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**PHYTOCHEMICAL CHARACTERIZATION AND ANTIOXIDANT ACTIVITY OF
 BALI PROPOLIS FOR DENTAL RESEARCH**

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ABSTRACT

Background: Propolis obtained from *Trigona* sp. has attracted attention as a potential source of bioactive compounds for oral-health research. However, the chemical profile and antioxidant capacity of propolis obtained in Bali remain insufficiently characterized.

Objectives: This study characterized the phytochemical composition and antioxidant activity of propolis obtained from *Trigona* sp. in Bali, Indonesia, to provide baseline information for subsequent dental research.

Methods: Raw propolis was extracted with 96% ethanol by maceration. Quantitative analyses were performed for total flavonoids, phenolic compounds, tannins, alkaloids, saponins, steroids, and sugars. Antioxidant radical-scavenging activity was determined by means of the DPPH assay, and the IC_{50} was derived from the concentration-response relationship. **Results:** The extract contained 1.87% w/w total flavonoids, 1.59% w/w phenolic compounds, 1.14% w/w tannins, 0.25% w/w alkaloids, 0.77% w/w saponins, 30.6% w/w steroids expressed as β -sitosterol equivalent, and 8.17% w/w sugars. DPPH testing yielded an IC_{50} of 147 μ g/mL, classified as moderate according to the criteria applied in this study. **Conclusion:** The study establishes a quantitative phytochemical and antioxidant profile for the investigated Bali propolis preparation. The observed DPPH activity provides preliminary biochemical evidence for further dental investigation but does not demonstrate clinical efficacy. Future studies should identify individual bioactive compounds and assess antimicrobial, antibiofilm, anti-inflammatory, cytotoxicity, formulation, and clinical outcomes.

Keywords: Antioxidant activity; Bali propolis; Dental research; Phytochemical characterization

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INTRODUCTION

Propolis is a bee-derived material composed of plant resins and exudates that are modified by bees. Its chemical composition varies according to botanical source, geographical origin, bee species, environmental conditions, and processing methods, resulting in differences in its biological activity.^{1,2} Propolis has been investigated in dental research because of its potential antioxidant, antimicrobial, and anti-inflammatory properties, including applications in periodontal and other oral-health fields.³⁻⁷

Antioxidant activity is particularly relevant because phenolic and flavonoid compounds in propolis can contribute to radical-scavenging activity. However, this activity varies among propolis samples from different geographical and botanical sources.^{8,9} Studies of Indonesian stingless-bee propolis have also demonstrated differences in chemical composition, bioactive constituents, antioxidant activity, and other biological properties according to bee species and geographical origin.^{10,11} Therefore, findings obtained from one propolis preparation cannot necessarily be extrapolated to another source.

Although research on Indonesian stingless-bee propolis has increased, information on the phytochemical composition and antioxidant activity of *Trigona* sp. propolis from Bali remains limited. Differences in botanical source, bee species, extraction procedures, and analytical methods further support the need for source-specific characterization before its potential dental applications can be investigated.

The present study therefore characterized the major phytochemical groups and antioxidant activity of a Bali-derived *Trigona* sp. propolis preparation using quantitative phytochemical analysis and the DPPH assay. The findings are intended to provide baseline information for subsequent biological, formulation, and dental research rather than to establish therapeutic or clinical efficacy.

MATERIALS AND METHODS

Study Design and Propolis Sample

A laboratory analytical study was conducted to characterize the phytochemical composition and antioxidant activity of propolis obtained from *Trigona* sp. in Bali, Indonesia. Raw propolis was processed into an ethanolic extract and subsequently analyzed for selected phytochemical constituents and antioxidant activity.

Preparation of Propolis Extract

For extraction, 1 kg of raw propolis was reduced into small pieces and macerated with 96% ethanol for 24 h. The mixture was filtered using a Buchner funnel, and the filtrate was evaporated under reduced pressure in a rotary evaporator at a water-bath temperature of 60°C. The concentrated extract was subsequently maintained in a porcelain dish in a water bath at 70°C with continuous stirring until a constant mass was obtained. The resulting raw propolis extract was weighed and stored for subsequent analyses.

Quantitative Phytochemical Analysis

Quantitative phytochemical analyses were performed at the Integrated Research and Testing Laboratory (LPPT), Universitas Gadjah Mada, Yogyakarta. The analyses included total flavonoids, total phenolic compounds, total tannins, total alkaloids, total saponins, total steroids, and total sugars. Results were expressed as percentage by weight (% w/w).

Total Flavonoid Content

Total flavonoid content was determined using a spectrophotometric method with quercetin as the reference standard. The extract was subjected to acid treatment and extraction, followed by reaction with sodium hydroxide and aluminum chloride. The absorbance values were recorded at a wavelength of 510 nm. Three analytical replicates were performed, and the results were expressed as % w/w quercetin equivalent.

Total Phenolic Content

Total phenolic content was determined using the Folin–Ciocalteu spectrophotometric method. The extract was reacted with Folin–Ciocalteu reagent and sodium carbonate, followed by incubation and spectrophotometric measurement at 755 nm. Three analytical replicates were performed, and the results were expressed as % w/w.

Total Tannin Content

Total tannin levels were quantified by spectrophotometric analysis using tannic acid as the calibration standard. Approximately 100 mg of extract was extracted with diethyl ether for 20 h. The resulting preparation was reacted with Folin–Ciocalteu reagent and sodium carbonate, followed by incubation for 30 min. Absorbance was measured at 760 nm. Three analytical replicates were performed, and the results were expressed as % w/w tannic acid equivalent.

Total Alkaloid Content

Total alkaloid content was determined spectrophotometrically using quinine as the reference standard. Approximately 100 mg of extract was treated with 2 N hydrochloric acid, followed by chloroform extraction and reaction with bromocresol green and phosphate buffer. The resulting chloroform fraction was analyzed spectrophotometrically at 470 nm. Two analytical measurements were reported, and the results were expressed as % w/w quinine equivalent.

Total Saponin Content

Total saponin content was determined using a spectrophotometric method with Quillaja bark saponin as the reference standard. Approximately 100 mg of extract was treated with sulfuric acid and subsequently reacted with anisaldehyde and sulfuric acid. After heating at 60°C, absorbance was measured at 435 nm. Two analytical measurements were reported, and the result was expressed as % w/w saponin equivalent.

Total Steroid Content

Total steroid content was determined using thin-layer chromatography followed by densitometric analysis. β -Sitosterol was used as the reference standard. The extract was applied to a silica gel 60 F254 plate and developed using the specified chromatographic solvent system. Following derivatization, densitometric measurement was performed at 340 nm. Two sample applications were analyzed for the undiluted preparation and two for the 10-fold diluted preparation. The result was expressed as % w/w β -sitosterol equivalent.

Total Sugar Content

Total sugar content was determined using the Luff–Schoorl method. Approximately 2 g of powdered extract was dissolved in water and filtered. The filtrate was reacted with Luff–Schoorl reagent and heated under reflux. After cooling, potassium iodide and sulfuric acid were added, and the solution was titrated with 0.2 N sodium thiosulfate. The procedure was also performed after acid hydrolysis to determine total reducing sugars. The total sugar content was expressed as % w/w.

DPPH Radical-Scavenging Activity

The antioxidant activity of the ethanolic propolis extract was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical-scavenging method, with UV–Vis spectrophotometric analysis performed at LPPT, Universitas Gadjah Mada. The analysis was performed according to the laboratory procedure IKU/5.4/TF-UV-01 (UV–Vis spectrophotometric method). Antioxidant activity was expressed as the

concentration of extract required to inhibit 50% of DPPH radicals (IC_{50}). Based on the classification described by Jun et al., IC_{50} values of <50 $\mu\text{g/mL}$, 50–100 $\mu\text{g/mL}$, 101–250 $\mu\text{g/mL}$, 250–500 $\mu\text{g/mL}$, and >500 $\mu\text{g/mL}$ are interpreted as very strong, strong, moderate, weak, and very weak antioxidant activity, respectively.²¹

The laboratory report provided an IC_{50} value of 0.147 mg/mL, equivalent to 147 $\mu\text{g/mL}$. Because the available laboratory report provided the final IC_{50} value but did not include the underlying concentration–response data, individual absorbance values, or regression equation, these parameters were not reconstructed in the present study.

4. Data Analysis

Phytochemical measurements were reported as percentage by weight (% w/w), whereas antioxidant activity was expressed as the DPPH IC_{50} in $\mu\text{g/mL}$. Findings from the Bali extract were considered alongside previously published data from propolis obtained in other Indonesian regions. Those published values were used for comparison only and were not treated as newly generated observations in the present study.

RESULTS

Phytochemical Composition and Antioxidant Activity

Quantitative analysis demonstrated the presence of several phytochemical constituents in the investigated Bali propolis extract. The extract contained total flavonoids, phenolic compounds, tannins, alkaloids, saponins, steroids, and sugars.

The total flavonoid content was 1.87% w/w, with three analytical values of 1.84%, 1.88%, and 1.89%. The total phenolic content was 1.59% w/w, with individual values of 1.71%, 1.62%, and 1.44%. The total tannin content was 1.14% w/w, with individual values of 1.11%, 1.21%, and 1.10%; the reported SD was 0.061 and the RSD was 5.32%.

The total alkaloid content was 0.25% w/w, whereas the total saponin content was 0.77% w/w. Steroid analysis yielded a reported total content of 30.6% w/w expressed as β -sitosterol equivalent. The total sugar content was 8.17% w/w.

DPPH radical-scavenging analysis yielded an IC_{50} of 0.147 mg/mL (147 $\mu\text{g/mL}$). This value corresponds to moderate antioxidant activity according to the IC_{50} classification applied in this study.²¹

Table 1. Quantitative phytochemical composition and antioxidant activity of Bali propolis extract

No.	Parameter	Result	Unit
1	Total flavonoid content	1.87	% w/w
2	Total phenolic content	1.59	% w/w
3	Total tannin content	1.14	% w/w
4	Total alkaloid content	0.25	% w/w
5	Total saponin content	0.77	% w/w
6	Total steroid content	30.6	% w/w
7	Total sugar content	8.17	% w/w
8	DPPH IC_{50}	147	$\mu\text{g/mL}$

Table note: Values represent laboratory-reported results for the investigated Bali propolis extract. Total flavonoid, phenolic, and tannin analyses were based on three analytical replicates; alkaloid and saponin analyses were based on two analytical measurements. Steroid analysis included two applications of the undiluted preparation and two applications of the 10-fold diluted preparation.

DISCUSSION

The present study demonstrated that the investigated Bali propolis extract contained measurable amounts of flavonoids, phenolic compounds, tannins, alkaloids, saponins, steroids, and sugars, together with measurable DPPH radical-scavenging activity. Because propolis composition is influenced by botanical source, geographical origin, bee species, environmental conditions, and extraction procedures, these findings should be interpreted as characteristics of the investigated preparation rather than as a universal profile of Bali or Indonesian propolis.^{1, 2, 18, 19}

The flavonoid and phenolic contents were 1.87% and 1.59% w/w, respectively. These compounds are commonly associated with antioxidant activity in propolis, although their concentrations may vary substantially among geographical sources.^{8, 9, 19} Indonesian studies have similarly demonstrated regional and species-related differences in the chemical and biological characteristics of stingless-bee propolis.^{10, 11} The present measurements therefore provide source-specific baseline information, but they do not identify individual compounds responsible for the observed activity. Further chromatographic and spectrometric analyses are required for more detailed chemical characterization.

Tannins, alkaloids, and saponins were detected at 1.14%, 0.25%, and 0.77% w/w, respectively. Their presence contributes to the overall phytochemical profile of the extract but should not be interpreted as evidence of specific antimicrobial, anti-inflammatory, or therapeutic effects, because these activities were not directly assessed in the present study. The steroid content was 30.6% w/w expressed as β -sitosterol equivalent. This result should also be interpreted cautiously because the assay provides a response relative to the reference standard and does not establish that β -sitosterol itself accounts for this proportion of the extract.

The DPPH assay yielded an IC_{50} of 147 μ g/mL, corresponding to moderate antioxidant activity according to the classification applied in this study.²¹ DPPH reflects chemical radical-scavenging capacity under defined in vitro conditions and therefore does not directly represent antioxidant efficacy in oral tissues or patients. The observed activity provides a biochemical basis for further investigation, but a direct causal relationship between individual phytochemical constituents and the measured IC_{50} cannot be established from the present data.

The dental relevance of these findings lies in the growing investigation of propolis as a potential oral-health material. Previous studies have explored propolis in periodontal care, endodontics, and delivery systems, with reported biological effects varying according to source, concentration, formulation, and experimental conditions.^{3–7, 12, 14, 16, 17} However, these findings cannot be directly attributed to the Bali preparation examined in the present study. Local characterization is therefore an important preliminary step before biological activity and formulation suitability are evaluated.

From a translational perspective, the present results provide baseline information for subsequent research rather than evidence of clinical efficacy. Further studies should identify individual bioactive compounds and evaluate antimicrobial and antibiofilm activity, anti-inflammatory effects, cytotoxicity, biocompatibility, stability, and formulation characteristics. Appropriate preclinical and clinical studies will also be required before potential dental applications can be established.

Several limitations should be considered. The assays quantified broad phytochemical groups rather than individual compounds, while DPPH is a chemical in vitro assay that does not reproduce the complexity of biological systems. Antimicrobial, antibiofilm, anti-inflammatory, cytotoxicity, tissue-healing, and clinical outcomes were not assessed. Comparisons with other propolis preparations should also be interpreted cautiously because differences in botanical source, bee species, extraction procedures, analytical methods, and reporting units may affect the results. Accordingly, the findings are limited to the Bali propolis preparation investigated in this study.

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