STANDARDIZATION OF ETHANOLIC EXTRACTS PROPOLIS FROM FOUR EAST KALIMANTAN STINGLESS BEES SPECIES

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Diajukan oleh:
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JANUARI 2024

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Diajukan Sebagai Salah Satu Persyaratan
Untuk Memperoleh Gelar Sarjana Farmasi
Fakultas Farmasi Universitas Muhammadiyah Kalimantan Timur

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

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Abstract: Propolis, a resinous substance collected by various bee species, exhibits diverse characteristics and bioactive properties depending on its source. Propolis standardization aims to establish standard and serve as a reference for further research in developing herbal products derived from propolis. 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 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 meeting 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 various propolis samples. Although all raw propolis samples met the requirements for low metal content and negative for microbial contamination.

Keywords: Standardization; Propolis; Stingless Bee; East Kalimantan

1. Introduction

The stingless bee, also known as the kelulut bee, is a species distinguished by its lack of a stinger and diminutive size [1]. Kelulut bees thrive in Indonesia, including the city of Samarinda. In Samarinda, beekeepers are driven by the revenue generated from the sale of kelulut honey [2]. Besides honey production, kelulut bees also manufacture 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. This material is mixed with pollen, wax, and enzymes, which are then produced by *Trigona sp* bees. Propolis has a blackish-green or blackish-brown color and has an astringent and bitter taste [4]. The composition of propolis in each region can vary depending on the plant source obtained by bees [5]. Propolis that comes from different types of bees has different compositions and biological abilities [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 propelled propolis into popularity, earning it widespread usage as a health supplement or alternative medicine across various

nations. Natural ingredients sourced for medicinal products hold promising potential for discovering raw medicinal materials. Researchers have underscored 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 [9, 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 [6], [12–15]. 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, fungi, and burns, containing flavonoids, phenolics, tannins, and saponins[10], [16–19].

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

Therefore, conducting a standardization process for extraction becomes imperative to ensure superior quality extracts before industrial-scale production. The standardization of herbal product ingredients derived from natural sources entails a comprehensive set of parameters, procedures, and measurement techniques aligned with elements pertinent to the pharmaceutical quality paradigm. This quality paradigm encompasses adherence to standard requirements for chemical, biological, and pharmaceutical aspects, along with assurances regarding stability limits similar to those expected of pharmaceutical products in general [21]. The dearth of research concerning propolis standardization underscores the necessity for researchers to engage in such endeavors. 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,* dan *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 reagent, Liebermann-Burchard reagent, NaOH, 5% ferric chloride solution, 10% Folin-Ciocalteu reagent

2.2. Sample preparation

The propolis underwent a crushing process to reduce its size. Subsequently, a portion of the propolis was subjected to maceration ethanol as the solvent. The propolis was combined with 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, followed by placement in a water bath at 60°C until a thick extract was obtained.

2.3. Organoleptic extratct

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 initial 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, wherein 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 [22].

2.5. Determination of drying shrinkage

1-2 grams of the extract were weighed into a closed shallow weighing bottle preheated at 105ºC for 30 minutes and previously weighed. The material in the bottle was leveled by shaking until the sample layer was ± 5-10 mm. 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 [22].

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 25ºC were determined. The pycnometer was filled with the liquid extract and weighed at 25ºC. 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 25ºC [22].

2.7. Determination of water content

Several propolis extracts were weighed in a balanced container, then dried at 105ºC for 5 hours in an oven and weighed. This process was repeated at 1-hour intervals until the difference between two consecutive weighings was no more than 0.25% [22].

2.8. Phytochemical test

The extract content test was conducted using a qualitative method based on color changes [23]. 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. A cloudy white or yellow precipitate formation 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 [24]. In the steroid/triterpenoid test, 2 mL of ethanol extract was added to 2 mL of n-hexane and shaken. Lieberman-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 added a few drops of 1% $FeCl_3$ solution. If the solution produces a brown-black color, then the extract contains tannins.

2.9. Microbial contamination

Microbial contamination analysis was conducted for the determination of 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, dilution was 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 NA (Nutrient Agar) 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 by 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 up 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 µg 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 [15].

2.12. Total flavonoid content test

The Total Flavonoid Content (TFC) was determined using the colorimetric method with aluminum chloride, with 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, with 750 µl of 2% aluminum chloride added to each. 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 [15].

3. Results and Discussion

This study used samples of *Heterotrigona itama, Tetragonula biroi, Geniotrigona thoracica, Tetragonula fuscobalteata* propolis from Samarinda City, East Kalimantan. The quality standardization carried out includes several specific and non-specific parameters. Specific parameters include organoleptic tests, water-soluble compound tests, ethanol-soluble compound levels (Table 1).

Table 1. Specific Parameter Standardization Results

Parameters		Propolis			
		H. itama	***T. biroi***	***G. thoracica***	***T. fuscobalteata***
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			
	H. itama	***T. biroi***	***G. thoracica***	***T. fuscobalteata***
Dry shrinkage	5,61%	2,91%	2,3827%	7,9441%
Specific gravity	0,9015 g/mL	0,9078 g/mL	1,2576 g/L	1,2209 g/mL
Water content	1,084%	1,117%	1,004%	1,011%
Ash content	3,06%	9,03%	0,59%	0,96%

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

Table 3. Metal and Microbial Contamination Propolis Results

Parameters		Units	Propolis				Terms
			H. itama	***T. biroi***	***G. thoracica***	***T. fuscobalteata***	
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							
	Escherichia coli	*	Negative	Negative	ND	Negative	Negative
	Yeast and mold	CFU/gr	0	100	ND	70	≤ 10^4
	Salmonella sp.	*	Negative	Negative	ND	Negative	Negative
	Shigella sp.	*	Negative	Negative	ND	Negative	Negative
	Pseudomonas aureoginosa	*	Negative	Negative	ND	Negative	Negative
	Staphylococcus aureus	*	Negative	Negative	ND	Negative	Negative

ND = Not Determinated

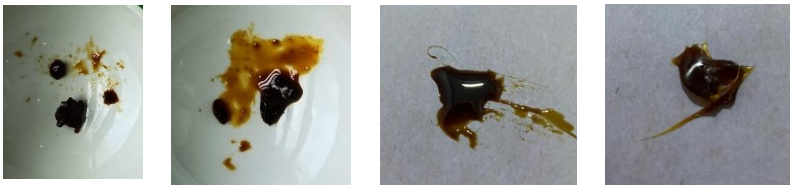
* = unit

< = Below Method Detection Limit (MDL)

3.1. Organoleptic

One of the specific parameters includes organoleptic determination, which serves as an initial assessment of the characteristics of propolis extract and is subjectively evaluated using the four senses to describe its form, color, odor, and taste. The organoleptic properties of the six types of propolis exhibit both similarities and differences. As shown in Table 1, the

organoleptic properties of the six types of propolis exhibit several differences, particularly in the color and taste of the extract. These variations may arise due to 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.



(a) **(b)** **(c)** **(d)**

Figure 1. Characterization of propolis color, (**a**) *H. itama*; (**b**) *T. biroi* ; (**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 offer an initial insight into the amount of a compound that can dissolve in the solvent employed. In Table 1, *fuscobalteata* propolis demonstrates superiority compared to other types of propolis in terms of extract solubility in water solvents. Conversely, when using ethanol solvent, *thoracica* propolis surpasses other types of propolis. In general, ethanol solvent was found to be superior to water solvent for most types of propolis. An important commonality among propolis types is the solubility of the extract, which is significantly higher in ethanol compared to water solvent. This indicates that the active compounds in the extract are more readily absorbed in ethanol due to its universal solvent properties, capable of attracting 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 number of compounds such as volatile compounds, thermolabile compounds, or water compounds that can be lost during the drying process. Generally, the drying shrinkage determination value for a good propolis extract is ≤10% [25]. Based on the results obtained from all propolis extracts, they meet the existing requirements with the drying loss value of propolis being below 10% (Table 2). In this instance, fimbriata propolis outperforms other types of propolis because 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 with the mass per volume value. Determination of specific gravity is carried out with the aim of providing a limit on the amount of mass per volume, which is a special parameter for liquid extract so that it becomes a thick extract that can still be poured. Determining specific gravity is also related to purity, extracting from contamination [26]. A high specific gravity value indicates a good concentration of the active compound in the extract,

while a low value indicates low solubility of the active compound in the solvent used. In standardizing propolis extracts, specific gravity functions 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 content present in the extract. A low water content in the extract can minimize or prevent the growth of microorganisms and mold (fungus) within it [27]. 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). The 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 [29, 30].

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 both propolis extracts fall within the category of good propolis extracts (≤10%) [25]. Of all propolis extracts, *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 not be suitable for consumption. However, it's 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 higher content 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 metal contamination results of all propolis (Table 3), indicate that both types of propolis have values below standard requirements and meet 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 [31]. Metal contamination is correlated with the ash content obtained; 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, representing examples of complications arising from the toxic effects of heavy metals [32–35]. 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 no contamination by *Escherichia coli,* molds and yeasts*, Salmonella sp., Shigella sp., Pseudomonas aeruginosa,* and *Staphylococcus aureus*. The presence of microbes in kelulut bee propolis is uncommon due to its potent antimicrobial properties. Additionally, propolis possesses antibacterial, antifungal, and antiviral properties that combat pathogenic microorganisms [36–38].

During standardization processes, ensuring the cleanliness and quality of materials is paramount. Ethanol possesses 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, ethanol usage 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). 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 these two propolis types. 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 also hold potential to affect propolis quality. Hence, a comprehensive understanding of all these factors is essential for comprehending and managing variations in phytochemical compound content among four stingless bee propolis, as well as for optimizing production conditions to attain propolis of desired quality.

Table 4. Phytochemical test results

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

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 measuring the gallic acid calibration curve, with the curve equation $y = 0.1378x + 0.0762$ ($R^2 = 0.9999$). The Total Flavonoid Content (TFC) of propolis ethanol extract was determined by utilizing the gallic acid calibration curve, with the equation $y = 0.0381x - 0.0856$ ($R^2 = 0.9929$). Research shows that there are differences in value between each type of propolis (Table 5).

Table 5. Total Phenolic and Total Flavonoid Content

Parameters	Propolis			
	H. itama	***T. biroi***	***G. thoracica***	***T. fuscobalteata***
TPC (mg GAE/g)	0,78 ± 0,005	0,86 ± 0,029	0,54 ± 0,005	1,39 ± 0,005
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 have an impact on the quality of the propolis produced, including the content of phenolic compounds. This variation can be attributed to several factors. Differences in geographic locations, where beehives are situated, impact the quality of produced propolis, including the content of phenolic compounds [39, 40]. 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 present [41, 42]. Moreover, disparities in bee species influence the propolis products obtained. Each bee 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 found within. Additionally, different bee species may possess varying enzymes and metabolic mechanisms utilized in the resin-to-propolis conversion process, influencing the types of compounds produced or transformed during propolis formation.

4. Conclusions

From this research, the results of the standardization of ethanol extracts from *H. itama, T. biroi, G. thoracica, T. fuscobalteata* were 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 and other values across each test. The research also revealed variations in phytochemical content, total polyphenol, and flavonoid content among the different propolis samples. In the metal contamination test, all raw propolis samples met the requirements with low levels of metals. The implications of this research extend to the herbal industry, public health, and product development. Further research is encouraged to delve deeper into the chemical differences between propolis samples and their impact on medicine and public health.

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 Health Laboratory of East Kalimantan, for their facilitation.

Conflicts of Interest

The authors declare no conflict of interest

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Menyatakan bahwa artikel ilmiah yang berjudul "*Standardization of Ethanolic Extracts Propolis from Four East Kalimantan Stingless Bees Species*" telah di submit pada Jurnal Bionterface Research in Applied Chemistry pada tahun 2024.
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Received: 1 May 2024
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