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ARAŞTIRMA MAKALESI / RESEARCH ARTICLE

ANTIOXIDANT ACTIVITIES AND PHYTOCHEMICAL COMPOSITION OF STINGLESS BEE *(Heterotrigona itama)* HONEY COLLECTED FROM *Calliandra calothyrsus* PLANTATION IN EAST KALIMANTAN, INDONESIA

Endonezya Doğu Kalimantan'daki *Calliandra calothyrsus* Bahçesinden Toplanan İğnesiz Arı (*Heterotrigona itama*) Balının Antioksidan Aktiviteleri ve Fitokimyasal Bileşimi

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ABSTRACT

Stingless bee honey is one of the most valuable insect products. The increasing popularity of stingless bee honey can be attributed to its composition, which has been linked to medicinal properties. Beekeeping with stingless bees is well-known in Indonesia, with *Heterotrigona itama* is the most popular stingless bee species cultivated in East Kalimantan, Indonesia. Stingless bees utilize various plant species as sustenance sources. Among those plants, *Calliandra calothyrsus* is popular planting in Indonesian bee plantations. This study analyzed the antioxidant (DPPH assay), phytochemical (qualitative method), water, and sugar content of *H. itama* stingless bee honey collected from a *C. calothyrsus* plantation. The results show that the water and sugar contents of the honey in this study were higher than in other research. Meanwhile, antioxidant capacity was also higher than in other studies. The phytochemical contents detected from honey in this study were carotenoids, coumarins, flavonoids, saponins, steroids, tannins, and triterpenoids. Even though the properties of stingless bee honey can differ based on vegetation and geographical origin, *H. itama* stingless bee honey collected from *C. calothyrsus* plantation in East Kalimantan, Indonesia, showed potential antioxidant activity and phytochemical content, which is advantageous to human health.

Keywords: Stingless bee honey, *Heterotrigona itama*, *Calliandra calothyrsus*, DPPH, Phytochemical

ARAŞTIRMA MAKALESI / RESEARCH ARTICLE

ÖZ

İğnesiz arı balı en değerli böcek ürünlerinden biridir. İğnesiz arı balının artan popülaritesi, tıbbi özelliklerle ilişkilendirilen bileşimine bağlanabilir. İğnesiz arılarla arıcılık Endonezya'da iyi bilinmektedir ve *Heterotrigona itama*, Endonezya'nın Doğu Kalimantan bölgesinde yetiştirilen en popüler iğnesiz arı türüdür. İğnesiz arılar, çeşitli bitki türlerini besin kaynağı olarak kullanırlar. Bu bitkiler arasında *Calliandra calothyrsus*, Endonezya arı plantasyonlarında popüler bir ekimdir. Bu çalışmada, *C. calothyrsus* plantasyonundan toplanan *H. itama* iğnesiz arı balının antioksidan (DPPH testi), fitokimyasal (kalitatif yöntem), su ve şeker içeriği analiz edilmiştir. Sonuçlar, bu çalışmadaki balın su ve şeker içeriğinin diğer araştırmalara göre daha yüksek olduğunu göstermektedir. Bu arada, antioksidan kapasitesi de diğer çalışmalara göre daha yüksek. Bu çalışmada baldan tespit edilen fitokimyasal içerikler karotenoidler, kumarinler, flavonoidler, saponinler, steroidler, tanenler ve triterpenoid. İğnesiz arı balının özellikleri bitki örtüsüne ve coğrafi kökene göre farklılık gösterebilse de, Endonezya'nın Doğu Kalimantan bölgesindeki *C. calothyrsus* plantasyonundan toplanan *H. itama* iğnesiz arı balı, insan sağlığı için avantajlı olan potansiyel antioksidan aktivite ve fitokimyasal içerik gösterir.

Anahtar kelimeler: İğnesiz arı balı, *Heterotrigona itama*, *Calliandra calothyrsus*, DPPH, Fitokimyasal

GENİŞLETİLMİŞ ÖZET

Giriş: İğnesiz arı balı en değerli böcek ürünlerinden biridir ve eski insanlar ona tıbbi özellikler atfetmişlerdir. İğnesiz arılar tarafından üretilen balın artan popülaritesi, antiseptik, antimikrobiyal, antikanser, anti-inflamatuar ve yara iyileştirici özelliklerle ilişkilendirilen bileşimine bağlanabilir. Küresel olarak, sıcak ve nemli ormanlar iğnesiz arılara ev sahipliği yapmaktadır. Bu nedenle, Endonezya gibi tropikal ülkelerde iğnesiz arılarla arıcılık daha iyi bilinen bir uygulamadır. Kalimantan'daki arıcılar tarafından birkaç iğnesiz arı türü yetiştirilmektedir ve *Heterotrigona itama* en popüler türdür. İğnesiz arılar, doğal ortamlarındaki bol bitki örtüsünden çiçek nektarları alır ve bunları kimyasal olarak maddeleriyle değiştirir, bunun sonucunda botanik kökeni, coğrafi bölgesi ve çevre koşullarından etkilenen kimyasal bileşim, lezzet ve aromaya sahip benzersiz bir bal elde edilir. Bu bitkiler arasında *Calliandra calothyrsus*, Endonezya arı plantasyonlarında popüler bir ekimdir çünkü bol miktarda çiçekten beslenen çok sayıda koloninin yetiştirilmesi yüksek bal verimine yol açmıştır.

Yöntemler: Bu çalışmada, *C. calothyrsus* plantasyonundan toplanan *H. itama* iğnesiz arı balının antioksidan aktivitesi ve fitokimyasal içeriği analiz edilmiştir. Antioksidan aktivite analizi için DPPH radikal temizleme aktivitesi testi yürütülmüştür. Önemli fitokimyasal içerik için tarama testleri standart nitel prosedürler kullanılarak gerçekleştirilmiştir.

Sonuçlar: Sonuçlar, bu çalışmadaki balın su (%28,91 ± %1,08) ve şeker (%66,68 ± %2,31) içeriğinin diğer araştırmalara göre daha yüksek olduğunu göstermektedir. Bu arada antioksidan kapasitesi de diğer çalışmalara göre daha yüksek olup 12,49 ile 90,85 µg/mL arasında değişmektedir. Bu çalışmada balda tespit edilen fitokimyasal içerikler karotenoidler, kumarinler, flavonoidler, saponinler, steroidler, tanenler ve triterpenoidlerdir. On iki aylık gözlem sırasında *H. itama* balında tespit edilen fitokimyasal içeriklerin esas olarak kumarinler ve flavonoidler olduğu görüldü.

Tartışma: Flavonoidler ve fenolikler balın aroması ve antioksidan aktivitesinden sorumlu bileşiklerdir. Bal örneklerinin flavonoid ve fenolik içeriğindeki farklılıklar arıların tükettiği nektarların çeşitli bitki örtüsüne ve coğrafi kökenlerine atfedilebilir. Flavonoidlerin ve fenolik bileşiklerin antioksidan, antitümör, antimikrobiyal, kardiyoprotektif ajan, antiinflamatuar ajan ve bağışıklık güçlendirici olarak etkili olduğu bildirilmiştir. İğnesiz arı balı, serbest radikalleri nötralize edebilen fenolik ve flavonoid içeriğinden dolayı yüksek bir antioksidan aktiviteye sahiptir. Daha önce belirtildiği gibi, fenolik bileşim bitki örtüsüne ve coğrafi kökene göre farklılık gösterebilir, ancak aynı zamanda her arı türünün bitki örtüsü tercihine göre de değişebilir.

Sonuçlar: İğnesiz arı balının özellikleri bitki örtüsüne ve coğrafi kökene göre farklılık gösterse de, Endonezya'nın Doğu Kalimantan bölgesindeki *C. calothyrsus* plantasyonundan toplanan *H. itama* iğnesiz arı balı, insan sağlığı için avantajlı olan

potansiyel antioksidan aktivite ve fitokimyasal içerik göstermiştir.

INTRODUCTION

Honey is the naturally sweet substance produced by honey bees from the nectar of plants (FAO 2019). Honey consists primarily of carbohydrates and other substances. It is abundant in flavonoids and phenolic acids, which are biologically active and function as natural antioxidants. There are currently two varieties of honey produced and sold globally: traditional honey from bees and honey from stingless bees. Honey produced by stingless bees is known by various names, including *Kelulut* honey, Meliponine honey, and pot honey (Amin et al. 2018).

Honey is one of the most valuable insect products, and ancient peoples attributed it with medicinal properties. The increasing popularity of honey produced by stingless bees can be attributed to its composition, which has been linked to antiseptic, antimicrobial, anticancer, anti-inflammatory, and wound-healing properties (da Silva et al. 2013). In contrast to the population of stingless bees, this honey is less widely distributed than the common honeybee due to the need for more information about this honey, making it less popular. Therefore, stingless bee honey needs further investigation (Abd Jalil et al. 2017).

Globally, warm and humid forests are home to stingless bees. Approximately 500 species of stingless bees and over 60 distinct genera of stingless bees have been identified. Therefore, in tropical countries such as Indonesia, beekeeping with stingless bees is a more well-known practice (Nordin et al. 2018). Ten species of stingless bees are cultivated by beekeepers in Kalimantan, with *Heterotrigona itama* being the most popular species (Syafrizal et al. 2020a). Stingless bee honey breeders are currently cultivating this species due to its larger size, adaptability, and increased honey production (Buchori et al. 2022). Honey produced by *H. itama* contains chemical compositions, including alkaloids, coumarins, flavonoids, saponins, and tannins (Syafrizal et al. 2020b). Stingless bees acquire floral nectars from the abundant vegetation of their native environments and chemically modify them with their substances, resulting in a unique honey with chemical composition, flavor, and aroma which are influenced by its botanical origin, geographic region, and environmental conditions (Avila et al. 2018).

Stingless bees utilize various plant species as sustenance sources (Juliasih et al. 2022). Among those plants, *C. calothyrsus* is popular planting in Indonesia bee plantations because the rearing of numerous colonies that could forage on the abundant blossoms led to a high honey yield (Suliasih et al. 2021; Ustadi et al. 2017). As fast-growing plants, *C. calothyrsus* produces more than 100 L honey per day per hectare (de Luna et al. 2020; Harianja et al. 2023). To our knowledge, no one has investigated the properties of stingless bee honey from the *C. calothyrsus* as the dominant vegetation. This study analyzed the antioxidant activity and phytochemical content of *H. itama* stingless bee honey collected from a *C. calothyrsus* plantation.

MATERIALS AND METHODS

Location of research

The location of the research was a stingless bee honey farm in the Lubuk Sawah Region of Samarinda, East Kalimantan Province, Indonesia, as shown in Figure 1. Samples of honey were collected from vegetation dominated by *C. calothyrsus*, as shown in Figure 2. Samples of honey were extracted using a vacuum device. Honey is packaged and shielded from light and moisture with aluminum sheeting before being placed in a styrofoam box. Before analyzing the honey according to the predetermined parameters, the honey was placed in a refrigerator room at 0° - 5°C temperature.

Figure 1. Lubuk Sawah Region in Samarinda, East Kalimantan, Indonesia as research location

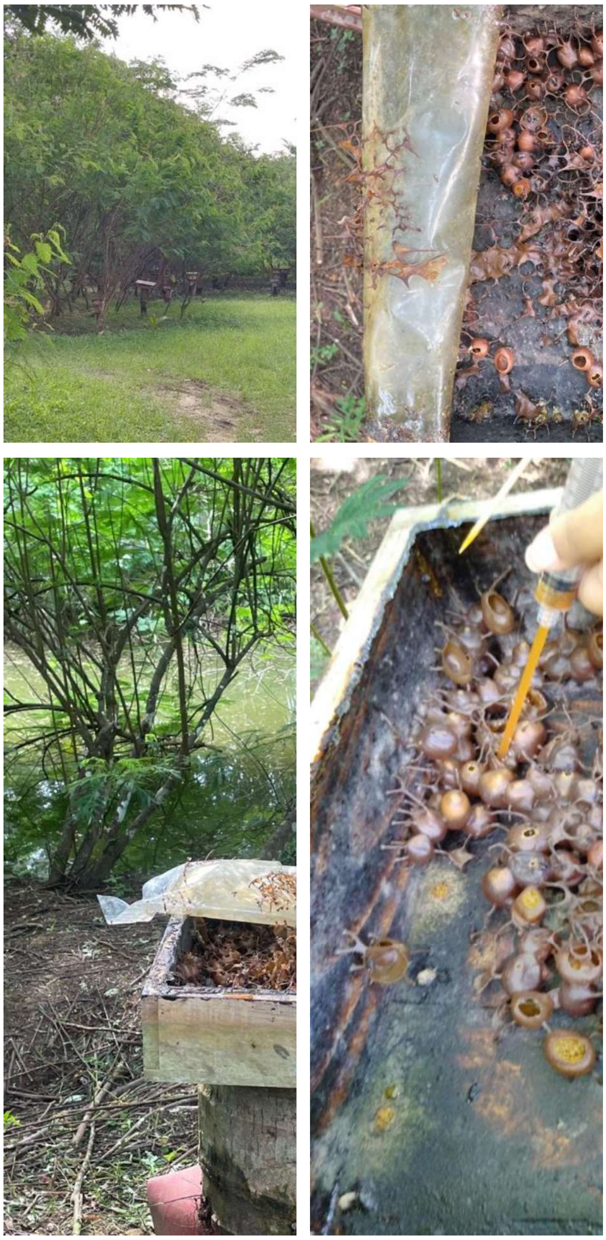

Figure 2. Samples of honey were collected from plantation with *C. calothyrsus* as dominant vegetation in Lubuk Sawah, Samarinda, East Kalimantan, Indonesia

Water and Sugar Contents

The water content was measured using a refractometer (Atago, Japan) according to Indonesian National Standard 8664-2018 for Honey (NSAI 2018). The sugar content was measured using Luff Schoorl methods for reducing sugar according to Indonesian National Standard 01-2892-1992 for Sugar Testing (NSAI 1992).

Antioxidant activity analysis

The antioxidant for radical scavenging activity assay was conducted using DPPH (2,2-diphenyl-1-picrylhydrazyl) according to Sukemi et al. (2021). As working solutions, DPPH and ethanol were used. The positive control for this assay was ascorbic acid. The effect of honey on scavenging free radicals on the measured concentration differed from the test using spectrophotometry. The radical scavenging activity was calculated using the following equation $[(A_c - A_s) / A_s] \times 100$. Where A_c is the absorbance of the control sample, and A_s is the absorbance that contains the test sample. The IC_{50} is the parameter used to express the relative antioxidant capacity. The IC_{50} was calculated by plotting the scavenging percentage against the test sample concentration, using µg/mL units. A linear regression analysis of the inhibition percentage as the honey concentration increased was used to estimate the IC_{50} value (Gulcin & Alwasel 2023).

Phytochemical analysis

Phytochemical assays were conducted to detect alkaloids, carotenoids, coumarins, flavonoids, saponins, steroids, tannins, and triterpenoids. The qualitative screening tests for these phytochemicals were conducted using standardized protocols with specific changes. The alkaloids were identified with HCl and Dragendorff reagents. Stingless bee honey (5 mL) was combined with hydrochloric acid (2 mL) in the tube, followed by Dragendorff reagent (1 mL). The appearance of a yellow substance indicated the alkaloid contents of the honey (Oscar et al. 2020).

Carotenoids were detected by chloroform and sulphuric acid. In a tube, honey (1 mL) was diluted with chloroform (5 mL), agitated briskly, and 85% sulphuric acid (4 drops) was added. The mixtures' blue substance suggested carotenoids (Viji, et al. 2013). Sodium hydroxide and ethanol detected coumarins, with sodium hydroxide (4 drops) and ethanol, added to stingless bee honey (1 mL). The solution's yellow color indicated coumarins (Rao et al. 2023).

Flavonoids were detected with sodium hydroxide and HCl. Honey (1 mL) was treated with 1% sodium hydroxide (5 drops). A colorless solution with 1% HCl turns vivid yellow, indicating flavonoids in honey (Oscar, et al. 2020). The saponins were found in acetone and HCl. Honey (60 mg) was combined with acetone (2 mL) and hot water (3 mL). After cooling, the solution was shaken for 10 seconds. Saponins in honey are indicated by foam bubbles 1-10 cm high for 10 minutes after adding one drop of HCl 2N (Dubale et al. 2023).

Acetic anhydride, sulphuric acid, and acetone identified steroids and triterpenoids. Acetic anhydride (10 drops) and sulphuric acid concentrated (2 drops) were added to acetone-diluted honey (1 mL). The mixture was shaken vigorously. Red-purplish color suggested triterpenoids, while blue-greenish suggested steroids (Rajkumar et al. 2022). The tannins were identified by lead acetate. The honey (1 mL) was combined with three new 1% lead acetate drops. Yellow precipitates indicated tannins (Rao et al. 2023).

RESULTS

The water contents of stingless bee honey from *H. Itama* is 28.91 ± 1.08%, ranging from 26.9 to 30.0%. The sugar content is 66.68 ± 2.31%, ranging from 62.2 to 69.6%. Meanwhile, the antioxidant capacity of stingless bee honey is 47.92 ± 25.06 µg/mL, ranging from 12.49 to 90.85 µg/mL. As shown in Table 1, during the twelve months of observation, the highest water contents were in August, September, and March, and the lowest was in January. The highest sugar content was in January, and the lowest was in September.

Table 1. Water, sugar contents, and antioxidant activity of *H. itama* honey from *C. calothyrsus* plantation during 12 months of observation

The Month of Observation	Water Content (%)	Sugar Content (%)	Antioxidant (IC_{50}, µg/mL)
Apr 2023	28.5	63.3	15.82
May 2023	27.3	68.0	34.26
Jun 2023	28.4	67.6	44.06
Jul 2023	29.9	67.7	43.95
Aug 2023	>30.0	63.5	28.09
Sep 2023	>30.0	62.2	52.56
Oct 2023	29.0	68.1	90.85
Nov 2023	28.5	68.3	83.64
Dec 2023	28.5	67.7	63.71
Jan 2024	26.9	69.6	69.74
Feb 2024	29.9	68.7	35.91
Mar 2024	>30.0	66.5	12.49
Mean ± SD	28.91 ± 1.08	66.68 ± 2.31	47.92 ± 25.06

Meanwhile, the highest antioxidant capacity of stingless bee honey was in March, and the lowest was in October. During twelve months of observation, as shown in Table 2, the phytochemical contents detected from *H. itama* honey were coumarins, flavonoids, tannins, triterpenoids, carotenoids, saponins, and steroids. Coumarins were detected in all months except January. Flavonoids were detected in all months except July and September.

Table 2. Phytochemicals of *H. itama* honey from *C. calothyrsus* plantation during 12 months observation

Month of observation	Stingless Bee Honey Phytochemicals							
	Alkaloid	Carotenoid	Coumarin	Flavonoid	Saponin	Steroid	Tannin	Triterpenoid
Apr 2023	-	-	+	+	-	-	-	-
May 2023	-	-	+	+	-	-	-	+
Jun 2023	-	-	+	+	-	-	-	-
Jul 2023	-	-	+	-	-	-	-	+
Aug 2023	-	-	+	+	-	+	+	-
Sep 2023	-	-	+	-	-	-	+	-
Oct 2023	-	-	+	+	-	-	+	-
Nov 2023	-	-	+	+	-	-	+	-
Dec 2023	-	-	+	+	-	-	+	-
Jan 2024	-	+	_	+	+	-	+	+
Feb 2024	-	-	+	+	-	-	+	-
Mar 2024	-	+	+	+	-	-	+	-

DISCUSSION

The study is the first to analyze stingless bee honey properties from *C. calothyrsus* plantation. Stingless bees utilize various plant species as sustenance sources: those that produce nectar and those that produce resin. The trees frequently planted in Indonesia because their nectar-producing are *Kaliandra* (*C. calothyrsus*), *Kelapa* (*Cocos nucifera*), *Karet* (*Hevea brasiliensis*), *Kapuk* (*Ceiba pentadra*), and *Rambutan* (*Nephelium lappaceum*) (Juliasih, et al. 2022). Among those plants, *C. calothyrsus* is widespread in Indonesian bee plantations because the rearing of numerous colonies that could forage on the abundant blossoms led to a high honey yield (de Luna, et al. 2020). As plants with rapid growth, *C. calothyrsus* produces more than 100 L of honey per day per hectare. During the flowering season, one colony produces more than 3 L of honey over two months (Harianja, et al. 2023).

Research in Java found that *C. calothyrsus* honey showed antioxidant activity higher than *H. brasiliensis* and *C. pentadra* honey. Flavonoid content from *C. calothyrsus* honey was also higher than other honey (Ustadi et al. 2017). The results indicate that *C. calothyrsus* honey has the maximum percentage of DPPH quenched and has the significant antioxidant capacity. These phenolic compositions in *C. calothyrsus* honey indicate that it is an excellent source of natural antioxidants due to its high antioxidant content (Suliasih et al. 2021). Regarding monofloral plantation for beekeeping, research in Turkiye found that lavender (*Lavandula* spp.) honey showed DPPH radical scavenging activity (Kolaylı et al. 2024). Chestnut (*Castania sativa*) honey, another monofloral honey from Turkiye, also showed antioxidant capacity using FRAP assay (Ucurum et al. 2024).

The water contents of the *H. itama* honey in this study (28.91 ± 1.08%) were higher than research results in Malaysia (Omar et al. 2019; Shamsudin, et al. 2019; Souza et al. 2021) and South Kalimantan, Indonesia (Adalina et al. 2020), ranging from 11.09 to 28.43%. Meanwhile, the water contents of honey in this study were lower than study in East Kalimantan, Indonesia (Saputra et al. 2021) and Malaysia (Fatima et al. 2018; Sujanto, et al. 2021), ranged from 30.80 to 33.67%. According to Indonesian National Standard (SNI) 8664-2018 for Honey, the maximum water content for stingless bee honey is 27.5% (NSAI 2018). Meanwhile, the Department of Standards Malaysia also has quality standards for honey from stingless bees. The department said that honey from stingless bees should have no more than 35% water (Department of Standards Malaysia 2017). Water is the second largest component in honey. Moisture is essential because it can change viscosity, specific weight, age, smell, and crystallization. Reports say stingless bee honey has more water than regular honey because the rainfall and humidity in a tropical rainforest (Cardona et al. 2019). Different floral behaviors and sources may also contribute to the variation in moisture content. Due to fermentation during storage, honey with a high moisture content has a shorter expiration life (Shamsudin et al. 2019). The Department of Standards Malaysia also has quality standards for honey from stingless bees. The department said that honey from stingless bees should have no more than 35% water (Department of Standards Malaysia 2017). Water is the second largest component in honey. Moisture is an important part because it can change the viscosity, specific weight, age, smell, and crystallisation. Reports say that stingless bee honey has more water than regular honey because it comes from the rainfall and humidity in a tropical rainforest (Cardona et al. 2019).

The sugar contents of stingless bee honey in this study (66.68 ± 2.31%) were higher than study results in South Kalimantan, Indonesia (62.97%) and Malaysia (47.25 – 55.61%) (Adalina, et al. 2020). According to Indonesian National Standard for Honey, the sugar content of stingless bee honey minimum is 55% b/b (NSAI, 2018). Meanwhile, the Department of Standards Malaysia stated that honey from stingless bees should have a maximum sugar content of 80 g/100g (Department of Standards Malaysia 2017). The composition of nectar is influenced by environmental factors and the floral origin of nectar (Shamsudin et al. 2019). Research shows that *C. calothyrsus* honey has the highest score for physicochemical quality, especially regarding sugar content, taste, and panelist preferences (Triwanto et al. 2021).

This study found that *H. itama* stingless bee honey had higher antioxidant activity than honey from East Kalimantan (Saputra et al. 2021; Saputra & Nurlina 2022), North Kalimantan (Syafrizal et al. 2020b) and Malaysia (Mahmood et al. 2021; Shamsudin et al. 2019), ranged from 105.53 to 59.91 µg/mL. The antioxidant activity in this study was higher than the results of *H. itama* propolis extract in East Kalimantan (Kustiawan et al. 2022). Meanwhile, the antioxidant activity in this study was lower than study in East Kalimantan (Saputra et al. 2021), and Malaysia (Mahmood et al. 2021; Shamsudin et al. 2019; Ya'akob et al. 2019) ranging from 25.0 to 43.54 µg/mL. Honey is often referenced for its potential antioxidant properties, which may benefit human health. The activity of antioxidants is generally ascribed to their capacity to neutralize free radicals (Rozman et al. 2022). Several healing properties, such as anti-inflammation, antibacterial, antitumor, and anti-obesity, are substantially correlated with antioxidant activity, given that antioxidants are well-established and involved in a diverse diseases (Al-Hatamleh et al. 2020). Honey contains numerous compounds with antioxidant properties. It has been reported that honey's antioxidant activity correlates with phenolics and flavonoids content (Abu Bakar et al. 2017). The phenolic compounds in honey are directly related to the nectars supplied to bees; consequently, honey from various vegetation origins has different bioactive properties (da Silva et al. 2013).

The phytochemical contents detected from *H. itama* honey in this study were carotenoids, coumarins, flavonoids, saponins, steroids, tannins, and triterpenoids. During twelve months of observation, the phytochemical contents detected from *H. itama* honey were mainly coumarins and flavonoids. Coumarins were detected in all months except January, and flavonoids were detected except in July and September. These results were similar to a study in East Kalimantan, which found coumarins and flavonoids as phytochemical compositions (Saputra et al. 2021; Saputra & Nurlina 2022). Another research for *H. itama* honey from East Kalimantan also found alkaloids, carotenoids, coumarins, flavonoids, saponins, tannins, and triterpenoids compositions (Arung et al. 2022; Saputra et al. 2021; Syafrizal et al. 2020b). These results differed from a study in East Kalimantan, which found alkaloids, terpenoids, and tannins as phytochemical constituents from the extract of *H. itama* propolis (Kustiawan et al. 2022). A study in North Kalimantan found alkaloids, coumarins, flavonoids, tannins, and triterpenoids in stingless bee honey, and a study in South Kalimantan found only flavonoids and saponin as phytochemical contents (Adalina et al. 2020; Syafrizal et al. 2020b). Meanwhile, research from Malaysia found flavonoids and carotenoids as chemical compositions from *H. itama* honey (Mahmood et al. 2021; Rozman et al. 2022; Shamsudin et al. 2019; Sujanto et al. 2021).

Stingless bee honey is a natural source of flavonoids, phenolic acids, and their derivatives. Stingless bee honey contains several phenolic acids, such as gallic, syringic, vanillic, *p*-coumaric, cinnamic, and salicylic acids, as well as some flavonoids, including luteolin, naringenin, and taxifolin (Al-Kafaween et al. 2023). Flavonoids are phenolic compounds responsible for the aroma and antioxidant activity of the honey. Variations in the flavonoid content of honey samples may be attributable to the various vegetation and geographical origins of the nectars bees consume (Mwangi et al. 2024; Nasir et al. 2019). Phenolics are a heterogeneous class of compounds produced by the secondary metabolism of plants, and they can be separated into flavonoids and non-flavonoids. Flavonoids are known as phenolic acids. Examples of non-flavonoids are tannins (Biluca, et al. 2020). It has been reported that flavonoids and phenolic compounds are effective as antioxidants, antitumors, antimicrobials, cardioprotective agents, anti-inflammatory agents, and immune boosters (Maringgal et al. 2019). Phenolic compounds were identified in stingless bee honey. Among the phenolic compounds, flavonoids and coumarin with *p*-coumaric acid are among the most prevalent compounds in the studied samples (Biluca et al. 2017). Honey has a high antioxidant activity due to its phenolic and flavonoid content, which can neutralize free radicals. Honey from stingless bees contains more flavonoids than honeybees (Cianciosi et al. 2018). As stated previously, the phenolic composition can differ based on vegetation and geographical origin, but it can also vary based on the vegetation preference of each bee species (Biluca et al. 2016).

Conclusions: The properties of *H. itama* honey from the *C. calothyrsus* plantation show that the water and sugar contents of the stingless bee honey in this study were higher than in other research. Meanwhile, the antioxidant activity of *H. itama* stingless bee honey from *C. calothrysus* was higher than in other studies. The phytochemical contents detected from *H. itama* honey in this study were carotenoids, coumarins, flavonoids, saponins, steroids, tannins, and triterpenoids. During twelve months of observation, the phytochemical contents detected from this stingless bee honey were mainly coumarins and flavonoids. Even though the properties of stingless bee honey can differ based on vegetation and geographical origin, *H. itama* honey collected from *C. calothyrsus* plantation in East Kalimantan, Indonesia, showed potential antioxidant activity and phytochemical contents, which is advantageous to human health.

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