Phytochemical test and physical chemical properties of rubber honey from three types of bees (Apis mellifera, Apis dorsata and Trigona itama)

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Abstract. The rubber plant is one type of plant as a source of nectar for honey bees. This study aims to determine the characteristics of rubber honey from Apis mellifera, Apis dorsata, and Trigona itama bees based on phytochemical screening and honey's physicochemical properties. The observed variables consisted of active compound content, water content, honey color, pH value, acidity, Hydroxyl Methyl Furfural (HMF) and reducing sugars. Rubber honey samples were taken by purposive sampling from A. mellifera honey beekeepers in Jepara Regency, Central Java Province, T. itama honey beekeepers in Tapin District, South Kalimantan Province and A. dorsata honey beekeepers in West Bangka Regency, Bangka-Belitung Province. The results showed that rubber honey from the three types of bees met the honey quality standard based on SNI 8664-2018, except for the water content. There are differences in honey's physicochemical characteristics from the three types of bees, which include differences in the value of color intensity, acidity, HMF levels, and reducing sugar levels. All honey samples contain active compounds saponins, flavonoids, and phenols but do not contain alkaloids, tannins, triterpenoids, and steroids. The need for post-harvest honey treatment reduces the water content of honey with a dehumidifier tool to comply with SNI standards.

1. Introduction
Honey contains many nutrients that are very beneficial for humans and are widely used in the food, pharmaceutical, and cosmetics industries [1]. Honey can be used as a traditional medicine because it does not cause harmful side effects and is widely used to cure various diseases. This is because of secondary metabolites found in honey, such as alkaloids, flavonoids, saponins, tannins, and essential oils. To find secondary metabolite compounds in honey through phytochemical screening [2].

Besides containing secondary metabolites, honey contains many compounds, including carbohydrates (primarily fructose and glucose), vitamins, minerals, enzymes, organic compounds, free amino acids, and volatile compounds that contribute to color, aroma, and taste [3]. The content of honey consists of 80-85% carbohydrates (glucose and fructose), 15-17% water, 0.1-0.4% protein, 0.2% ash, small amounts of amino acids, enzymes, vitamins, and other substances [4]. The composition and quality of honey are very diverse and depends on the source of honey bee feed as a source of nectar, climate differences, honey maturity and processing and storage [3,5,6]. The honey content significantly affects
the physicochemical properties of honey [1]. Honey has physicochemical and microbiological characteristics that can be used as honey quality parameters [7]. In Indonesia the honey quality standard is regulated in the Indonesian National Standard (SNI) 8664-2018 [8].

Various studies have been conducted to determine the quality of honey, among others [9], about local honey from several regions in Temanggung Districts and Wulandari [10] concerning storage on honey quality. Several studies related to rubber honey characteristics, among others, Harjo et al. [11] stated that Malang rubber honey from A. mellifera bees has a water content of 21.59%, diastase enzyme activity of 11.5 DN and HMF 17.23 mg/kg. The results of research by Evahelda et al. [12] show that rubber honey from forest bees (A. dorsata) from Central Bangka Regency has a moisture content of 24.25%, HMF 0 mg/kg, a pH value of 3.92, the total sugar content of 74.77%. The study results by Ridoni et al. [13] that the rubber honey from the Trigona sp. originating from Banjar Regency has a moisture content of 25%, 54.13% reducing sugar, 146.79 mL of NaOH 1N/kg acidity, and 5% water-insoluble solids. However, research on honey from rubber tree nectar from various bees has not been done much. Therefore, it is necessary to test honey's physicochemical properties to determine the quality of rubber honey from three different types of bees.

This study aims to determine the secondary metabolite compounds contained in honey and honey's physicochemical properties with the source of rubber tree nectar (Hevea brasiliensis (Willd. ex A. Juss.) Müll. Arg) from three different types of honey bees. This study's results are expected to provide information about the quality of rubber honey produced from various types of honey bees and active compounds contained in rubber honey.

2. Materials and Methods

2.1. Materials
The material used in this study was honey from rubber tree nectar (H. brasiliensis) from three types of bees, namely A. mellifera and T. itama bees obtained from honey beekeepers, while from A. dorsata bees were obtained from forest honey collectors. The chemicals used in this study include glacial acetic acid, sulfuric acid, aquades, ethanol, Mayer reagents, Dragendorf reagents, Wagner reagents, HCl, H₂SO₄, chloroform, ammonia, FeCl₃, Mg powder, NaOH, 1% PP indicator and Oxalic acid. The equipment used includes a spectrophotometer, shaker, analytical balance, hotplate, oven, vortex mixer refractometer, and honey color analyzer.

Three rubber honey samples were taken by purposive sampling and measurements were carried out 3 times. Rubber honey from A. mellifera bee species was obtained from Telogowungu District, Pati Regency, Central Java Province; A. dorsata bee honey from Coconut District, West Bangka Regency, Bangka Belitung Province, and T. itama bee honey from Lok Paikat District, Tapin Regency, South Kalimantan Province.

2.2. Methods
This research approach uses a quantitative approach with the support of a qualitative approach. The research method was a survey and interview with A. dorsata forest honey collectors and A. mellifera and T. itama honey beekeepers.

Qualitative analysis of phytochemical constituents refers to Harborne [14]. Phytochemical testing parameters consist of alkaloids, tannin, flavonoid, saponin, phenol, quinone, steroid, and triterpenoid. The physico-chemical characteristics of the analyzed honey consist of color intensity, water content (SNI 8664: 2018) [8], referring to [15], pH [16], acidity [17], hydroxymethylfurfural (HMF) according to AOAC [15], and sugar was reducing methods DNS [18]. Phytochemical analysis was carried out at the Biopharmaca laboratory Bogor and honey physicochemical analyses were carried out at the Forest Products Research and Development Laboratory, Bogor. The results of the analysis are compared with the Indonesian National Standard (SNI) 8664-2018 [8] concerning honey. The results of data analysis are presented descriptively.
3. Results and Discussion

3.1. Phytochemical constituents

3.1.1. Analysis of alkaloids compounds
The analysis results showed that rubber honey from the three types of bees with Dragendorff, Mayer, and Wagner reagents did not form sediment. This is indicated by the formation of orange-red precipitates with Dragendorff reagents, not forming white deposits with Mayer reagents, and the formation of brown-red precipitates with Wagner reagents. The analysis showed that all negative honey samples contained alkaloids (Table 1). This study's results are in line with Adalina and Kuntadi [19] that various types of honey monofloral (rubber honey, kapok honey, and mango honey) from A. mellifera bees originating from Java islands negative contain alkaloids.

3.1.2. Analysis of steroid compounds and triterpenoids
The steroid analysis results are not formed by greenish-blue or triterpenoid analysis with no brownish color, which indicates that all negative honey samples contain steroids and triterpenoids. Rubber honey is a monofloral honey, honey, with the dominant source of nectar from one plant type. This study's results are in line with Adalina and Kuntadi [19] that various types of monofloral honey from the A. mellifera species from Java Island are adverse containing steroids and triterpenoids. Likewise, the study results by Gunawan et al. [20], Sumarlin et al., [21] and Emmasitah et al. [22] that Trigona honey was negative for steroids, while Adalina [23] forest honey (A. dorsata) from South Kalimantan which was negative for steroids. In this test, acetic acid and sulfuric acid will bind to terpenoids/steroids so that the color changes [24].

3.1.3. Analysis of flavonoid compounds
The principle of the method analysis flavonoid compounds by reducing benzopyrene with the addition of HCl and Mg metal, the formation of dark red color on flavonoids [25]. The results of the flavonoid analysis showed that all samples of positive research honey contained flavonoid compounds (Table 1), which were marked by the formation of deep red color. Rubber honey from all three types of bees has flavonoid content with weak positive flavonoid results. This research is in line with Adalina and Kuntadi [19]. That honey monofloral (kapok, mango, and rubber) from the native Java Island A. mellifera types have the same flavonoid that weak positive results. Mahardika [26] suggested that flavonoid compounds can function as antioxidants.

3.1.4. Analysis of tannin compounds
The results of the analysis showed that all negative honey samples contained tannins (Table 1). The 1% FeC13 reaction results with one of the hydroxyl groups present in tannin compounds showed no dark blue or greenish-black formation. Tannins function as secondary antioxidants because they can bind iron ions and slow down oxidation [27].

3.1.5. Analysis of saponin compounds
The results obtained from the analysis of saponin compounds showed that all positive honey samples contained saponins characterized by the formation of foam or stable foam with moderate positive saponin results (Table 1). Ningsih et al. [28] suggested that the formation of foam in the saponin test was due to glycosides, which can form foam in water and hydrolyze into glucose and other compounds. Prasetyo et al. [29] stated that saponin is a secondary metabolic compound with antibacterial ability.

3.1.6. Analysis of phenol compounds
The principle of the method of analysis of phenol compounds by adding 1% FeCL3 reagent in ethanol extract, if a green or blue-green color is formed, indicates a positive phenol compound. The analysis results that all rubber honey from the three types of positive bees contains phenolic compounds marked by the formation of green color (Table 1). Rubber honey from A. mellifera and T. itama bees with actual phenolic content is weak, whereas A. dorsata is moderately positive. According to Alvárez-Suárez et al. [30] and Biluca et al. [31] reported the presence of individual phenolic compound in their stingless bee honey sample. Whereas plants pollinated by bees produce
nectar that is used for the production of honey, it is possible that the bioactive compounds synthesized by the parent plants are transferred to the honey. Thus, the location of the apiaries, and or the geographical origin where the bees collect the nectar can influence the composition of the polyphenols of the honey, since these compounds act in the plant as protectors of environmental stress, in the change of temperature, light level, water content, UV exposure and mineral nutrient deficiency. These compounds, may therefore vary due to the exposure of the plant to the environment. According to Da Silva et al. [32] there are differences in honey phenol content from the same bee species because it influenced by geographical differences related to variations in the flowers found at each location, climate, and other factors. Likewise, there are differences in honey phenol content from different bee species at the same beekeeping location with the same harvest period.

| Testing parameters | Type of bee | Alkaloid | Wagner | Mayer | Dragendorf | Steroid | Triterpenoid | Saponin | Phenol | Flavonoid |
|--------------------|-------------|----------|--------|-------|------------|---------|-------------|---------|--------|----------|
|                    | Apis mellifera | Apis dorsata | Trigona itama |
| Alkaloid           | -           | -        | -      |
| Wagner             | -           | -        | -      |
| Mayer              | -           | -        | -      |
| Dragendorf         | -           | -        | -      |
| Steroid            | -           | -        | -      |
| Triterpenoid       | -           | -        | -      |
| Saponin            | ++          | ++       | ++     |
| Phenol             | +           | ++       | +      |
| Flavonoid          | +           | +        | +      |

Remarks: - negative; + weak positive; ++ positive medium; +++ strong positive; ++++ very positive

### 3.2. Analysis of the physicochemical properties of honey

#### 3.2.1. Honey color

Honey has a different color, aroma, and taste, depending on the type of plant as a nectar source. Honey color, aroma, and taste are essential indicators for consumers [33]. However, they cannot directly determine honey quality because honey color depends on the type of flower nectar [5]. Nevertheless, consumers often judge honey based on their color [12].

Honey classification can be distinguished by color. There are seven color calcifications of honey, from extra white to dark amber [12]. The study results show that rubber honey has extra light amber - light amber color criteria with a color intensity value of 36-73 mmP fund (Table 2). There is a difference in the color of honey from *A. mellifera* bees and the two other types of bees, namely *A. dorsata* and *T. itama*. The lowest color intensity values were obtained from *A. dorsata* bee species and the highest from *A. mellifera* bee species. This is because honey's color depends on the type of nectar that is dominant and the variety of plants as a source of nectar consumed by bees at each location where honey bees nest. Kek et al. [34] state that the color of honey is affected by mineral content and also geographical and botanical origin. Diverse condition may produce different honey color either white, colorless, pale yellow, golden or amber. Storage duration and light exposure are affecting the color of honey. By the opinion of Wibowo et al. [5], and Nayik and Nanda [33], that the type of plant influences the color of honey as the dominant source of nectar consumed by honey bees. Besides nectar as a source of honey, honey's color is also influenced by the length of storage and processing or heating [35].

#### 3.2.2. Water content

One of the parameters that determine the quality of honey is moisture content. The water content in honey determines the durability of honey. Honey with high water content is natural to ferment. In Indonesia, the quality of honey refers to SNI 8664-2018 [8] concerning honey, which limits the water content of honey of *A. dorsata* and *A. mellifera* bees to a maximum of 22% and maximum stingless bees
The water content of stingless bee honey depends on various factors such as fruit or flower season, the geography that affect the temperature, bee species and maturity of the honey [36].

The results showed that rubber honey's water content from the three types of bees was above the limit of the Indonesian National Standard (SNI). High levels of honey water are affected by the humidity of the surrounding air, ambient temperature, and early harvest by the opinion of Kahraman et al. [37]. The factors that influence the water content of honey include the humidity in the surrounding air, harvest season and the level of maturity of honey in the comb.

There are differences in the water content of rubber honey from the three types of bees. This is because of differences in harvest location. According to the statement of Sjamsiah [1], each honey from a different area has a different water content because the ambient temperature influences it. The study results showed that the water content of T. itama honey was higher than the honey content of A. mellifera and A. dorsata. This is in line with Tanuwidjaya [38] that the water content of Trigona honey is higher than A. mellifera and A. dorsata bees. The high-water content of T. itama honey is due to the cell storage in open pot-shaped contact with air, the water from the surrounding environment will be absorbed by honey while Apis bees store honey in wax-covered cells.

The water content of rubber honey bee type A. mellifera is 22.5%. However, compared to Savitri et al. [9], it shows that the water content of rubber honey is 20.9%, and that meets SNI standards, as well as Harjo et al. [11] with a moisture content of 21.59%. This difference is due to various factors that affect the water content of honey. Savitri et al. [9] state that the high and low levels of water content are caused by the influence of environmental temperature and way of production and time of harvest. Wulandari [10] states that honey has hygroscopic properties, which is easy to absorb water. The higher the environmental humidity, the higher the honey water content. Likewise, in Hilmanto [39] low water levels are caused by low humidity levels. Handling honey harvest that is too early, most honeycombs are still not covered by wax, also affects honey's water content [40]. Honey harvested with wax-covered nests has lower water content than honey that is harvested. Most honeycombs are still not covered by wax [10].

The water content in honey determines the quality of honey. The higher the water content of honey, the lower it is quality [41]. The water content in honey determines the durability of honey. Microbes cannot live in honey with low water content. Harjo et al. [11] explained that honey with high water content would be easily fermented by the yeast of the genus Zygomascharomyces, which is resistant to high sugar concentrations to grow and develop in honey.

3.2.3. PH value
The study results that the rubber honey from the three types of bees has a low pH of 3.80 - 4.50 (Table 2). Although from the same dominant source of nectar, rubber honey from the three types of bees has a different honey pH. The difference in pH values is due to differences in mineral and acid content in honey [42]. However, the type of bee has no significant effect on honey pH [43].

The lowest pH value of T. itama bee is 3.80 and has a more acidic taste than rubber honey from A. dorsata and A. mellifera. All of the honey types are characterized as acidic. The results showed that although with different geographical and botanical source of the honey samples, their pH was almost within the same range. The finding may suggest that stingless bee honey has lower range of pH regardless their origin. By the opinion of Khalil et al. [44], the pH of honey can affect honey's flavor and aroma.

3.2.4. Acidity level
The acidity of honey is one of the parameters used to determine the quality of honey. Acidity shows the amount of free acid per kg of honey [10]. The analysis results all samples showed acidity levels, quality requirements were a maximum of 50 mL of 0.1N/kg NaOH for A. mellifera and A. dorsata bees and a maximum of 200 mL of 0.1N/kg NaOH for Trigona bees (Table 2). The acidity of honey from the three different bees is 2.94 mL NaOH 0.1N/kg A. mellifera bee; 5.92 mL NaOH 0.1N/kg A. dorsata bee and equal to 11.29 mL NaOH 0.1N/kg T. itama (Table 2). This difference is due to the soil's biophysical
conditions and the environment of the different harvest locations so that it affects the acidity of the honey, by the opinion of Savitri et al. [9] that the acidity of honey significantly affects different regions. The highest acidity level of honey was obtained from *T. itama*. This shows that the type of bee influences the acidity. By Tanuwidjaya [38] statement, bee species have a very significant effect on acidity. SNI standard stipulates the maximum acidity of honey reaches only 50 mL NaOH/kg. Due to the higher acidity of *Trigona* bees, the SNI reference used is based on the type of bees. The acidity of *Trigona* honey is not caused by fermentation but due to the content of free acids, acidic minerals, and amino acids of honey.

The acidity of rubber honey in this study is different from [10], where the average acidity of rubber honey is 45 mL NaOH 0.1N/kg, while Savitri *et al.* [9] is 34.59 mL NaOH 0.1 N/kg. As stated by Savitri *et al.* [9], acidity levels significantly affected different regions. Some factors that influence honey's acidity are the biophysical conditions of the honey harvesting location, climate, honey maturity, and the way it is processed and stored.

### 3.2.5. Hidroksi Metil Furfural (HMF)

HMF content is widely recognised as a parameter of honey freshness. HMF is a breakdown product of simple sugars, especially fructose. Ultimately, fructose in the honey is decomposing and reacting with acid. HMF levels are one indicator of honey damage by overheating or due to counterfeiting with inverted sugar and duration of storage [12]. HMF in honey is produced from an overhaul of honey monosaccharides (glucose and fructose) in an acidic atmosphere and with the help of heat [11].

The results of the study show that rubber honey from the three types of bees has a low HMF content of 0.0897 - 0.4590 mg/kg and meets SNI 2018 [8] standards. This shows that the honey has no heating or counterfeiting treatment with invert sugar. HMF levels of *A. mellifera* bee species results of this study were lower than research by Harjo *et al.* [11] with HMF levels of rubber honey by 17.23 mg/kg. Following the statement of Harjo *et al.* [11] that storage temperatures caused an increase in HMF levels. Low HMF levels indicate that the honey has just been harvested. According to Al-Diab *et al.* [45], newly harvested honey contains HMF with HMF levels below 1 mg/kg.

| Parameters                | Type of bee               |
|---------------------------|---------------------------|
|                           | *Apis mellifera* | *Apis dorsata* | *Trigona itama* | SNI 8664-2018 |
| Color criteria            | light amber           | extra light amber | extra light amber | -               |
| Color intensity (mmP fund)| 73                      | 36                | 49               | -               |
| Water content (%)         | 22.5                   | 30.0              | 28.4             | *A. mellifera* dan *A. dorsata* maks 22%; *T. itama* maks 27.5% |
| pH acidity (mL N NaOH/kg) | 4.50                   | 4.00              | 3.80             | -               |
| Reducing sugar (%)        | 65.76                  | 65.60             | 62.97            | *A. mellifera* dan *A. dorsata* maks 50(mL N NaOH/kg); *T. itama* maks 200 (mL N NaOH/kg) |
| HMF (mg/kg)               | 0.0897                 | 0.2372            | 0.4590           | Maks 50 (mg/kg) |
|                           |                         |                   |                  | *A. mellifera* dan *A. dorsata* Min 65%; *T. itama* min 55% |

**Table 2.** The results of the physicochemical analysis of rubber honey
3.2.6. Reducing sugar

The honey quality can be determined from the sugar content contained in it [5]. The results showed that the sugar reducing levels of rubber honey from bee species A. mellifera, A. dorsata, and T. itama met the SNI 8664-2018 [8] standard, namely 65.76%, 65.60%, and 62.97%. The three types of bees have different reducing sugar levels. Rubber honey from T. itama bee species has lower reducing sugar levels than A. dorsata and A. mellifera bees but meets SNI 2018 standards. According to Tanuwidjaya [38] statement that bee species have a very significant effect on reducing sugar levels.

Factors affecting sugar composition include bee food types, regional differences, and climatic conditions [46]. Temperature can affect the nectar production of feed source plants. The higher the ambient temperature, the less nectar produced by feed sources, but the nectar's sugar content is increasing and vice versa. The drier the air, the lower the nectar produced [47]. The difference in humidity affects honey glucose levels. High humidity causes more nectar production but low sugar content. When the air is dry, the nectar production is low, but the sugar content increases [49].

4. Conclusion

The phytochemical analysis of honey from the three positive bee species has active compounds of phenol and flavonoid saponins. Nevertheless, it does not contain alkaloid compounds, steroids, triterpenoids, tannins, and quinones. Rubber honey from A. dorsata bees has higher phenol active compounds compared to A. mellifera and T. itama bees.

All rubber honey samples from A. mellifera, A. dorsata, and T. itama species have physicochemical characteristics with honey quality in accordance with the SNI 8664-2018 based on hydroxymethylfurfural parameters, reducing sugar content and acidity but have water content above SNI standards. Rubber honey has extra light amber color criteria to light amber.

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