram of the ethanol-derived propolis extract with 5 mL of purified water in a test tube. To induce the formation of foam, a key indicator of saponin presence, the mixture is subjected to vigorous agitation. Specifically, the test tube is shaken intensely for 10 seconds, promoting the interaction between any potential saponins and the aqueous medium. Following this agitation, the test enters a critical phase with the introduction of hydrochloric acid. A measured amount of 2 N HCl is carefully added to the foamy mixture. The persistence of the foam upon acid addition serves as the definitive marker for saponin presence. The hallmark of a positive result in this assay is the stability of the generated foam even after the acidification step. If the foam remains intact and does not dissipate upon HCl addition, it strongly indicates the presence of saponin compounds within the propolis extract under examination. (Nugraha et al., 2024).

Total Flavonoid Content

Quantification of the overall flavonoid content was accomplished through spectrophotometric analysis. The specific instrument employed for this purpose was a Shimadzu UV-Vis spectrophotometer, model 1240, manufactured in Tokyo, Japan. This advanced analytical tool allowed for precise measurement of light absorption by the sample solutions. To ensure accuracy and provide a basis for comparison, quercetin was utilized as the reference standard in this assay. This well-established flavonoid served as a calibration point, allowing for the conversion of absorption readings into meaningful concentration values for the total flavonoids present in the propolis extracts.

Determination of Calibration Curve

The process began with the creation of a quercetin stock solution at a concentration of 1000 µg/mL in a 50 mL volume. From this stock, a series of dilutions were prepared to achieve a range of lower concentrations: 10, 8, 6, 4, and 2 µg/mL, each in a 10 mL volume. For the spectrophotometric analysis, a specific mixture was prepared using each concentration. The procedure involved extracting 1 mL from each diluted solution and combining it with several reagents: 3 mL of ethanol, 2 mL of aluminum chloride ($AlCl_3$) at 10% concentration, and 2 mL of 1 molar sodium acetate (CH_3COONa). This mixture was then brought to a final volume of 10 mL in a volumetric flask by adding distilled water. Following preparation, the solutions were left to incubate for 30 minutes, allowing for complete reaction development. To determine the optimal wavelength for subsequent measurements, the two µg/mL solution was utilized. Its absorbance was measured across a spectrum ranging from 350 to 450 nanometers. This scan allowed for the identification of the wavelength at which maximum absorption occurred, which would serve as the reference point for future analyses (Hasan et al., 2023).

Determination of Total Flavonoid Content

The analytical process began with the preparation of a concentrated propolis extract solution. A 10,000 µg/mL concentration was achieved by dissolving an appropriate amount of propolis extract in 10 mL of 96% ethanol. From this concentrated solution, a 4 mL aliquot was extracted for further analysis. This sample then underwent a series of chemical additions to facilitate the spectrophotometric measurement of flavonoids. Specifically, 2 mL of aluminum chloride ($AlCl_3$) at a 10% concentration and 2 mL of 1 M sodium acetate (CH_3COONa) were introduced to the propolis extract. To standardize the volume for analysis, the mixture was then diluted with additional ethanol in a volumetric flask, bringing the total volume to precisely 10 mL. This step ensures consistency in concentration across samples. Following preparation, the solution was allowed to incubate for 30 minutes. This incubation period allows for the complete development of the color reaction between the flavonoids in the propolis extract and the added reagents. The final step involved the quantitative analysis of the prepared sample. Using a UV-Vis spectrophotometer, the absorbance of the solution was measured at a specific wavelength of 420 nm. This wavelength was likely chosen based on its optimal sensitivity for detecting the flavonoid-aluminum complex formed during the reaction (Hasan et al., 2023).

The total flavonoid content was calculated using a standard calibration curve based on quercetin, a common flavonoid reference compound. The results were expressed as milligrams of quercetin equivalents per gram of propolis extract (mg QE/g). The calibration curve was constructed by plotting the absorbance of various known concentrations of quercetin solutions (2–10 µg/mL) at 420 nm, allowing for the interpolation of flavonoid concentrations in the propolis samples (Hasan et al., 2023)

Antioxidant Activity (DPPH)

Preparation of DPPH Solution

Weigh 8 mg of DPPH and place it into a 100 mL volumetric flask to make an 80 µg/mL solution, which is then dissolved using 96% ethanol up to the mark (Hasan et al., 2023).

Preparation and Determination of Sample Extract Solution

The antioxidant potential of propolis was evaluated through a series of meticulously designed experimental procedures. Initially, a concentrated propolis extract solution was formulated by precise gravimetric measurement of 1 g propolis extract, which was subsequently transferred to a volumetric apparatus of 100 mL capacity. The vessel was then filled to its calibration mark with 96% ethanol, yielding a solution with a nominal concentration of 10,000 µg/mL. From this stock, a gradient of concentrations was systematically prepared, encompassing 1000,

2500, 5000, and 10,000 µg/mL. The protocol then dictated the transfer of 1 mL aliquots from each concentration into separate reaction vessels, followed by the introduction of an equivalent volume of 2,2-diphenyl-1-picrylhydrazyl (DPPH) reagent. These binary mixtures were subjected to a 30-minute incubation period under light-exclusion conditions. Post-incubation, the samples underwent spectrophotometric analysis using a Shimadzu UV-Vis spectrophotometer model 1240 (Tokyo, Japan). The absorbance of each concentration was quantified at the predetermined optimal wavelength of 517 nanometers, corresponding to the absorption maximum of DPPH. This analytical approach enabled the assessment of the propolis extract's capacity to neutralize free radicals, thus providing insights into its antioxidant efficacy (Hasan et al., 2023).

Preparation of Control Solution

The control solution is made by adding 1 mL of 96% ethanol into a vial, then adding 1 mL of DPPH, and incubating for 30 minutes. After incubation, the wavelength is measured at a maximum wavelength of 517 nm, sing a Shimadzu UV-Vis spectrophotometer model 1240 (Tokyo, Japan) (Hasan et al., 2023).

Antioxidant Activity (FRAP)

Antioxidant testing was conducted using the *Ferric Reducing Antioxidant Power* (FRAP) method. The preparation of materials consisted of propolis extract, ascorbic acid, distilled water, 0.1% FeCl3, phosphate buffer with a concentration of 0.2 and a pH of 6.6, 96% ethanol, 10% trichloroacetic acid (Merck), and 1% potassium ferricyanide.

Determination of Calibration Curve

To generate an ascorbic acid standard curve, a 1000 µg/mL solution was prepared using distilled water and subsequently diluted to 4, 6, 8, 10, and 12 µg/mL. Equal volumes (1 mL) of each diluted sample and phosphate buffer were combined. This mixture was then added to 1 mL potassium ferricyanide and vortexed for 2 minutes. Following incubation at 50 °C for 20 minutes, 1 mL trichloroacetic acid was introduced, and the solution was centrifuged (3000 rpm, 10 minutes). The resulting supernatant (1 m) was mixed with an equal volume of $FeCl_3$, and absorbance was measured at 650-730 nm using a Shimadzu UV-Vis spectrophotometer model 1240 (Tokyo, Japan) (Nasir et al., 2021).

Antioxidant Activity Test on Propolis Extract

Propolis antioxidant activity was evaluated using a stock solution (10,000 µg/mL) prepared in 96% ethanol. This stock was diluted to 2500, 5000, and 8000 µg/mL. Equal volumes (1 mL) of each dilution and phosphate buffer were combined, then mixed with 1 mL of potassium ferricyanide and vortexed for 2 minutes. After incubation (50 °C, 20 minutes), 1 mL trichloroacetic acid was added, and the mixture was centrifuged (3000 rpm, 10 minutes). The supernatant (1 mL) was then combined with an equal volume of $FeCl_3$, and absorbance was measured at 700 nm using a Shimadzu UV-Vis spectrophotometer model 1240 (Tokyo, Japan). The absorbance obtained from the measurements was extrapolated using the linear regression equation derived from the ascorbic acid standard curve, yielding the antioxidant value expressed in units of Ascorbic Acid Equivalent (AAE) (Nasir et al., 2021).

FTIR

The Fourier Transform Infrared (FTIR) analysis was conducted using a Cary 630 FTIR (California, USA). Before sample analysis, a background scan was performed without the sample to establish accurate baseline measurements. For the analysis, the propolis extract was first dissolved in ethanol, and two µL of the resulting solution was then placed in the FTIR sample holder. Each sample was subjected to 32 scans at a resolution of 4 cm^{-1}, covering the spectral range from 4000 to 500 cm^{-1}. The analysis was performed in multiple replications for each propolis extract solution to ensure consistency and reliability. The data obtained from the FTIR analysis were then processed and analyzed using OriginLab software to identify and interpret the functional groups present in the propolis extract

Data Analytic

The yield value and total flavonoid content of propolis extracts obtained using 70% and 96% ethanol as solvents were compared using a t-test. This statistical analysis was conducted to determine whether there were significant differences in the flavonoid content extracted by the two solvents. The results were reported as mean ± standard deviation, and a significance level of $p < 0.05$ was used to evaluate the statistical differences.

RESULTS AND DISCUSSION

The data presented in Table 1 highlights the significant impact of solvent type on the extraction yield and phytochemical content of stingless bee propolis from dammar forest vegetation (p-value < 0.05). The comparison between 70% ethanol and 96% ethanol solvents reveals important differences in extraction efficiency and the resulting phytochemical content, which are crucial for understanding the bioactive potential of the extracts.

Table 1. Characterisation of Propolis Extract

Test	Solvent	
	Ethanol 70%	**Ethanol 96%**
Yield value	2.05 %	56%*
Phytochemical screening		
Flavonoid	+	+
Tannin	+	+
Saponin	+	+
Alkaloid	+	+
Total Flavonoid Content**	0.015 ± 0.002	0.147 ± 0.002*

* p Value < 0.05
** mg Quarcetin Equivalent (QE) /g dry propolis

The yield value, which indicates the efficiency of the extraction process, shows a stark contrast between the two solvents (Table 1). The 96% ethanol solvent resulted in a substantially higher yield of 56%, compared to only 2.05% for the 70% ethanol solvent. This difference suggests that the higher ethanol concentration is much more effective at extracting the bioactive components from propolis, likely due to its greater ability to dissolve non-polar compounds that are abundant in propolis. Previous research on the extraction of propolis has demonstrated a wide range of yield values, heavily influenced by the solvents used and the specific extraction methods employed. Trusheva et al. (2007) utilized ultrasonic extraction with a solvent-to-solid ratio of 1:10, using ethanol as the solvent for 30 minutes, achieving a yield value of 53%. This highlights the efficiency of ultrasonic extraction combined with high-concentration ethanol in extracting bioactive compounds from propolis. Similarly, Pramono and Puspitasari (2015) achieved a high yield value of 51.76% by using maceration with 96% ethanol over 120 hours (5 days), where the prolonged extraction time and strong solvent likely contributed to the substantial yield (Pramono & Puspitasari, 2015). Chong and Chua (2020) employed ultrasound-assisted extraction with different solvents, including 96% ethanol, water, 20% PEG-400-added ethanol, and 20% PEG-400-added water using a solid-to-solvent ratio of 1:10. They observed yield values ranging from 35.7% to 42.6%, depending on the solvent system. The use of ultrasound enhanced the extraction efficiency, although the yield varied based on the solvent's polarity and composition (Chong & Chua, 2020). In contrast, Lim et al. (2023) utilized a milder extraction method by soaking propolis in either distilled water or 20% aqueous ethanol at room temperature for 7 days, resulting in lower yields between 4% and 5.5% (Lim, Chua, & Soo, 2023).

Both ethanol concentrations were effective in extracting key phytochemicals, as evidenced by the positive results (+) for flavonoids, tannins, saponins, and alkaloids in both extracts, as shown in Table 1. This suggests that, regardless of the solvent concentration, these compounds are sufficiently polar to be extracted by ethanol. However, the presence of these phytochemicals alone does not provide complete insight into their concentration or bioactivity, which is why further quantitative analysis, such as total flavonoid content, is necessary.

A more detailed examination of the total flavonoid content, as provided in Table 1, highlights the significant impact of solvent concentration on the extraction of specific bioactive compounds from propolis, with 96% ethanol resulting in a much higher total flavonoid content of 0.147 mg QE/g dry propolis compared to just 0.015 mg QE/g in the 70% ethanol extract. This nearly tenfold increase underscores the efficiency of 96% ethanol in extracting flavonoids, which are crucial antioxidants contributing to the bioactivity of propolis. These findings align with Fikri et al. (2019), who found that 75% ethanol extracted a higher total flavonoid content (3.39 mg/g) compared to water (1.5 mg/g), indicating that ethanol, particularly at higher concentrations, is generally more effective in extracting flavonoids than more polar solvents like water. This can be attributed to the fact that some flavonoids are non-polar or less polar (Mello et al., 2010), making them more soluble in higher concentrations of ethanol. For example, flavonoids like kaempferol and apigenin, which have fewer hydroxyl groups and more non-polar characteristics, are better extracted with less polar solvents like 96% ethanol. However, this study's findings contrast with those reported by Kolaylı and Birinci (Kolaylı & Birinci, 2024), who found that a 70% ethanolic extract contained the highest levels of phenolic compounds and demonstrated the greatest antioxidant capacity, suggesting that a more polar solvent might be more effective for extracting certain bioactive compounds. Similarly, Woźniak et al. (2020) reported that quercetin content was higher in 70% ethanol extracts (2.3 mg/g) compared to 96% ethanol extracts (1.3 mg/g), indicating that 70% ethanol might be more efficient for extracting specific flavonoids like quercetin (Woźniak et al., 2020). Based on the results in Table 1, 96% ethanol demonstrated superior extraction efficiency, yielding higher amounts of bioactive compounds, especially flavonoids. Therefore, 96% ethanol has

been selected as the solvent for further testing, specifically for antioxidant activity assays using DPPH and FRAP, as well as FTIR analysis, to fully assess the bioactive potential of stingless bee propolis.

According to the results in Table 1, the yield value, which indicates the efficiency of the extraction process, shows a stark contrast between the two solvents. The 96% ethanol solvent resulted in a substantially higher yield of 56%, compared to only 2.05% for the 70% ethanol solvent. This difference suggests that the higher ethanol concentration is much more effective at extracting the bioactive components from propolis, likely due to its greater ability to dissolve non-polar compounds that are abundant in propolis. Previous research on the extraction of propolis has demonstrated a wide range of yield values, heavily influenced by the solvents used and the specific extraction methods employed. Trusheva et al. (2007) utilized ultrasonic extraction with a solvent-to-solid ratio of 1:10, using ethanol as the solvent for 30 minutes, achieving a yield value of 53%. This highlights the efficiency of ultrasonic extraction combined with high-concentration ethanol in extracting bioactive compounds from propolis. Similarly, Pramono and Puspitasari (2015) achieved a high yield value of 51.76% by using maceration with 96% ethanol over 120 hours (5 days), where the prolonged extraction time and strong solvent likely contributed to the substantial yield (Pramono & Puspitasari, 2015). Chong and Chua (2020) employed ultrasound-assisted extraction with different solvents, including 96% ethanol, water, 20% PEG-400-added ethanol, and 20% PEG-400-added water, using a solid-to-solvent ratio of 1:10. They observed yield values ranging from 35.7% to 42.6%, depending on the solvent system. The use of ultrasound enhanced the extraction efficiency, although the yield varied based on the solvent's polarity and composition (Chong & Chua, 2020). In contrast, Lim et al. (2023) utilized a milder extraction method by soaking propolis in either distilled water or 20% aqueous ethanol at room temperature for 7 days, resulting in lower yields between 4% and 5.5% (Lim, Chua, & Soo, 2023).

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A more detailed examination of the total flavonoid content, as provided in Table 1, highlights the significant impact of solvent concentration on the extraction of specific bioactive compounds from propolis, with 96% ethanol resulting in a much higher total flavonoid content of 0.147 mg QE/g dry propolis compared to just 0.015 mg QE/g in the 70% ethanol extract. This nearly tenfold increase underscores the efficiency of 96% ethanol in extracting flavonoids, which are crucial antioxidants contributing to the bioactivity of propolis. These findings align with Fikri et al., (2019), who found that 75% ethanol extracted a higher total flavonoid content (3.39 mg/g) compared to water (1.5 mg/g), indicating that ethanol, particularly at higher concentrations, is generally more effective in extracting flavonoids than more polar solvents like water. This can be attributed to the fact that some flavonoids are non-polar or less polar (Mello et al., 2010), making them more soluble in higher concentrations of ethanol. For example, flavonoids like kaempferol and apigenin, which have fewer hydroxyl groups and more non-polar characteristics, are better extracted with less polar solvents like 96% ethanol. However, this study's findings contrast with those reported by Kolaylı and Birinci (Kolaylı & Birinci, 2024), who found that a 70% ethanolic extract contained the highest levels of phenolic compounds and demonstrated the greatest antioxidant capacity, suggesting that a more polar solvent might be more effective for extracting certain bioactive compounds. Similarly, Woźniak et al. (2020) reported that quercetin content was higher in 70% ethanol extracts (2.3 mg/g) compared to 96% ethanol extracts (1.3 mg/g), indicating that 70% ethanol might be more efficient for extracting specific flavonoids like quercetin (Woźniak et al., 2020).

Based on the results in Table 1, 96% ethanol demonstrated superior extraction efficiency, yielding higher amounts of bioactive compounds, especially flavonoids. Therefore, 96% ethanol has been selected as the solvent for further testing, specifically for antioxidant activity assays using DPPH and FRAP, as well as FTIR analysis, to fully assess the bioactive potential of stingless bee propolis.

Antioxidant Activity (DPPH)

The IC-50 value of 6001.33 µg/mL for stingless bee propolis from dammar forest vegetation, obtained using the DPPH method, is the result of extrapolation from the regression equation derived from the curve shown in Figure 1. This figure presents the graphical representation of the antioxidant activity, where the DPPH radical scavenging effect is plotted against the concentration of the propolis extract. The regression equation obtained from this curve was used to calculate the IC-50 value, which represents the concentration required to inhibit 50% of the DPPH radicals. In this case, the extrapolated IC-50 value of 6001.33 µg/mL

falls well outside the typical range for strong or even moderate antioxidant activity, indicating an extremely weak potential for antioxidant action in this propolis sample.

The extremely weak antioxidant activity observed in the stingless bee propolis from dammar forest vegetation, as indicated by the high IC-50 value of 6001.33 µg/mL, can likely be attributed to the unique botanical origin of this propolis. Specifically, the high resin content from the dammar gum, which is the primary source of propolis in this region, plays a significant role in this outcome. This result contrasts sharply with the higher antioxidant potentials reported in various studies for propolis from other stingless bee species and regions. For example, research on propolis from *T. biroi* demonstrated strong antioxidant activity, achieving 82.31% DPPH radical scavenging capacity at a concentration of just 6.25 µg/mL (Arung et al. 2023). This stark difference in antioxidant capacity suggests that the propolis from *T. biroi* contains higher concentrations of active compounds, such as flavonoids and phenolic acids, which are known contributors to antioxidant activity. In contrast, the dominance of dammar resin in the propolis from dammar forest vegetation likely dilutes the concentration of these more effective antioxidant compounds, leading to the observed low bioactivity.

Similarly, *Heterotrigona* items propolis has shown significantly higher bioactive compound content in ethanolic extracts compared to aqueous extracts. These extracts exhibited a phenolic content of 17.043 mg GAE/g and a flavonoid content of 0.83 mg QE/g, which correlated with enhanced antioxidant activity (Lim et al., 2023). The extraction method, therefore, plays a critical role in determining the bioactivity of propolis, with ethanolic extracts generally yielding more potent antioxidant properties.

It is important to note that not all samples of stingless bee propolis exhibit high antioxidant activity. For instance, propolis from Brazilian stingless bees such as *M. quadrifasciata* and *T. angustula* demonstrated low antioxidant effects, with DPPH IC-50 values greater than 1000 µg/mL (Santos et al. 2017). These findings are more consistent with the results observed for propolis from the dammar forest, indicating that environmental and botanical factors may lead to a reduced concentration of antioxidant compounds.

In comparison, the propolis from *T. apicalis* in Peninsular Malaysia, particularly its ethanolic extract, exhibited moderate antioxidant activity, with an IC-50 value of 1.68 mg/mL for ABTS+ (1680 µg/mL) and 1.78 mg/mL for DPPH (1780 µg/mL), along with relatively high total phenolic and flavonoid contents (Mohamed et al. 2020, 2022). Although these values are higher than the 200 µg/mL threshold for weak antioxidant activity, they still indicate a better antioxidant potential compared to the propolis from dammar forest vegetation.

The weak antioxidant activity observed in the propolis from dammar forest vegetation could be attributed to several factors, including the specific plant species available in the dammar forest, the extraction method used, or the inherent chemical profile of the propolis itself. This variability highlights the necessity for comprehensive studies to explore the full spectrum of bioactive compounds in propolis from diverse ecological contexts and to optimize extraction techniques that maximize the yield of antioxidants.

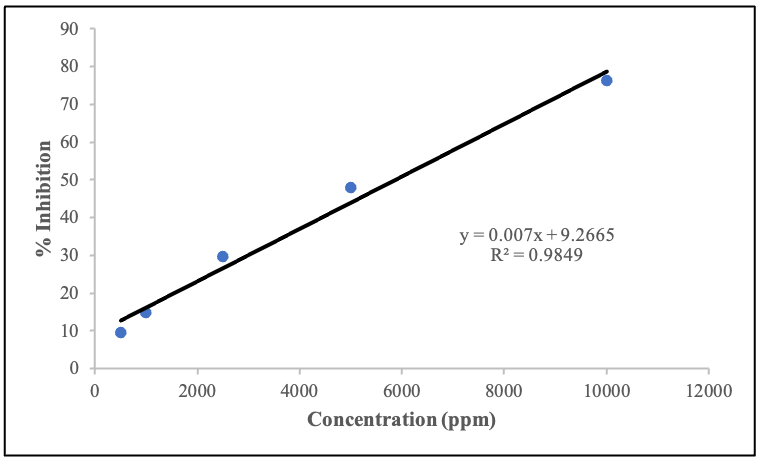

Figure 1. Diagram of antioxidant activity (DPPH) on samples of ethanol 96% extract of Propolis

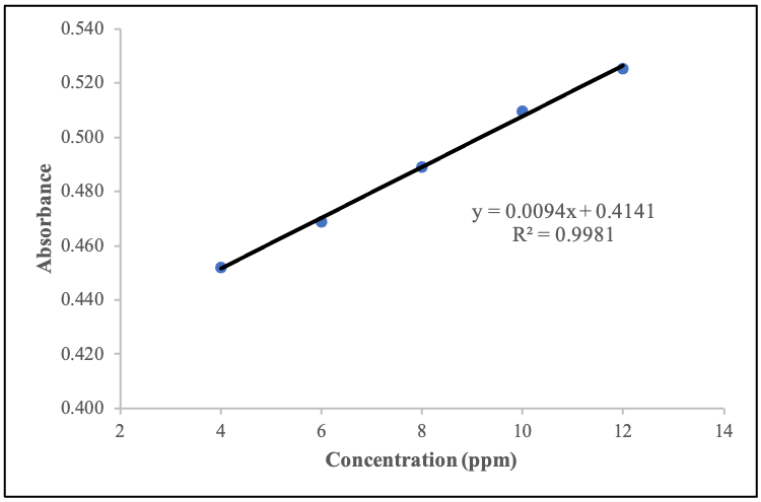

Figure 2. Calibration curve for determination of FRAP value using ascorbic acid (AA) standards

Antioxidant Activity (FRAP)

The antioxidant activity of stingless bee propolis from dammar forest vegetation, measured in this study, reveals significantly lower values compared to the propolis samples investigated by Pratama et al. (2018). The calibration curve provided in Figure 2 was used to determine the equivalence of ascorbic acid (vitamin C) in the antioxidant activity measured by the FRAP method.

This curve allowed for the precise calculation of antioxidant capacity, which was then applied to the propolis extracts to quantify their total antioxidant capacity in terms of ascorbic acid equivalents. In Pratama's study, the antioxidant activities of propolis samples circulating in Makassar were determined using the FRAP (Ferric Reducing Antioxidant Power) method, with reported values ranging from 49.3 mg AAE/g to 97.1 mg AAE/g (Pratama et al. 2018). These values are substantially higher than the antioxidant activities obtained in this study, where the propolis extract demonstrated an antioxidant activity of 0.067 mg AAE/g of extract and 0.0378 mg AAE/g of dry propolis, as shown in Table 2.

Table 2. Total antioxidant capacity of ethanol 96% extract of propolis using FRAP method

Antioxidant Capacity	
mg AAE/g extract	mg AAE/g dry propolis
0.067 ± 0.009	0.0378 ± 0.005

FTIR

The infrared (IR) spectral analysis of propolis, as seen in Figure 3, reveals a complex composition characterized by various functional groups, including O–H stretching vibrations at 3342 cm^{-1}, indicative of alcohols and phenols commonly found in flavonoids and phenolic acids (Galeotti et al., 2018). The presence of C–H stretching vibrations at 2917 and 2850 cm^{-1} suggests aliphatic hydrocarbons, likely from fatty acids or waxes (Bankova et al., 2014). An unusual peak at 2125 cm^{-1}, potentially corresponding to C≡C or C≡N stretching, may indicate trace amounts of alkynes or nitriles, although such groups are not typically dominant in propolis (Pasupuleti et al., 2017).

The carbonyl group, identified by the C=O stretching vibration at 1691 cm^{-1}, suggests the presence of ketones, aldehydes, esters, or carboxylic acids (Silva-Carvalho et al., 2015). Further, the peaks at 1458 cm^{-1} and 1376 cm^{-1} correspond to C–H bending vibrations in $–CH_2$, $–CH_3$ groups, and methyl groups, respectively, pointing to the presence of aliphatic chains, likely from lipid components (Bonvehí et al., 1994; Kalogeropoulos et al., 2009; Nedji & Loucif-Ayad, 2014; Woźniak et al., 2022). Lastly, the peak at 1021 cm^{-1} corresponds to C–O stretching vibrations, potentially from alcohols, esters, or ethers, which may be associated with flavonoids, phenolic acids, or glycosides (Santos et al., 2017). These findings align with the known composition of propolis, comprising flavonoids, phenolic acids, waxes, and fatty acids, which contribute to its diverse biological activities, including antioxidant, antimicrobial, and anti-inflammatory properties.

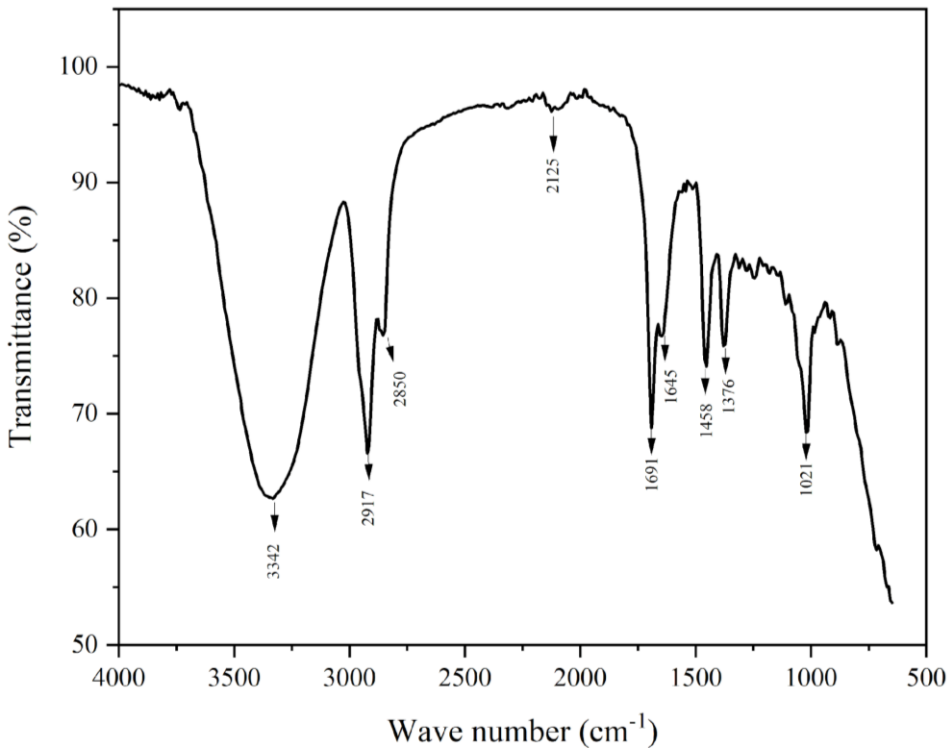

Figure 3. Fourier transform infrared spectroscopy (FTIR) spectra of the propolis extract

CONCLUSION

Ethanol 96% was more effective than 70% ethanol in extracting bioactive compounds, yielding higher total flavonoid content. Despite this, the flavonoid content and antioxidant activity were lower compared to other studies, likely due to the unique botanical origin of the propolis, particularly the high resin content from dammar trees. FTIR analysis supported the phytochemical screening by confirming the presence of functional groups indicative of flavonoids, phenolic acids, and aliphatic hydrocarbons. These findings highlight the need for further isolation or fractionation to enhance the extraction of potent bioactive compounds and maximize the antioxidant potential of dammar forest propolis.

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