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Indonesian honey bioactivity profile linking composition to antioxidant and antibacterial potency

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Abstract. *Fahmi AG, Hasan AEZ, Hanif N, Rukayadi Y, Shaari K. 2025. Indonesian honey bioactivity profile linking composition to antioxidant and antibacterial potency. Biodiversitas 26: 4991-5002.* Indonesian honey, sourced from diverse tropical flora, is valued for its antimicrobial and antioxidant properties, yet comparative multi-parameter studies are limited. This study evaluates five Indonesian honey types (Akasia/*Acacia carpa* honey, Rambutan/*Nephelium lappaceum* honey, Randu/*Ceiba pentandra* honey, Flores honey, and Kelulut honey) for physicochemical traits, bioactive compounds, antioxidant capacity, and antibacterial activity against *Escherichia coli* and *Salmonella* Typhi. Physicochemical analysis determined pH, moisture, ash, and color. Total phenolic content (TPC) and total flavonoid content (TFC) were measured spectrophotometrically. Bioactives were identified via LC-MS. Antioxidant activity was assessed by DPPH radical scavenging and antibacterial efficacy was tested using disk diffusion. Data were analyzed using ANOVA and principal component analysis (PCA). All samples adhered Codex and SNI (RSNI3 8664:2024) quality standards. Flores honey had the highest TPC (4526.12 mg GAE/100 g) and TFC (3810.96 QE/100 g), yielding superior antioxidant activity (IC_{50}: 2.11±0.03 mg/mL). Kelulut honey showed the strongest antibacterial effect (inhibition zone: 13.50 mm against *E. coli* and 9.42 mm against *Salmonella* Typhi), linked to amino acids (L-proline, leucine) and phenolic acids (caffeic, quercetin). PCA revealed distinct clustering by bioactive profile: phenolic/flavonoid-rich honeys (e.g., Flores honey) aligned with antioxidant potency, while amino acid-rich honeys (e.g., Kelulut honey) correlated with antibacterial activity. Indonesian honey exhibits diverse and potent bioactivities, surpassing or matching Southeast Asian and Manuka honeys in key metrics. Flores honey represents a promising dietary antioxidant source, while Kelulut honey shows potential for antimicrobial applications such as topical formulations. Future work should isolate and characterize specific bioactives to support functional food and therapeutic development.

Keywords: Antimicrobial properties, antioxidant activity, Indonesian honey, flavonoids, PCA analysis

INTRODUCTION

Honey is a complex natural product with recognized nutritional and therapeutic properties. Its antimicrobial activity results from a synergistic combination of high sugar concentration, low pH, hydrogen peroxide, and diverse secondary metabolites (Koochak et al. 2010; Almasaudi 2021). These factors create conditions unfavorable for microbial growth and inhibit pathogenic bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Staphylococcus aureus*, and *Propionibacterium acnes* (Deng et al. 2018; Mama et al. 2019; Djakaria et al. 2020; Nayaka et al. 2020; Kaya and Yıldırım 2021; Ben Amor et al. 2022; Fadhil et al. 2020; Mustafa et al. 2022). Honey's antibacterial effect is enhanced by its acidic pH, which inhibits bacterial survival at values between 4.0 and 4.5 (Koochak et al. 2010). As a natural alternative to synthetic antibiotics, honey demonstrates broad-spectrum antibacterial potential (Saputra et al. 2021) without promoting microbial resistance, making it a promising complementary therapeutic agent (Hassan et al. 2022; Nobel et al. 2022). In addition to its antimicrobial activity, honey is rich in phenolic and flavonoid compounds that scavenge free radicals, reduce oxidative stress, and contribute to its antioxidant and anti-inflammatory potential (Luvanda and Lyimo 2018.

In Indonesia, several studies have examined the chemical and biological properties of honey from different bee species and regions. Agussalim et al. (2021) identified 17 amino acids, including glutamic acid, lysine, histidine, and arginine, with Sleman and Lombok honeys showing superior quality. Indonesian honeys also exhibit high vitamin C (6.49-13.58 mg/100 g), total phenolic content (0.65-2.30% GAE/100 g), total flavonoids (0.28-1.00 mg QE/g), and strong antioxidant activity (61-90% DPPH inhibition) (Agussalim et al. 2022). A subsequent study found that Lombok honey contained higher protein, phenolics, flavonoids, organic acids, and amino acids, correlating with stronger antioxidant performance (Agussalim et al. 2023). Honey from stingless bees (*Tetragonula laeviceps*, *Heterotrigona itama*) in Yogyakarta, South Sulawesi, and West Java also showed high levels of phenolics, flavonoids, and amino acids, contributing to potent antioxidant and antimicrobial activities

(Agus et al. 2019; Noor et al. 2019; Agussalim et al. 2021; Misrahanum et al. 2023). Meanwhile, *Apis dorsata* honey from Sumatra and Kalimantan exhibited higher moisture and acidity and demonstrated antibacterial activity against *S. aureus* and *E. coli* (Mustafa et al. 2022). In contrast, *Apis mellifera* honey, widely cultivated in Java and Bali, showed lower flavonoid and bioactivity levels than stingless bee honeys (Adalina et al. 2020; Huyop et al. 2024).

Collectively, these studies highlight the remarkable chemical diversity of Indonesian honeys, shaped by geographical origin, bee species, and floral source. However, most prior research has focused narrowly on *T. laeviceps*, leaving *A. dorsata* and *A. mellifera* honeys underexplored. Comprehensive multi-species and multi-region comparisons remain scarce. This gap underscores the novelty and importance of the present research.

Globally, honeys such as New Zealand's Manuka and Yemen's Sidr have gained recognition through systematic profiling that links specific chemical markers—such as methylglyoxal in Manuka honey—to distinct bioactivities (Tananaki et al. 2024). Similar integrated approaches in Malaysia and Thailand have connected physicochemical traits, phytochemical content, antioxidant and antimicrobial properties (Mustafa et al. 2022; Wongsa et al. 2023). Despite its biodiversity, Indonesia's honey industry lacks such holistic characterization, limiting its global recognition and market value.

The rise of multidrug-resistant pathogens has intensified the search for natural antimicrobials to complement or replace antibiotics (Hassan et al. 2022; Nobel et al. 2022). Understanding which constituents drive antioxidant and antibacterial functions is crucial. Polyphenols such as quercetin and kaempferol are well-known for antioxidant and antimicrobial effects, while amino acids like L-proline serve as precursors of proline-rich antimicrobial peptides (Mardirossian et al. 2019). Yet, these mechanisms remain underexplored in Indonesian honey. This study addresses these gaps through a comprehensive, multi-parameter assessment of five Indonesian honeys—Flores honey (FH), Randu honey (RH), Akasia honey (CH), Kelulut honey (KH), and Rambutan honey (RaH)—representing diverse botanical and geographical origins. This study advances understanding of the functional potential of Indonesian honey and supports its development as a valuable natural product for nutrition, medicine, and trade.

MATERIALS AND METHODS

Honey sample collection

Five Indonesian honey types were obtained directly from certified local beekeepers between April 2022 and March 2023, covering both dry and wet seasons to capture potential seasonal variation. The selected honeys included Akasia (*Acacia carpa*) honey (CH) from Dumai, Riau; Rambutan (*Nephelium lappaceum*) honey (RaH) from Depok, West Java; Randu (*Ceiba pentandra*) honey (RH) from Lebak, Banten; multifloral Flores honey (FH) from East Nusa Tenggara; and multifloral Kelulut honey (KH) from Sumedang, West Java (Figure 1 and Table 1). Each honey was collected in triplicate (n=3 per type) from separate hives to account for biological variability. Samples were collected using sterile food-grade equipment, filtered to remove wax debris, and stored in sterile amber glass bottles at 25±2°C, protected from light until analysis, as per Codex and RSNI recommendations. Botanical origin was confirmed through beekeeper records and local flora surveys.

Figure 1. A. Appearance, and B. Geographical location of honey sample. FH: Flores honey, CH: Akasia honey, KH: Kelulut honey, RaH: Rambutan flower honey, RH: Randu flower honey

Table 1. Classification of honey samples, types and regional sources

Honey code	Sample	Type	Coordinate point		Regional source
			Latitude	Longitude	
CH (n=3)	Akasia honey	*Acacia crassicarpa*	0.66902	101.57615	Dumai, Riau
RH (n=3)	Randu flower honey	*Ceiba pentandra*	-6.34807	106.26146	Lebak, Banten
RaH (n=3)	Rambutan flower honey	*Nephelium lappaceum*	-6.37542	106.88786	Depok, West Java
FH (n=3)	Flores honey	Multifloral	-8.30191	123.01907	Flores, East Nusa Tenggara
KH (n=3)	Kelulut honey	Multifloral	-6.93819	107.83729	Sumedang, West Java

Physicochemical analysis

Physicochemical parameters including pH, moisture content, ash content, and color were analyzed using standardized methods. All measurements were conducted in triplicate. pH was measured using a calibrated digital pH meter (Milwaukee-MW180 Max) by dissolving 1 g of honey in 10 mL of distilled water (1:10 w/v) (Agussalim et al. 2021). Moisture content of each honey sample was dried in an oven (Labtech Universal Drying Oven LDO 100E) for 1 h at 105°C until a consistent mass was measured to estimate the moisture content (Agussalim et al. 2021). Ash content was determined by incinerating 5 g of honey at 600°C in a muffle furnace until a constant weight was obtained (AOAC 2006). Color was evaluated using the Pfund scale with a spectrophotometer. Samples of honey were homogenized and diluted with distilled water to a 50% concentration. The measurements were performed at room temperature (28°C). A spectrophotometer (Shimadzu Spectro UV-VIS 1900i) was used to measure the absorbance at 635 nm, and the Pfund scale was used to calculate the color intensity using the following formula (Al-Farsi et al. 2018).

$$\text{Pfund} = -38.70 + 371.39 \times \text{Abs}$$

Phytochemical screening and quantification

Utilize qualitative analytical techniques to identify phytochemical components and secondary metabolites found in honey. Based on selection of Pham et al. (2022) techniques, honey samples were subjected to qualitative testing with three replicates (Table 2). Standard phytochemical tests were performed to detect the presence of alkaloids, flavonoids, phenolics, saponins, and terpenoids. The positive control for each test is a pure compound of each secondary metabolite, while the negative control is distilled water. All tests were performed in triplicate.

Total Phenolic Content (TPC)

The modified Folin-Ciocalteu technique was employed to ascertain the total polyphenol content (Pham et al. 2022). Initially, honey was diluted to a 20% concentration. In a test tube, 0.1 mL of this diluted honey was combined with 1.5 mL of a 10% Folin-Ciocalteu solution. This mixture was homogenized carefully and thoroughly using a vortex mixer. After allowing the solution to stabilize for five minutes, 1.2 mL of 7.5% Na_2CO_3 was added and mixed well. The mixture was incubated at room temperature for 30 minutes and absorbance was measured at 765 nm using a UV-Vis spectrometer (Spectro UV-VIS 1900i Shimadzu Japan). All assays were performed in triplicate. Results were expressed as mg gallic acid equivalent (GAE) per 100 g of honey. To determine the polyphenol concentration (mg GAE/100 g sample), the equation y = 0.29x - 0.0752 from the gallic acid standard curve was utilized.

Total Flavonoid Content (TFC)

The modified colorimetric technique using aluminum chloride was employed to quantify the total flavonoid content (Pham et al. 2022). First, the honey was diluted to a 20% concentration solution. Subsequently, a test tube was prepared by adding 2 mL of the diluted honey, 0.1 mL of a 10% aluminum chloride ($AlCl_3$) solution, and 0.1 mL of a 0.1 mM potassium acetate solution, which was then homogenized using a vortex mixer. Absorbance was read at 415 nm using UV-Vis spectrometer (Spectro UV-VIS 1900i Shimadzu Japan). All assays were performed in triplicate. Results were expressed as mg quercetin equivalent (QE) per 100 g of honey. The flavonoid concentration (mg QE/100 g sample) was calculated using the standard curve for Quercetin, represented by the equation y = 0.283x - 0.0592.

Secondary metabolite identification using LC-MS

A mass spectrometer (Xevo G2-S QTof, Waters, USA) and an ultra-performance liquid chromatography (UPLC) unit (ACQUITY UPLC® H Class System, Waters, USA) were utilized for high-resolution mass spectrometry investigations. A C18 column (1.8 µm, 2.1 x 100 mm, ACQUITY UPLC® HSS, Waters, USA) was employed, with the room and column temperatures maintained at 25°C and 50°C, respectively. The LC analysis utilized a mobile phase composed of water with 5 mM ammonium formate (A), acetonitrile, and 0.05% formic acid (B). An injection volume of 5 µL was filtered through a 0.2 µm syringe filter, and the flow rate was set to 0.2 mL/min using a step gradient over 23 minutes (refer to the slide on the moving phase) Ismed et al. (2021). Electrospray ionization (ESI) in positive mode was implemented for the mass spectrometry (MS) analysis with a source and desolvation temperature of 100°C and a mass range of 50-1200 m/z. Additionally, a collision energy range from 4 to 60 eV was applied, with cone and desolvation gas flow rates of 0 L/hr and 793 L/hr, respectively. Data collection, analysis, and instrument control were carried out using Masslynx version 4.1 software, a reliable and accurate solution in this field. To further ensure the accuracy of the data analysis, the molecular weight values obtained from the chromatograms were matched with the NIST Chemistry WebBook.

Table 2. Procedures for identifying qualitative phytochemical substances

Parameter	Reagents	Observations
Alkaloid	Sample + chloroform + Mayer	Cream colored precipitate (+)
Phenolic	Sample + $FeCl_3$	Blue or violet color complex (+)
Tannin	Sample + $FeCl_3$	Brown until black color (+)
Steroids	Sample + Acetic acid glacial + H_2SO_4	Blue or purple or green (+)
Flavonoid	Sample + Mg powder + HCl (conc.)	Red or yellow or brown (+)
Saponin	Sample + HCl + distilled water shaken	Appearing foam that lasts 5 minutes (+)

Antioxidant activity (DPPH Assay)

The DPPH method is the most widely recognized technique for assessing antioxidant capacity due to its affordability, simplicity, speed, and sensitivity. The DPPH radical, characterized by a single unpaired electron located on a nitrogen atom, imparts a distinctive deep purple color to the alcoholic DPPH solution. The measurement of radical scavenging is determined by the decrease in absorbance at 517 nm, which occurs as the radical transitions to a non-radical yellow form.

A modified approach based on Dżugan et al. (2018) was employed to evaluate the total antioxidant capacity (TAC) of the honey samples. Specifically, 1 mL of the sample was added to 2.9 mL of a 1 mmol/l DPPH methanol solution (Sigma-Aldrich, Steinheim, Germany). The mixture was then agitated and allowed to incubate in the dark at room temperature for 30 minutes. Absorbance was measured at 517 nm using a Shimadzu Spectro UV VIS 1900i spectrophotometer (Shimadzu, Japan). The antioxidant capacity was expressed in IC_{50}. All tests were performed in triplicate. Pearson correlation coefficients (r) were calculated to evaluate the relationships between antioxidant activity (IC_{50} values), total phenolic content (TPC), and total flavonoid content (TFC). Correlations were considered strong when $|r| \geq 0.7$, moderate for $0.5 \leq |r| < 0.7$, and weak for $|r| < 0.5$ ($p < 0.05$).

Antibacterial activity assay

The antibacterial activity of the honey samples was evaluated using the disk diffusion method, following the Hossain et al. (2022) method with slight modifications. Three bacterial strains were used as test organisms: *Escherichia coli* ATCC 8739 and *Salmonella* Typhi IPBCC b 11 669 (both Gram-negative). Sterile paper disks (6 mm diameter, Oxoid, UK) were impregnated with 20 µL of undiluted honey and allowed to stand for 30 minutes at room temperature to ensure uniform absorption. The bacterial inoculum was standardized to 0.5 McFarland ($\approx 1.5 \times 10^8$ CFU/mL) and uniformly spread over the surface of Mueller-Hinton agar (MHA) plates using sterile cotton swabs. The honey-loaded disks were then placed onto the inoculated agar, and the plates were incubated at 37°C for 24 h. After incubation, the diameters of inhibition zones were measured in millimetres. All assays were performed in triplicate, and results were expressed as mean ± standard deviation. Clindamycin (100 mg/L) was used as the positive control because it is a standard antibiotic effective against Gram-positive cocci. To ensure proper benchmarking for Gram-negative bacteria as it represents a clinically relevant broad-spectrum antibiotic. Sterile distilled water served as the negative control. The inclusion of both antibiotics enabled appropriate comparison of honey activity with reference antimicrobial agents for each bacterial group. After normalizing all data, we employed Pearson correlation to assess the similarity of the samples. Statistical analysis using one-way ANOVA followed by Tukey's HSD post-hoc test to compare treatments ($p < 0.001$).

Data analysis

All experiments were performed in triplicate, and data are presented as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was used to assess significant differences among honey samples for total phenolic content (TPC), total flavonoid content (TFC), antioxidant activity (IC_{50}), and antibacterial inhibition zones. When significant differences were found ($p < 0.05$), Tukey's honestly significant difference (HSD) post hoc test was applied to identify pairwise differences between means. Pearson correlation analysis was performed to examine the relationships among TPC, TFC, and IC_{50} values. All statistical analyses were carried out using Minitab 16 Software.

Based on the findings from our physicochemical investigation, we conducted statistical studies to confirm significant distinctions or similarities among plants and geographical areas. With three replicates and a standard deviation, the data were presented at a significance level of $p < 0.05$, highlighting the importance of our results. We used Principal Component Analysis (PCA) in conjunction to evaluate the variations in physicochemical profiles and chemical compounds of Indonesian honey derived from various botanical origins, particularly in relation to their antimicrobial activity. Multivariate data analysis was conducted using Minitab 16 Software. The results of the analysis are represented in score plots to illustrate the clustering effects and in loading plots to identify the characterizing compounds.

RESULTS AND DISCUSSION

Physicochemical analysis

The physicochemical characteristics of five Indonesian honey types are presented in Table 3. All samples met the quality requirements outlined by Codex Alimentarius (2001) and Indonesian National Standard (RSNI3 8664:2024). The moisture content of Indonesian honey is more than 22%, ranging from 23.43±2.23 to 26.16±1.21%. The highest moisture content was found in Kelulut honey (26.16±1.21%), because it is a type of stingless bee known for its high moisture content, and the samples used have been processed for sale in commercial stores compared to other types of honey. The moisture content of honey in this study was significantly different from the results of previous studies conducted by Agussalim et al. (2021) due to differences in location and the influence of season, climate, and plant type (mono-flower or multi-flower) around the apiary site. Different geographical origins of honey can affect the composition of honey because there are other plant species and environmental conditions as sources of nectar and pollen. One of the most important aspects of honey quality is its moisture content, which correlates with the viscosity of honey. As the moisture content increases, the viscosity of honey decreases (Afshari et al. 2022). In addition, the stability of honey against crystallization and fermentation depends on its moisture content (Singh and Singh 2018). Therefore, premium honey is generally viscous and has a low moisture content.

Table 3. The physicochemical parameters of Indonesian honey samples

Parameter	FH	RH	CH	KH	RaH	Codex limits	SNI standard
Moisture content (%)	24.34±0.60	25.93±0.80	24.88±1.23	26.16±1.21	23.43±2.23	Not exceed 20% for blossom honey	Max. 22% for *Apis mellifera* and Max. 27.5% for stingless bee
pH	4.62±0.06	3.93±0.05	3.69±0.06	3.28±0.03	4.19±0.04	-	3.4-6.1
Ash content (%)	0.45±0.01	0.13±0.02	0.12±0.01	0.35±0.01	0.20±0.02	≤0.6%	≤0.5%
Color (mm Pfund)	491.70±0.43 Dark amber	46.99±0.27 Extra light amber	277.08±0.65 Dark amber	148.93±1.04 Dark amber	69.11±0.11 Light amber	-	-

Note: FH: Flores honey, RH: Randu honey, CH: Akasia honey, KH: Kelulut honey, RaH: Rambutan honey

All Indonesian honey samples in our study were acidic, with pH values ranging from 3.28±0.03 to 4.62±0.06. These values are consistent with previous studies on Indonesian honey, which is acidic (Agussalim et al. 2021). At this pH level, it is sufficient to inhibit the growth of some bacteria, as most bacteria thrive under neutral pH conditions (6.5-7.5). The acidity level in honey is an important factor, particularly regarding its antibacterial properties against pathogenic bacteria such as *Salmonella* spp., which require a minimum pH of 4.0 to grow (Pauliuc et al. 2020). Additionally, high acidity levels may indicate the occurrence of sugar fermentation in honey, which significantly affects the organoleptic characteristics and overall quality of honey (Alvarez-Suarez et al. 2018). According to the SNI standard, Kelulut honey from all types of honey samples below the lower bound of the standard (3.40-6.10). The pH of all honey in this study aligns with previous research conducted by Huyop et al. (2024), ensuring ideal conditions for long-term storage.

The color of the five types of Indonesian honey were evaluated using the Pfund scale method, resulting in varying color intensities ranging from 46.99±0.27 to 491.70±0.43 mm Pfund (Figure 1). Flores honey has the darkest color among the other samples, at 491.70±0.43. Color intensity is one of the sensory attributes because it is easily visible, and therefore can influence consumer preference when purchasing. Many factors influence the color of honey, including the age of the honey, temperature during processing, handling and storage, moisture content, floral and geographical origin, age, secondary metabolites (phenolics, carotenoids, sugars, minerals, and pollen), and measurement conditions for color measurement (Bodor et al. 2021). Darker honey has higher nutritional content and is generally used in industry, while lighter honey is more commonly used for direct consumption (Pham et al. 2022). Color intensity can also be influenced by processing and storage conditions. Therefore, considering consumer concerns and the widespread impact of honey's physicochemical properties, producers need to pay attention to storage locations.

Phytochemical screening and quantification

The qualitative phytochemical profiles of the five Indonesian honey provide further insights into the diversity of secondary metabolites and their contribution to functional properties such as phenolics, tannins, steroids, flavonoids, alkaloids, and saponins (Table 4). All five honey types contained phenolics and flavonoids, which are universal bioactive groups in honey and known to contribute to antioxidant potential. Tannins were unique to Flores honey, which may explain its superior antioxidant performance since tannins are potent radical scavengers and metal chelators (Becerril-Sánchez et al. 2021). Alkaloids were detected only in Kelulut honey. Alkaloids are less common in honey and may reflect contributions from specific stingless bee foraging plants. Their presence could be linked to Kelulut honey's strong antimicrobial activity, as alkaloids disrupt bacterial cell membranes and protein synthesis (Saeed et al. 2021). Saponins were present in most honeys, except Randu honey, suggesting plant source variability. This finding aligns with previous research noting the presence of saponins in Indonesian honey (Adalina et al. 2020). Saponins have surfactant properties that can contribute to antibacterial and antifungal activity. Steroids/ terpenoids were absent in all samples, which may reflect either low concentrations below detection thresholds or their degradation during honey maturation. This condition indicates that all Indonesian honey samples contained beneficial phytochemical components.

Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)

The results of measuring the total phenolic content (TPC) in the five Indonesian honey samples ranged from 620.99±0.78 to 4526.12±35.22 mg GAE/100 g (Table 5). These levels are higher compared to previous Indonesian studies, such as Djakaria et al. (2020), which reported values between 495 and 673 mg GAE/100 g. They are also higher than the ranges reported for stingless bee honeys by Agussalim et al. (2022), who found TPC values between 650 and 2300 mg GAE/100 g in *Tetragonula laeviceps* honeys from Lombok, Depok, and Gunungkidul. More recent work by Agussalim et al. (2023) confirmed significant geographical variability in Indonesian stingless bee honeys, with samples from Lombok containing higher phenolic content than those from Magelang and Purworejo. Flores honey, with TPC above 4500 mg GAE/100 g, therefore

exceeds previously reported Indonesian values and demonstrates exceptional phenolic richness.

International comparisons further emphasize this distinction. Indonesian honey had higher TPC values than those reported for Thailand (89.9 mg GAE/100 g) (Wongsa et al. 2023), Vietnam (1110 mg GAE/100 g) (Pham et al. 2022), the Philippines (0.2475 mg GAE/100 g) (Belina-Aldemita et al. 2019), and even New Zealand Manuka honey (179.5±33.3 mg GAE/100 g) (Tananaki et al. 2024). According to Becerril-Sánchez et al. (2021), the floral source of honey strongly determines its phenolic content, since polyphenols are largely derived from pollen and nectar. Indonesia's extraordinary floral biodiversity and tropical climate likely underpin the unusually high phenolic concentrations observed in Flore honey and Kelulut honey, positioning them as valuable sources of bioactive compounds that contribute to honey's functional and organoleptic qualities.

The total flavonoid content (TFC) also varied significantly among samples, ranging from 510.63±0.70 mg QE/100 g in RH to 3810.96±40.13 mg QE/100 g in FH (Table 4). These values exceed those reported for *T. laeviceps* stingless bee honeys in Indonesia by Agussalim et al. (2019, 2022), where TFC values were generally between 28 and 100 mg QE/100 g. Agussalim et al. (2023) similarly documented regional variability, but our FH and KH samples surpass even their upper ranges. Compared to other Asian honeys, Indonesian honey again stands out Thailand (58.09 mg QE/100 g) (Wongsa et al. 2023), Philippines (0.3504 mg QE/100 g) (Belina-Aldemita et al. 2019), Vietnam (75.54±3.95 mg QE/100 g) (Pham et al. 2022), and Yemeni honey (400.9±0.36 mg QE/100 g) (Saeed et al. 2021).

The consistently higher flavonoid levels in Indonesian honey reflect the tropical environment, where abundant flowering plants supply a wide spectrum of flavonoid compounds, including hesperetin, isorhamnetin, apigenin, luteolin, pinobanksin, kaempferol, luteocembrin, and galangin (Becerril-Sánchez et al. 2021). These compounds are known to support antioxidant, anti-inflammatory, and antimicrobial activities, reinforcing the multifunctional health potential of Indonesian honey. The alignment of our results with, and in some cases extension beyond, prior Indonesian studies demonstrates both continuity and novelty, thereby filling an important research gap and situating Indonesian honey as a regional leader in phenolic and flavonoid content.

Secondary metabolite identification using LC-MS

In the honey samples studied, a rich array of 11 polyphenolic compounds, 3 vitamins, and 5 amino acids were identified (Table 6). These polyphenolic components, including citric acid derivatives, kaempferol, quercetin, caffeic acid, eupatorine, and ibogaine, were found in all honey samples at varying concentrations. However, certain types of honey contained unique polyphenolic compounds such as chrysin, pinocembrin, hesperetin, azelaic acid, and coumaric acid derivates. The diverse polyphenolic constituents in Indonesian honey present a promising range of potential health benefits. The unique combination of these substances in Indonesian honey holds significant promise for improving human health, sparking optimism for further in-depth research into each compound.

Building on previous research, citric acid derivatives in honey have shown moderate antimicrobial qualities and contribute to the honey's acidic flavors. For instance, Akasia honey contains approximately 44.89±2.32 mg/kg of these organic acids, which can be utilized for bacteriostasis, antioxidant, and other potential purposes in honey (Sun et al. 2023). Moreover, honey's organic acids can serve as a unique marker, aiding in the distinction between various honey varieties. This compound likely plays a crucial role in the antimicrobial activity of honey, a fascinating aspect that warrants further exploration and engagement.

Potent antioxidants are kaempferol, hesperetin, and quercetin, two types of flavonoids. Due to their antioxidant and anti-platelet properties, kaempferol and quercetin may reduce the risk of cardiovascular diseases (CVDs) in several ways, including by reducing oxidative stress and blood platelet activation (Olas 2020). This compound likely plays an essential role in honey's antioxidant activity.

Table 4. Results of phytochemical identification of several honey varieties

Qualitative indicators	FH	RH	CH	KH	RaH
Phenolic	(+)	(+)	(+)	(+)	(+)
Tannin	(+)	(-)	(-)	(-)	(-)
Steroid/Terpenoid	(-)	(-)	(-)	(-)	(-)
Flavonoid	(+)	(+)	(+)	(+)	(+)
Alkaloid	(-)	(-)	(-)	(+)	(-)
Saponin	(+)	(-)	(+)	(+)	(+)

Note: FH: Flores honey, RH: Randu honey, CH: Akasia honey, KH: Kelulut honey, RaH: Rambutan honey

Table 5. Total Phenolic Content (TPC), Total Flavonoid Content (TFC), and antioxidant activity (IC_{50}) of Indonesian honey

	FH	RH	CH	KH	RaH
TPC (mg GAE/100 g sample)	4526.12±35.22	620.99±0.78	2991.98±42.94	829.84±0.04	761.13±0.55
TFC (mg QE/100 g sample)	3810.96±40.13	510.63±0.70	2467.79±70.85	697.63±0.02	626.61±10.31
IC_{50} (mg/mL)	2.11±0.03	9.40±0.12	3.49±0.10	3.31±0.01	11.86±0.22

Note: FH: Flores honey, RH: Randu honey, CH: Akasia honey, KH: Kelulut honey, RaH: Rambutan honey

Another noteworthy compound found in honey is caffeic acid, an anti-inflammatory antioxidant that may also aid in blood sugar regulation. While it has been associated with cancer, caffeine has also been shown to reduce colon tumor growth in mice when combined with other antioxidants. This underscores the potential of combining compounds like quercetin, kaempferol, pinocembrin, caffeic acid, chrysin, and eupatorine, which have emerged as prospective pharmacological agents with antiproliferative actions in various cancer cell lines (Lima et al. 2023). The synergistic effect of these compounds offers inspiration and motivation for further research into addressing health problems.

Natural occurring azelaic acid may be helpful in the treatment of acne and other skin disorders. Approximately 0.83±0.10 mg/kg azelaic acid in akasia honey comprises these organic acids (Sun et al. 2023). Its effectiveness in treating various cutaneous disorders, including papulopustular rosacea and acne vulgaris, is believed to correlate with its anti-inflammatory and antioxidant qualities. For various dermatological disorders, azelaic acid, either alone or in combination, is a well-tolerated and safe alternative or first-line treatment option (Hollinger et al. 2018). It can potentially be developed as a new natural cosmetic if honey contains high azelaic acid compounds.

Derivatives of coumaric acid: these might be anti-inflammatory and antioxidant. The type and quantity of polyphenols in the honey determine which particular health benefits they offer. More research is necessary to completely comprehend these drugs' efficacy in people. Taking honey should not be the only way you get these nutritious chemicals; it should be a part of your diet.

All honey samples were detected to contain vitamin C. Vitamin B6 was found in the FH and KH honey. Moreover, vitamin E was found in all honey samples except RaH honey. Pyridoxine (Vitamin B6) is the highest component of vitamin B compared to other B-group vitamins (Popkova et al. 2021). Vitamins C and E are other water-soluble vitamins that can significantly affect the level of antioxidant activity (Sawicki et al. 2020). The vitamin content in honey depends on the nectar and pollen of the plant species, as well as many other factors. The presence of vitamins in honey increases its value as a food product, including non-food products.

There are five types of amino acids in all honey samples. Leucine was identified in all kinds of honey. Proline, glycine, alanine, and tryptophan were only identified in some honey types. These essential and non-essential amino acids indicate that Indonesian honey is rich in secondary metabolites that can be utilized as dietary supplements. Noor et al. (2019) identified 16 types of amino acids in Sulawesi honey, much more than the analysis results of our five samples. Each amino acid has its role in the growth of bees, whose origin is also influenced by the surrounding environment. For example, Proline supplementation aided the rapid development of honey bee larvae and pupae, resulting in the quickest total bee brood development time of 20.1 days. Proline supplementation enhanced larval survival (93.8%) and weight (Noor-ul-Ane and Jung 2022). Therefore, the presence of these identified amino acids can be the basis for further development of honey-derived products.

Table 6. Bioactive compounds identified in Indonesian honey samples by LC-MS

Compound name	Group	RT (min)	RI (Kovats)	Biological activity	References
L-Proline	Amino acid	1.19	1140	Osmo protection, protein synthesis	Alvarez-Suarez et al. 2018
Eupatorine	Polyphenol	1.61	1875	Anti-inflammatory, anticancer	Wongsa et al. 2023
Triethyl citrate	Polyphenol	2.11	1722	Antioxidant, preservative	Misrahanum et al. 2023
Ibogaine	Polyphenol	2.93	2021	Neuroprotective, anti-addiction potential	Ben Amor et al. 2022
Azelaic acid	Polyphenol	3.94	1850	Antibacterial, anti-inflammatory	Djakaria et al. 2020
Coumarin-3-carboxylic acid	Polyphenol	4.92	2065	Antioxidant, anticoagulant	Becerril-Sánchez et al. 2021
Pyridoxine	Vitamin	5.85	1785	Coenzyme, antioxidant	El-Wahed et al. 2023
Glycine	Amino acid	6.27	1107	Neurotransmitter, anti-inflammatory	Pham et al. 2022
Kaempferol	Polyphenol	7.17	2745	Antioxidant, antibacterial, anticancer	Huyop et al. 2024
Alanine	Amino acid	9.02	1275	Energy metabolism	Almasaudi 2021
L-Tryptophan	Amino acid	9.43	2571	Precursor of serotonin, antioxidant	Mărgăoan et al. 2021
Chrysin	Polyphenol	9.56	2648	Antioxidant, antimicrobial, anticancer	Nayaka et al. 2020
Pinocembrin	Polyphenol	10.02	2732	Antibacterial, anti-inflammatory	Wongsa et al. 2023
Leucine	Amino acid	10.88	1389	Essential amino acid, muscle synthesis	Misrahanum et al. 2023
Dehydroascorbic acid dimer	Vitamin	12.83	1895	Antioxidant (Vitamin C derivative)	Dżugan et al. 2018
δ-Tocopherol	Vitamin	13.12	3274	Vitamin E activity, antioxidant	Becerril-Sánchez et al. 2021
Hesperetin	Polyphenol	13.45	2857	Antioxidant, antibacterial, anti-inflammatory	Ben Amor et al. 2022
Quercetin	Polyphenol	17.01	2750	Strong antioxidant, antiviral, anticancer	Wongsa et al. 2023
Caffeic acid	Polyphenol	20.37	1920	Antioxidant, antibacterial	Becerril-Sánchez et al. 2021

Antioxidant activity (DPPH free radical scavenging ability)

The DPPH free radical scavenging activity was measured using the IC_{50} value. Table 5 shows the IC_{50} values of the honey samples, which indicate significant variation ($p<0.05$), indicating the concentration required to scavenge 50% of the free radicals. Lower IC_{50} values indicate higher antioxidant activity. The antioxidant activity of honey samples ranged from 2.11±0.03 to 11.86±0.22 mg/mL. Notably, Flores honey exhibited the highest antioxidant activity at 2.11±0.03 mg/mL, comparable to the standard value of ascorbic acid (2.03±0.09 mg/mL). Conversely, Rambutan honey (11.86±0.22 mg/mL) and Randu honey (9.40±0.12 mg/mL) exhibited relatively low IC_{50} values. In comparison with honey studies conducted in other Asian countries, the IC_{50} values of Indonesian honey samples were higher than those of Thai honey at 43.996±0.377 mg/mL (Wongsa et al. 2023), Philippine honey at 43.04±1.07 mg/mL (Belina-Aldemita et al. 2019), Vietnamese honey at 89.10 to 1,285.24 mg/mL (Pham et al. 2022), Indian honey at 46.10±0.71 mg/mL, and Yemeni honey at 28.06±0.15 mg/mL (Saeed et al. 2021). These observations indicate that the antioxidant quality of honey is due to the presence of phenolic compounds and flavonoids. Higher phenolic and flavonoid content is positively correlated with lower IC_{50} values. According to Olas (2020), quercetin and kaempferol are the main contributors to radical scavenging activity. The antioxidant potential (IC_{50}) of Flores honey is superior to honey samples from other Asian countries and comparable to Manuka honey (Tananaki et al. 2024).

Pearson correlation analysis revealed a strong negative relationship between antioxidant activity (IC_{50} DPPH) and both total phenolic content ($r = –0.97$, $p<0.01$) and total flavonoid content ($r = –0.96$, $p<0.01$) (Table 7). The combined correlation (TPC + TFC vs IC_{50}) remained strongly negative ($r = –0.97$, $p<0.01$). These results indicate that higher phenolic and flavonoid concentrations are closely associated with stronger antioxidant capacity (lower IC_{50}).

The strong negative Pearson correlations between IC_{50} and both TPC ($r = –0.97$) and TFC ($r = –0.96$) confirm that phenolic compounds are the dominant contributors to antioxidant activity in Indonesian honey, with flavonoids acting in a supportive role. This finding aligns with reports from Vietnamese (Pham et al. 2022) and Algerian honeys (Ben Amor et al. 2022), where phenolic levels most strongly predicted radical scavenging capacity. The close correlation of TPC and TFC with antioxidant potential reinforces the role of Indonesia's floral diversity in producing honeys rich in polyphenolic antioxidants.

Antibacterial activity

Five samples of pure honey were analyzed for antimicrobial activity against *E. coli* and *S.* Typhi, as shown in Table 8 and Figure 2. The results of the antimicrobial activity test of the five samples showed that all honey tested in this study were active against Gram-negative bacteria. Kelulut honey is the type of honey with the best antimicrobial activity, having an inhibition zone value of 13.50±0.02 mm for *E. coli* and 9.42±0.10 mm for *S.* Typhi, compared to the positive control. This is stronger than the activity reported for Thai honey of ~8-9 mm (Wongsa et al. 2023) and even comparable to Manuka honey of 8-12 mm (Deng et al. 2018). The effectiveness of Kelulut honey in overcoming the outer membrane of Gram-negative bacteria indicates the presence of strong small-molecule bioactive compounds capable of penetrating the lipopolysaccharide layer. The presence of proline, which is a precursor of proline-rich antimicrobial peptides (PrAMPs), possibly contributes to strong antibacterial activity (Mardirossian et al. 2019). Interestingly, PCA analysis showed a negative correlation between polyphenol content and antibacterial activity, but a positive correlation with amino acids, particularly proline and alanine (Figure 4). This suggests the presence of different antimicrobial mechanisms compared to polyphenol-dominated honeys like Manuka, where methylglyoxal (MGO) is the primary component.

Although MIC and MBC values were not determined in this study, previous research has shown MIC values for kelulut honey ranging from 12.5-50% (v/v) against Gram-negative bacterial species (Mama et al. 2020). Future studies should use liquid microdilution techniques to accurately measure bacteriostatic and bactericidal effects. Similar efficacy against Gram-negative pathogens has also been reported for Malaysian *Geniotrigona thoracica* honey, with MIC values ranging from 6.25-25% (v/v) against *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* (Mustafa et al. 2022). Further testing against Gram-positive bacteria is needed to confirm the broader antimicrobial spectrum of Indonesian honey.

Table 7. Pearson correlation analysis of Total Phenolic Content (TPC), Total Flavonoid Content (TFC), and antioxidant activity (IC_{50}) of Indonesian honey

Correlation pair	r (Pearson)	Direction	Interpretation
TPC vs IC_{50}	–0.972	Strong negative	Higher phenolic content strongly associated with higher antioxidant activity (lower IC_{50})
TFC vs IC_{50}	–0.955	Strong negative	Higher flavonoid content also closely associated with higher antioxidant activity
(TPC + TFC) vs IC_{50}	–0.966	Strong negative	Combined phenolic + flavonoid content explains overall antioxidant performance

Principal Component Analysis (PCA)

Honey sample discrimination was successfully conducted the first principal component (PC1) 41.7% indicating correlation with antimicrobial activity and the second principal component (PC2) 66.7% indicating correlation with polyphenol and flavonoid components. Figure 3 presents a principal component analysis biplot, a crucial tool used in this study to visualize and interpret the relationships between the examined parameters: physicochemical properties, chemical compounds, total phenolic content (TPC), total flavonoid content (TFC), antioxidant, and antibacterial activity. This biplot aids in the discrimination of the honey samples based on their parameters versus inhibition zone. Polyphenol and flavonoid variables had the most significant length and a tiny angle between these two vectors, suggesting that honey with high polyphenol content also has high flavonoid content (Figure 3). Flores honey and Akasia honey samples in the second quadrant (PC1 negative and PC2 positive) showed the highest TPC and TFC compared to other honey types. However, Akasia honey is located near the bottom center of the plot, indicating that the chemical profile of Akasia honey tends to be more moderate. Rambutan honey in third quadrant samples have a high moisture content, with lower polyphenol and flavonoid contents than Flores honey. Randu honey stands in fourth quadrant with positive correlation with antibacterial activity. Kelulut honey stands out with its unique profile, characterized by positive PC1 and PC2 values, by being in the first quadrant, indicating that this honey has high antibacterial activity characteristics that distinguish it from other honey, a remarkable feature that leaves a lasting impression.

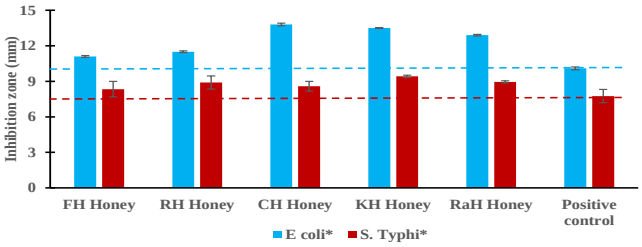

Figure 2. Antimicrobial activity test of the five honey samples on the test microbe *Escherichia coli* and *Salmonella* Typhi; positive control is clindamycin 100 mg/L; and negative control is distilled water. FH: Flores honey, RH: Randu honey, CH: Akasia honey, KH: Kelulut honey, RaH: Rambutan honey

Table 8. Mean inhibition zones (mm) of Indonesian honey samples against *Escherichia coli* and *Salmonella* Typhi (mean ± SD, n=3)

Sample	Inhibition zone (mm)	
	Escherichia coli*	***Salmonella* Typhi***
Florest honey (FH)	11.10±0.08a	8.34±0.65a
Randu honey (RH)	11.50±0.07a	8.90±0.55b
Akasia honey (CH)	13.80±0.11b	8.58±0.41a
Kelulut honey (KH)	13.50±0.02b	9.42±0.10b
Rambutan honey (RaH)	12.90±0.06b	8.95±0.09b
Positive control (Clindamycin 100 mg/mL)	10.10±0.12c	7.75±0.56c
Negative control (Distilled water)	0	0

Note: *One-way ANOVA revealed highly significant differences ($p<0.001$) in inhibition zones among honey types for both bacterial species (*E. coli*: $F_{5,14} = 33.98$, $p = 2.38\times10^{-7}$; *Salmonella*: $F_{5,12} = 31.37$, $p = 1.72\times10^{-6}$). Post-hoc Tukey's HSD test indicated that Kelulut honey exhibited significantly larger inhibition zones compared to the control antibiotic and most other honey types, with the exception of Akasia honey (*E. coli*) and Rambutan honey (both bacteria)

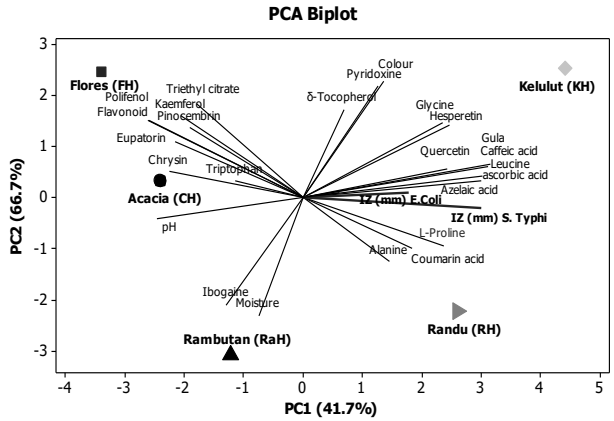

Figure 3. Principal component analysis biplot obtained for the examined physicochemical properties, chemical compounds, total phenolic content (TPC), total flavonoid content (TFC), and antibacterial activity

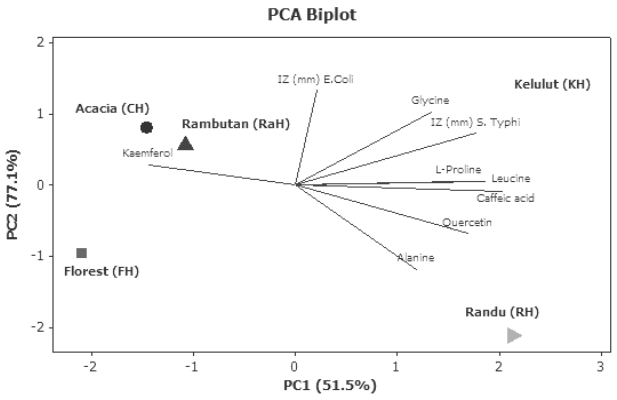

Figure 4. Principal component analysis (PCA) biplot obtained for the examined parameters amino acid compounds, polyphenol, and antibacterial activity

Table 9. Comparative bioactive profile and antimicrobial activity of Indonesian and Southeast Asian honeys

Honey type	Country	TPC (mg GAE/100g)	TFC (mg QE/100g)	Antioxidant (IC50 mg/mL)	Antimicrobial activity (*E. coli*, mm)	Dominant bioactives	References
Kelulut honey	Indonesia	829.84±0.04	697.63±0.02	3.31±0.01	13.50±0.02	L-Proline, Caffeic acid, Quercetin, Azelaic acid	Present study
Flores honey	Indonesia	4526.12±35.22	3810.96±40.13	2.11±0.03	11.10±0.08	Kaempferol, Pinocembrin, Eupatorine	Present study
Manuka	New Zealand	179.5±33.3	NA	1.4-2.5	8-12	Methylglyoxal (MGO)	Deng et al. 2018
Stingless bee	Thailand	89-495	58.09±0.29	43.99±0.38	~8-9	Flavonoids	Wongsa et al. 2023
Multifloral	Vietnam	495-1110	75.54±3.95	43.04±1.07	7.2-10.4	Flavonoids, Phenolic acids	Pham et al. 2022
Stingless bee	Philippines	495-673	35.04±0.10	43.04	~8.5	Flavonoids, Phenolics	Belina-Aldemita et al. 2019

The principal component analysis (PCA) results in Figure 4 determined the components that most influence the antibacterial activity of Indonesian honey samples from different regions. This analysis was instrumental in revealing the potential of Indonesian honey regarding its amino acid profile and antibacterial properties. The identification of a strong correlation between the amino acid L-proline and the antibacterial activity against *E. coli* and *Salmonella* Typhi, characterized by a slight angle between the two, opens up exciting possibilities within microbiological research. As noted by Mardirossian et al. (2019), Proline-rich antimicrobial peptides (PrAMPs) are regarded as promising agents for tackling multidrug-resistant pathogens due to their significant antimicrobial activity combined with low cytotoxicity. The antibacterial properties observed in most Indonesian honey can be attributed to the high concentration of amino acid derivatives; a distinctive characteristic not typically found in honey sourced from other countries. This uniqueness could stimulate further research and the development of novel antibacterial agents.

The PCA biplot reveals that illustrates distinct correlations between bioactive components and biological activities. Amino acids (L-Proline, Leucine) showed strong positive correlations with antibacterial activity (IZ against *Escherichia coli* and *Salmonella* Typhi), while phenolic and flavonoid contents were closely associated with antioxidant capacity (IC_{50}). This dichotomy underscores differing mechanisms of action between Flores honey (antioxidant-rich) and Kelulut honey (antibacterial-rich), and highlights Indonesian honey's diverse bioactive matrix compared to regional honeys (Table 9).

In conclusion, this study demonstrated that Indonesian honeys, particularly Flores honey and Kelulut honey, possess strong antioxidant and antibacterial activities associated with their high total phenolic and flavonoid contents. Pearson correlation analysis confirmed that phenolic compounds were the major contributors to antioxidant activity, with flavonoids playing a complementary role. Although these honeys exhibited higher bioactive compound levels compared to some regional reports, such comparisons should be interpreted cautiously due to unit differences among published studies.

Overall, the findings highlight Indonesia's potential as a source of bioactive honeys with distinct physicochemical and functional properties. However, this study has certain limitations, including a small number of replicates (n = 3 per honey type), testing against only two bacterial species, and the absence of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) determinations. Future research should therefore include larger sample sets, additional microbial targets, and quantitative antibacterial assays to further validate these findings and explore mechanistic pathways.

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