

Standardization of Ethanolic Extracts of Propolis from Four East Kalimantan Stingless Bees Species

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Received: 1.05.2024; Accepted: 1.01.2025; Published: 6.09.2025

Abstract: Propolis, a resinous substance collected by various bee species, exhibits diverse characteristics and bioactive properties depending on its source. The standardization of propolis aims to establish a benchmark and serve as a reference for further research in developing herbal products derived from it. The samples analyzed in this study included propolis sourced from bees such as *H. itama*, *T. biroi*, *G. thoracica*, and *T. fuscobalteata*, collected from Samarinda, East Kalimantan, Indonesia. The testing process involved both specific and non-specific parameters, phytochemical tests, and the determination of total phenolics and flavonoids. This study presents the results of standard ethanol extracts for each propolis, covering both specific and non-specific parameters. Overall, the standardization tests yielded results that met the established criteria. The research findings revealed variations in organoleptic tests and values in other assessments. Additionally, differences in phytochemical content, total polyphenols, and flavonoid content were observed among the various propolis samples. All raw propolis samples met the requirements for low metal content and were negative for microbial contamination. This study underlines the importance of propolis standardization, making it a valuable recommendation for the manufacture of derivative products from this natural material.

Keywords: standardization; propolis; stingless bee; East Kalimantan.

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1. Introduction

The stingless bee, also known as the kelulut bee, is a species distinguished by its small size and lack of a stinger [1]. Kelulut bees thrive in Indonesia, particularly in the city of Samarinda. In Samarinda, beekeepers are motivated by the revenue generated from the sale of kelulut honey [2]. In addition to honey production, kelulut bees also produce propolis, commonly referred to as bee glue. This substance, characterized by a thin brown layer enveloping the honey and pollen sacs of bees, serves as a self-protective measure against predators, leading to a higher production of propolis compared to honey [3].

Propolis is a resinous substance obtained from the shoots, leaves, and exudates of trees and plants. It is mixed with pollen, wax, and enzymes produced by *Trigona* species bees. Propolis has a blackish-green or blackish-brown color and has an astringent and bitter taste [4]. The composition of propolis can vary by region, depending on the plant sources collected by

the bees [5]. Propolis derived from different types of bees has varying compositions and biological properties [6].

Propolis boasts numerous benefits and multifaceted activities, serving as an antioxidant, antifungal, anticancer, antiviral, and antibiotic agent. Beyond these roles, propolis harbors a plethora of metabolite compounds, including flavonoids, alkaloids, phenols, tannins, and saponins [7]. Its diverse array of beneficial ingredients has contributed to the growing popularity of propolis as a health supplement or alternative medicine in various countries. Natural ingredients sourced for medicinal products hold promising potential for discovering raw medicinal materials. Researchers have highlighted propolis as one such natural ingredient with immense potential due to its myriad health benefits [8].

Propolis, a resinous substance collected by various bee species, exhibits diverse characteristics and bioactive properties depending on its source. Among these bees, *Heterotrigona itama* can produce a sticky variant of propolis with high antioxidant activity [9]. In terms of composition, it contains alkaloids, phenols, flavonoids, terpenoids, saponins, and steroids [10]. On the other hand, propolis produced by *Tetragonula biroi* bees is rich in polyphenols, flavonoids, phenolics, alkaloids, steroids, and anthraquinones, which exhibit antioxidant, antifungal, and anticancer properties [11–15]. Additionally, propolis from the *Geniotrigona thoracica* and *Tetragonula fuscobalteata* variants has antioxidant, antibacterial, anti-inflammatory, antifungal, antiviral, antidiabetic, and anticancer activities, containing secondary metabolites such as terpenoids, tannins, saponins, and flavonoids. *Tetragonula fuscobalteata* propolis shows activity against bacteria and fungi, and is effective for treating burns, containing flavonoids, phenolics, tannins, and saponins [16–19].

The composition of bioactive compounds in propolis varies based on geographic location and bee species. Differences in bee characteristics and types can influence the resulting products. Additionally, the foraging ability of bees impacts propolis quality as they collect resin from trees surrounding their nests or apiaries. Geographic factors also play a pivotal role in determining the bioactive compound content of propolis, as the flora in each area varies, influencing its bioactivity [20].

Therefore, conducting a standardization process for extraction is imperative to ensure high-quality extracts before industrial-scale production [21,22]. Polar solvents such as methanol and ethanol can be used in extraction due to their high efficiency for a wide range of compounds [23]. The standardization of herbal product ingredients derived from natural sources involves a comprehensive set of parameters, procedures, and measurement techniques aligned with pharmaceutical quality standards. This quality paradigm includes adherence to requirements for chemical, biological, and pharmaceutical aspects, along with assurances regarding stability limits similar to those expected of pharmaceutical products in general [24]. The lack of research on propolis standardization underscores the need for researchers to pursue this area. Standardizing propolis can establish a benchmark for subsequent research aimed at developing herbal products derived from this substance. Therefore, this research focuses on standardizing the ethanol extracts of propolis from *H. itama*, *T. biroi*, *G. thoracica*, and *T. fuscobalteata*, employing specific and non-specific parameters, and comparing the chemical content of the extracts through phytochemical tests, as well as determining the total phenolic and flavonoid content.

2. Materials and Methods

2.1. Materials.

The materials utilized in this study comprised propolis sourced from *Heterotrigona itama*, *Tetragonula biroi*, *Geniotrigona thoracica*, and *Tetragonula fuscobalteata* bees collected in Samarinda. Additionally, distilled water, 70% ethanol, 96% ethanol, chloroform, quercetin, gallic acid, 10% sodium carbonate solution, 10% aluminum chloride solution, Mayer's reagent, Liebermann-Burchard reagent, NaOH, 5% ferric chloride solution, and 10% Folin-Ciocalteu reagent were used.

2.2. Sample preparation.

The propolis underwent a crushing process to reduce its size. Subsequently, a portion of the propolis was subjected to maceration in ethanol as the solvent. The propolis was combined with an ethanol solvent until fully submerged. After stirring and pressing the mixture, it was left to macerate for 24 hours, with subsequent remaceration every 24 hours. The resulting macerate was then filtered and placed in a water bath at 60°C until a thick extract was obtained.

2.3. Organoleptic evaluation of extracts.

Organoleptic determination of the extracts encompasses evaluating their shape, color, odor, and taste.

2.4. Content of dissolved compounds.

To determine the levels of water-soluble compounds, a specified amount of propolis extract was macerated for 24 hours using 50 ml of chloroform water in a stoppered flask, with intermittent shaking for the first 6 hours, followed by resting for 18 hours. The resulting macerate was then filtered, and 20 ml of the filtrate was evaporated to dryness in a pre-weighed flat-bottomed shallow dish. The residue was heated at 105°C until a constant weight was achieved. The percentage content of water-soluble compounds was calculated based on the initial extract weight.

For compounds soluble in ethanol, a similar procedure was followed, in which a certain amount of propolis extract was macerated for 24 hours using 50 ml of 96% ethanol in a stoppered flask, with agitation for the initial 6 hours and subsequent incubation for 18 hours. The resulting macerate was filtered, and 20 ml of the filtrate was evaporated to dryness in a pre-weighed flat-bottomed shallow dish. The residue was heated at 105°C until a constant weight was attained, and the percentage content of ethanol-soluble compounds was calculated relative to the initial extract weight [25].

2.5. Determination of drying shrinkage.

1-2 grams of the extract were weighed into a closed, shallow weighing bottle that had been preheated at 105°C for 30 minutes and previously weighed. The material in the bottle was leveled by shaking until the sample layer was approximately 5-10 mm thick. It was then placed in a drying room (oven) with the lid opened until a constant weight was achieved. Before each

drying cycle, the bottles were sealed and allowed to cool in a desiccator to room temperature [26].

2.6. Determination of specific gravity.

The specific gravity of the liquid extract was measured using a calibrated, empty, and dry pycnometer. The weight of the empty pycnometer and the weight of water at 25°C were determined. The pycnometer was filled with the liquid extract and weighed at 25°C. The specific gravity of the liquid extract was calculated as the ratio of the weight of the extract-filled pycnometer to the weight of water at 25°C [27].

2.7. Determination of water content.

Several propolis extracts were weighed in a balanced container and then dried at 105°C for 5 hours in an oven before being weighed again. This process was repeated at 1-hour intervals until the difference between two consecutive weighings was no more than 0.25% [28].

2.8. Phytochemical test.

The extract content test was conducted using a qualitative method based on color changes [29]. For the alkaloid test, the test filtrate was placed into a test tube, followed by the addition of 1 – 2 drops of Mayer's reagent. The formation of a cloudy white or yellow precipitate in the solution indicated a positive result for alkaloids. In the flavonoid test, a few drops of NaOH were added to the liquid extract in a test tube. A change in the solution's color to dark yellow indicated a positive result for flavonoids. For the phenolic test, the extract was dissolved in 5 mL of distilled water, and a few drops of neutral 5% ferric chloride were added. A dark green to blackish coloration of the solution indicated a positive result for phenolics. The steroid and saponin tests were conducted using a qualitative method based on color changes. In the steroid/triterpenoid test, 2 mL of ethanol extract was added to 2 mL of n-hexane and shaken. Liebermann-Burchard reagent was then added. Steroid-positive extracts were indicated by a change in color to greenish blue, while triterpenoid-positive extracts showed a color change to red-purple. Tannin was determined by adding a few drops of 1% FeCl₃ solution; if the solution produced a brown-black color, then the extract contained tannins.

2.9. Microbial contamination.

Microbial contamination analysis was conducted to determine the total plate count. A specific quantity of propolis was mixed with phosphate buffer (pH 7.2) to achieve a volume of 10 mL. Subsequently, dilutions were performed until reaching a dilution factor of 10⁻⁶. From each dilution, 1 mL was transferred to sterile petri dishes in triplicate. To each petri dish, 15-20 mL of Nutrient Agar (NA) seeding medium at a temperature of 45 ± 10°C was added. The number of colonies that developed after incubating the petri dishes inverted for 24 hours at a temperature of 35-37°C was counted to determine the total plate count. Similarly, the process for determining the total yeast and mold count was carried out using potato dextrose agar (PDA) seed media.

2.10. Metal contamination.

The heavy metal content was analyzed using spectrophotometry and light scattering, with a standard lead solution as a reference. Metal contaminants such as mercury, arsenic, lead, and cadmium were examined.

2.11. Total phenolic content test.

The total phenolic content (TPC) was quantitatively tested using Folin-Ciocalteu reagent, with gallic acid as a standard reference. A primary gallic acid standard was prepared by weighing 10 mg of gallic acid and placing it into a 10-ml volumetric flask. Next, 1 ml of methanol was added and stirred until dissolved. Distilled water was then added to the mark and homogenized. A standard series ranging from 0.5 to 16 µg/ml was prepared in 10 ml volumetric flasks. To each flask, 0.5 ml of 10% Folin-Ciocalteu reagent was added and left to stand for 3-8 minutes. Subsequently, 4 ml of 10% sodium carbonate reagent was added, followed by stirring with a vortex mixer until homogeneous. The mixture was then left for 2 hours while protected from light. The absorbance of the standard solutions was measured at a wavelength of 754 nm.

For the test solution, 0.1 g of the extract was weighed and placed into a 10 ml volumetric flask. Distilled water was added to the mark and homogenized. Next, 500 µl of the solution was pipetted and transferred into a 10 ml test tube, while being protected from light. Then, 0.5 ml of 10% Folin-Ciocalteu reagent was added and allowed to stand for 3 – 8 minutes. Following this, 3 ml of 10% sodium carbonate reagent was added and stirred with a vortex mixer until homogeneous. Similar to the standard procedure, the mixture was left for 2 hours while protected from light, and the absorbance was measured at a wavelength of 754 nm [30].

2.12. Total flavonoid content test.

The total flavonoid content (TFC) was determined using the colorimetric method with aluminum chloride and quercetin serving as a reference standard. The test solution was prepared by weighing 0.1 g of the extract and placing it into a microtube. Then, 1 ml of methanol was added and stirred with a vortex mixer until homogeneous. The mixture was filtered into a 10-ml measuring flask, and ethanol was added to reach the mark. A comparison solution was prepared by weighing 5 mg of quercetin, placing it into a microtube, and adding 1 ml of methanol. A series of dilutions of the comparison solution were made with concentrations of 5, 10, 20, 40, and 80 µg/ml. Subsequently, the test solution and each series of comparison solutions were separately pipetted into appropriate containers, to which 750 µl of 2% aluminum chloride was added. The containers were then shaken and allowed to sit for 1 hour in the dark. Absorbance was measured at the maximum absorption wavelength. A calibration curve was constructed, and the concentration of the test solution was calculated based on the curve.

3. Results and Discussion

This study used samples of *Heterotrigona itama*, *Tetragonula biroi*, *Geniotrigona thoracica*, *Tetragonula fuscobalteata* propolis from Samarinda, East Kalimantan. The quality standardization includes several specific and non-specific parameters. Specific parameters

comprised organoleptic tests, water-soluble compound tests, and ethanol-soluble compound levels (Table 1).

Table 1. Specific parameter standardization results.

Parameters		Propolis			
		<i>H. itama</i>	<i>T. biroii</i>	<i>G. thoracica</i>	<i>T. fuscobalteata</i>
Organoleptic	Form	Viscous	Viscous	Viscous	Viscous
	Color	Dark brown	Light brown	Dark brown	Dark brown
	Odor	Distinctive propolis scent	Distinctive propolis scent	Distinctive propolis scent	Distinctive propolis scent
	Taste	Bitter, slightly astringent	Bitter, slightly sweet	Slightly chewy	Tasteless
Water soluble compounds (%)		39.97%	26.21%	89%	92%
Ethanol soluble compounds (%)		73.76%	95%	97%	88%

Non-specific parameters include determination of drying shrinkage, moisture content, specific gravity, and ash content (Table 2).

Table 2. Non-specific parameter standardization results.

Parameters	Propolis			
	<i>H. itama</i>	<i>T. biroii</i>	<i>G. thoracica</i>	<i>T. fuscobalteata</i>
Dry shrinkage	5.61%	2.91%	2.38%	7.94%
Specific gravity	0.90 g/mL	0.91 g/mL	1.26 g/mL	1.23 g/mL
Water content	1.08%	1.12%	1.00%	1.01%
Ash content	3.06%	9.03%	0.59%	0.96%

Additionally, identification of metal and microbial contamination was conducted to determine the characterization and potential of propolis (Table 3).

Table 3. The metal and microbial contamination of propolis results.

Parameters		Units	Propolis				Terms
			<i>H. itama</i>	<i>T. biroii</i>	<i>G. thoracica</i>	<i>T. fuscobalteata</i>	
Metal Contaminants	Mercury (Hg)	mg/L	0.00225	0.00122	0.00105	0.00060	≤ 0.5
	Arsenic (As)	mg/L	< 0.00002	< 0.00002	< 0.00002	< 0.00002	≤ 5
	Lead (Pb)	mg/L	< 0.0001	< 0.0001	0.0002	< 0.0001	≤ 10
	Cadmium (Cd)	mg/L	< 0.00002	< 0.00002	< 0.00002	< 0.00002	≤ 0.3
Microbial Contaminants	<i>Escherichia coli</i>	*	Negative	Negative	ND	Negative	Negative
	Yeast and mold	CFU/gr	0	100	ND	70	≤ 10 ⁴
	<i>Salmonella sp.</i>	*	Negative	Negative	ND	Negative	Negative
	<i>Shigella sp.</i>	*	Negative	Negative	ND	Negative	Negative
	<i>Pseudomonas aureoginosa</i>	*	Negative	Negative	ND	Negative	Negative
	<i>Staphylococcus aureus</i>	*	Negative	Negative	ND	Negative	Negative

ND = Not Determined; * = unit; < = Below Method Detection Limit (MDL).

3.1. Organoleptic.

One of the specific parameters is organoleptic determination, which serves as an initial assessment of the characteristics of propolis extract. This evaluation is subjectively conducted using the four senses to describe its form, color, odor, and taste. The organoleptic properties of the four types of propolis exhibit both similarities and differences. As shown in Table 1, these properties display several differences, particularly in the color (Figure 1) and taste of the extract. These variations may arise from differences in manufacturing methods or metabolic processes among different bee species. Additionally, the bees' preference for particular plants can also influence the taste of the propolis they produce.

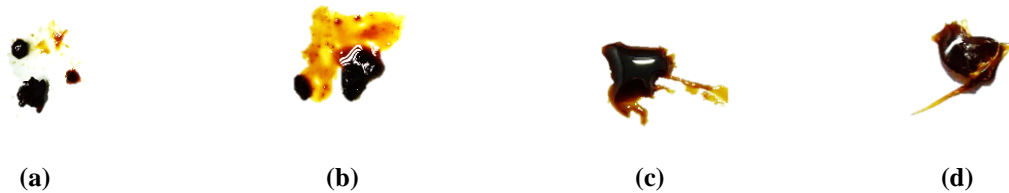


Figure 1. Characterization of propolis color (a) *H. itama*; (b) *T. biroii*; (c) *G. thoracica*; (d) *T. fuscobalteata*.

3.2. Levels of compounds soluble.

Assessing the solubility level of a compound in a specific solvent aims to provide initial insight into the amount of the compound that can dissolve in the solvent used. In Table 1, *T. fuscobalteata* propolis demonstrates superiority compared to other types of propolis in terms of extract solubility in water. Conversely, when using ethanol as the solvent, *G. thoracica* propolis surpasses the other types of propolis. Generally, ethanol was found to be superior to water for most types of propolis. An important commonality among the different species of propolis is that the solubility of the extract is significantly higher in ethanol compared to water. This indicates that the active compounds in the extract are more readily absorbed in ethanol due to its universal solvent properties, which allow it to attract both polar and non-polar compounds. In contrast, water can only attract polar compounds, resulting in partial extraction of certain compounds.

3.3. Determination of drying shrinkage.

One of the standardization parameters required for extracts from natural ingredients is the determination of drying losses. This parameter aims to illustrate the maximum limit or range of values for the loss of compounds such as volatile compounds, thermolabile compounds, or water during the drying process. Generally, the drying shrinkage determination value for a good propolis extract is $\leq 10\%$ [31]. Based on the results obtained from all propolis extracts, they meet the established requirements, with the drying loss value of propolis being below 10% (Table 2). In this instance, *H. fimbriata* propolis outperforms other types of propolis, as a smaller percentage of drying shrinkage indicates that the sample better retains its nutritional content and active compounds during the drying process.

3.4. Determination of specific gravity.

Determining specific gravity is defined as the ratio of the density of a substance to the density of air, expressed as mass per unit volume. The determination of specific gravity is carried out to establish a limit on the amount of mass per volume, which is a specific parameter for liquid extract. This ensures that the extract remains thick enough to be pourable. Specific gravity is also related to purity, as it helps identify contamination [32]. A high specific gravity value indicates a good concentration of active compounds in the extract, while a low value suggests low solubility of the active compound in the solvent used. In standardizing propolis extracts, specific gravity serves as an important parameter that ensures the consistency and quality of the resulting extract product, as well as guiding its application in various scenarios.

3.5. Determination of water content.

The determination of water content is conducted to ascertain the minimum limit or range of values for the amount of water present in the extract. Low water content in the extract

can minimize or prevent the growth of microorganisms and mold (fungus) within it. According to the Indonesian Herbal Pharmacopoeia (2017), the required water content is generally less than 10%. The results obtained from each propolis extract indicated low water content (Table 2). Water content plays a crucial role in determining the stability of an extract; typically, a water content exceeding 10% poses risks, as higher water content can diminish the biological activity of the extract during storage and hasten the growth of microorganisms [31, 33].

3.6. Determination of ash content.

The total ash content can serve as a reference for assessing the quantity of minerals present both internally and externally throughout all stages of extract production, from the initial to the final stages. The percentage results of the ash content (Table 2) indicate that four propolis extracts fall within the category of good propolis extracts ($\leq 10\%$) [28]. Among all propolis extracts, *H. fimbriata* propolis exhibits a superior value compared to other propolis, with a lower percentage of ash content. The ash content obtained is also linked to metal contamination. If the sample contains metals, they can remain in the ash. Consequently, if the extract has an excessively high ash content, it may pose risks and may not be suitable for consumption. However, it is important to note that not all minerals or metals present have negative impacts; some minerals, such as calcium, iron, zinc, and sodium, are beneficial for the body. The concern lies in the fact that a higher ash content value indicates a greater presence of inorganic compounds, including heavy metals such as mercury (Hg), arsenic (As), lead (Pb), and cadmium (Cd). Thus, if the extract's ash content is excessively high, it may pose risks and not be suitable for consumption.

3.7. Metal contaminants.

The results of metal contamination analysis for all propolis samples (Table 3) indicate that four species of stingless bee propolis have values below the standard requirements and meet the appropriate standards regarding metal contamination, including mercury (Hg), arsenic (As), lead (Pb), and cadmium (Cd). Heavy metals such as Pb and Cd should not be consumed in excess, as they can lead to poisoning, neurotoxicity, and even death [34]. Metal contamination is correlated with the ash content; excessive ash content in the extract can render it unsafe for consumption. Heavy metals can have acute and chronic toxic effects on various body organs, including gastrointestinal and kidney dysfunction, nervous system disorders, skin lesions, blood vessel damage, immune system dysfunction, birth defects, and cancer. These represent examples of complications arising from the toxic effects of heavy metals [35]. Therefore, propolis must adhere to standard requirements to prevent metal contamination above permissible limits.

3.8. Microbial contamination.

Products derived from natural ingredients must be free from microbial contamination if they are to be utilized as fundamental components in medicines, cosmetics, or food. However, achieving this can sometimes be challenging. Natural products intended for consumption, whether as essential components for herbal medicines or cosmetic products, are strictly prohibited from containing pathogenic microorganisms such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Clostridia* sp., *Shigella* sp., and *Salmonella* sp. Specific values or ranges regarding purity and permissible contamination levels are established.

The results of the microbial contamination test on the propolis samples (Table 3) show that there was no contamination by *Escherichia coli*, molds and yeasts, *Salmonella* sp., *Shigella* sp., *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. The presence of microbes in stingless bee propolis is uncommon due to its potent antimicrobial properties. Additionally, propolis possesses antibacterial, antifungal, and antiviral properties that combat pathogenic microorganisms [36–39].

During standardization processes, ensuring the cleanliness and quality of materials is paramount. Ethanol has effective antimicrobial properties that eliminate microbes and pathogens present in natural ingredients like propolis during extraction. It serves as a natural preservative, enhancing the product's resistance to microbial growth. However, the use of ethanol must be carefully managed to ensure efficiency and safety, with proper procedures in place to minimize residual ethanol content in the final product, making it safe for consumption.

3.9. Phytochemical test.

The extract content test aims to qualitatively identify the metabolite compounds present in the extract of each propolis, as shown in Table 4.

Table 4. Phytochemical test results of four species of stingless bee propolis.

Compound	Propolis			
	<i>H. itama</i>	<i>T. biroi</i>	<i>G. thoracica</i>	<i>T. fuscobalteata</i>
Alkaloid	+	+	+	+
Flavonoid	+	+	+	+
Phenolic	+	+	+	+
Steroid/Triterpenoid	- / -	- / -	- / +	- / +
Saponin	+	+	-	-
Tanin	+	+	+	+

Flavonoid, terpenoid, and phenolic compounds were also found in *H. itama* propolis from Malaysia [40]. Aromatic compounds such as phenols, flavonoids, and terpenes were also found in the propolis of *G. thoracica* bees from North Sumatra (Indonesia) and Phattalung (Thailand), which are different locations from the propolis used in this study [41,42]. The stingless bee species *T. biroi*, from Nigeria, also shows the presence of key compounds, including flavonoids, isoflavones, and phenolics [43]. Propolis from these locations did not show any alkaloids, while our findings detected them. This difference may be due to the varying sources of resin collected by the bees. Some tree species contain alkaloids, which can affect the composition of compounds in propolis.

However, variations in phytochemical compound content in propolis can occur due to diverse factors, both natural and associated with environmental and production processes. Factors such as the plant species serving as the resin source, geographical location, season, and climate can introduce variations in the profile of phytochemical compounds between among different types of propolis [44-46]. Moreover, the bee species and genetic factors also influence resin collection preferences and processes. Additionally, the methods employed for collecting, processing, and storing propolis can impact the phytochemical compound content. Differences in beekeeping practices and the environmental quality in which bees thrive can also affect propolis quality [47]. Therefore, a comprehensive understanding of all these factors is essential for comprehending and managing variations in phytochemical compound content among the four types of stingless bee propolis, as well as for optimizing production conditions to achieve propolis of desired quality.

3.10. Determination of total phenolic and flavonoid content.

The determination of the Total Phenolic Content (TPC) levels in propolis ethanol extract was performed by using a gallic acid calibration curve, with the curve equation $y = 0.1378x + 0.0762$ ($R^2 = 0.9999$). Similarly, the Total Flavonoid Content (TFC) of propolis ethanol extract was assessed using another gallic acid calibration curve, which provided the equation $y = 0.0381x - 0.0856$ ($R^2 = 0.9929$). Research indicates that there are differences in the values of TPC and TFC among the various types of propolis, as shown in Table 5. These differences may reflect the diverse botanical sources and environmental conditions affecting the bees.

Table 5. Total phenolic and total flavonoid content.

Parameters	Propolis			
	<i>H. itama</i>	<i>T. biroi</i>	<i>G. thoracica</i>	<i>T. fuscobalteata</i>
TPC (mg GAE/g)	0.78 ± 0.01	0.86 ± 0.03	0.54 ± 0.01	1.39 ± 0.01
TFC (mg QE/g)	11.04 ± 0.29	3.07 ± 0.01	13.71 ± 0.36	13.50 ± 0.41

When examining total phenolics and flavonoids, variations in the TPC and TFC values of propolis extracts sourced from different types of bees in Samarinda were observed. This variation can be attributed to several factors. Differences in the geographical location of beehives impact the quality of the propolis produced, including its phenolic compound [48, 49]. Propolis, obtained by bees from plant sap or resin, varies depending on the diversity of plant species in a region, resulting in different types of phenolic compounds [50, 51]. Moreover, disparities in bee species influence the propolis products obtained, as each species may exhibit specific preferences for the types of plants or trees from which they collect resin. Various plants produce resins with distinct chemical compositions, affecting the compounds present in the propolis. Additionally, different bee species may possess varying enzymes and metabolic mechanisms used in the resin-to-propolis conversion process, influencing the types of compounds produced or transformed during propolis formation.

4. Conclusions

The standardization of ethanol extracts from *H. itama*, *T. biroi*, *G. thoracica*, and *T. fuscobalteata* was obtained, both specifically and non-specifically. Overall, the specific and non-specific standardization tests yielded results that met the standards, with variations observed in organoleptic properties and other values across each test. The research also revealed differences in phytochemical content, total polyphenol levels, and flavonoid content among the various propolis samples. In the metal contamination test, all raw propolis samples met the requirements, showing low levels of metal contamination. The implications of this research extend to the herbal industry, public health, and product development. Further research is encouraged to explore the chemical differences between propolis samples and their impact on medicine and public health.

Author Contributions

Conceptualization, P.M.K.; methodology, M.N.I., R.P., M.A.S.; software, P.H.S., K.A.A.K.; validation, E.M., and P.M.K.; formal analysis, P.M.K., M.N.I., R.P., and M.A.S.; investigation, P.M.K.; resources, P.M.K.; data curation, E.M., P.H.S. and P.M.K.; writing—original draft preparation, M.N.I., R.P., and M.A.S.; writing—review and editing, P.M.K. and I.A.M.; visualization, M.N.I.; supervision, P.M.K.; project administration, P.M.K.; funding

acquisition, P.M.K. All authors have read and agreed to the published version of the manuscript.

Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.

Data Availability Statement

Data supporting the findings of this study are available upon reasonable request from the corresponding author.

Funding

This research was funded by KEDAIREKA 2023 (Contract ID: 62/E1/HK.02.02/2023).

Acknowledgments

We gratefully acknowledge the Ministry of Education, Culture, Research, and Technology of the Republic of Indonesia for funding this research through the Kedaireka Kampus Merdeka program in 2023. Special thanks are extended to Rendri Arista Avimaro (Sahabat Kelulut Samarinda), the Laboratory of the Faculty of Pharmacy, Universitas Muhammadiyah Kalimantan Timur, and the Health Laboratory of East Kalimantan, for their facilitation.

Conflicts of Interest

The authors declare no conflict of interest.

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