The content of propolis's flavonoid from two species of stingless bee in Lombok

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Abstract. Today's stingless bees are in great demand by beekeepers in Lombok. Besides producing honey, it also produces propolis which has more value. Propolis or bee glue is a substance produced by honey bees collected from the top young leaves mixed with the saliva, patch, and hive sterilization. This substance could be used for antibacterial, antiviral, antifungal, and anti-protozoa. In Lombok, apiculture for stingless bees has spread in almost every district. Northern Lombok, West Lombok, and East Lombok were the first three locations that developed stingless bees. This study aimed to identify the species of stingless bees and their propolis productivity and characteristics produced by each species. There were two species of stingless bees cultivated by the people of Lombok, *Trigona clypearis* and *Trigona sapiens*. The development of science, genus *Trigona* changed nomenclature into *Tetragonula*. The yield of propolis through extraction using water or Aqueous Extraction Propolis (AEP) produced by *T. clypearis* was 34-55% and *T. sapiens* was in the range of 3-24%. The flavonoid content equivalent to quercetin in propolis produced by *T. sapiens* as vary as *T. clypearis*. The results of this study could be used as a baseline for other propolis extraction activities to produce propolis more marketable.

1. Introduction
Apiculture characteristics in West Nusa Tenggara consists of two types: hunting forest’s honey on Sumbawa island and beekeeping honey on Lombok island. The product produced on Sumbawa island is not only honey but also beeswax and bee pollen. It is different from beekeeping in Lombok island that has produced honey only.

Beekeeping, in principle, is to provide permanent housing with complete facilities that a strong colony of bees formed. However, beekeeping activity in West Nusa Tenggara is still straight forward. Primarily just for hobbies. The product obtained from bees is honey and other products such as bees wax, royal jelly, propolis, bee pollen, and bee bread. The diversification of the product and increasing the added value of beekeeping will improve the skills and incomes of the beekeeper.

Mostly, the species of bees that are cultivated throughout the world are tame bees. In general, there are two types of bees, with sting and without sting (stingless bee). The species of sting bees that can be cultivated so far are *Apis cerana* and *Apis mellifera*. Stingless bees around the world more than 500 species [1]. One genus of stingless bees is *tetragonula*, which is *Meliponini ordo*. One feature of *Meliponini* is its ability to produce propolis, and *tetragonula* produce it.

Propolis or bee glue is a substance produced by honey bees, collected by bees from the buds of young leaves then mixed with saliva, is used for patching and sterilize the hive. Propolis is an antibacterial that
kills all the germs that enter the hive. Propolis is reported to function as an antibacterial, antiviruses, antifungal, and antiprotozoal. Propolis also stimulate the immune system and repair tissue damage in various organs [2]. The uses of propolis are for cosmetic, medicinal materials, traditional, food technology, and others [3].

Extraction of propolis has been widely carried out on *Apis* sp. Several methods of extraction of propolis have been done [3]. Many researchers also have done some propolis extraction methods, namely maceration extraction, ultrasound extraction, and microwave-assisted extraction [4]. Extraction is not only on *Apis* sp. propolis, but currently, propolis extractions also have to do with the *Trigona* spp. A patent on propolis extraction from *Trigona* spp. and extracted propolis to inhibit bacteria growth [5].

Some people in Lombok island are also cultivating *Trigona* spp., even though it is not as much as *Apis cerana*. Supported by various potential benefits of propolis and propolis *Trigona* spp. development on Lombok island, this study learned about the production of propolis species *Trigona* spp. in particular on Lombok island, West Nusa Tenggara. The first goal of this research is for the public to obtain alternative types of *Trigona* spp. that can produce propolis, and the production could be increased and could encourage farmers to develop beekeeping of *Trigona* spp. because it does not have the kind of sting that is relatively safe and easy to cultivate.

Hotnida Siregar, a bee expert from the IPB University, said worker bees processing the propolis from various materials such as the leaf, sap of plants, and a variety of plants [6]. The fact resulted in the need to identify the types of feed consumed by producing bee propolis to increase production. This study aimed to identify the species of stingless bees and their propolis productivity and characteristics produced by each species.

2. Materials and Methods

2.1. Colony of *Trigona* spp.

Identification of *Trigona* spp. was conducted to determine the species of trigona that has been cultivated in Lendang Nangka, East Lombok; Karang Bayan, West Lombok; Sigar Penjalin dan Gangga, North Lombok. The bee sample takes by trapping the drones and workers bees from some artificial hive. Subsequently, the sample species were identified in the zoology laboratory field, biology research center LIPI, Cibinong.

2.2. Raw propolis

Raw propolis was collected from identified bee colonies. Propolis production was extracted with the water extraction method and become an aqueous (water) extracted propolis (AEP). AEP method will be treated on a long soaking time, which are 7 and 14 days. The propolis production process through the water extraction method carried out with the following preparations [3].

The content of propolis in hive measures by scale the weight of propolis in each hive. Each piece was weighed heavily nest initially (B0). The part is then extracted or soaked in water to obtain the content. The content of propolis is then scaled (B1). The yield content of propolis calculated by the formula:

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\text{The yield content of propolis (%) } = \frac{B1}{B0} \times 100\%
\] (1)

Then propolis extracts were analyzed for physical and chemical properties. The physical properties of propolis which is the color observed. The chemical analysis of propolis held in Bogor with the flavonoid parameters.

2.3. Data analysis

Data collected in this research consisted of primary data and secondary data. Primary data consist of species *Trigona* spp. cultivated by the people of Lombok, temperature, humidity, vegetation (feed bees), the propolis content in hives *Trigona* spp., yield propolis, and flavonoid content analysis. Secondary data includes data on flowering season bee forage plants. The data tabulated, processed, and then analyzed descriptively and presented in the form of graphs, tables, and images.
3. Results and Discussion

3.1. Identification of Trigona from several locations
Sampling was conducted on 12 artificial hives taken from beekeeping in Lombok. Based on the identification, species of stingless bees are two, *Trigona sapiens* and *Trigona clypearis*. With the development of science, genus Trigona changed nomenclature into tetragonula [7]. Here's a description of the two types of tetragonula.

3.1.1. *T. clypearis*. *T. clypearis* included in the genus tetragonula families Meliponini, Apinae order, class Hymenoptera and kingdom Apidae. This tetragonula has physical characteristics in the abdomen yellow when viewed with the naked eye. The size of the females ranged from 3.2-3.7 mm, and a male worker body size 3-3.6 mm. The physical form can be seen clearly in Figure 1.

![Figure 1. Trigona clypearis Friese, 1909.](image1)

3.1.2. *T. sapiens*. *T. sapiens* included in the genus Tetragonula, families Meliponini, Apinae order, class Hymenoptera and kingdom Apidae. This tetragonula type has physical traits in its abdomen black when viewed with the naked eye. Female body size larger than *T. clypearis* ranged between 3.6-4.2 mm, and a male worker body size is also larger than *T. clypearis* 4.2-4.5 mm. The physical form can be seen clearly in Figure 2.

![Figure 2. Tetragonula sapiens Cockerell, 1911.](image2)

The life and development of bees are influenced by environmental factors [8]. Widhiono [9] also supported this statement, which stated that in addition to the availability of feed bees, environmental factors such as temperature, humidity, rainfall, and altitude also determine the honey bee development. Identifying environmental conditions is needed to determine the environmental conditions around the artificial hive honey bee *Trigona* spp. cultivated. Data taken on identifying environmental conditions include temperature, humidity, and type of feed around cultivation. Some locations where cultivation is *Trigona* spp. observed in the Village of East Lombok Lendang Nangka, Sigar Penjalin Genggelang Village of North Lombok Regency, and Village of Karang Bayan West Lombok District.

Trigona preferred an ideal temperature range of 18-24°C and 60-70% humidity [10]. Meanwhile, 33-34°C is the optimum temperature for bees [11]. The present study captured data on temperature and humidity. Note the temperature of an environment as the temperature could determine the activity of bee honey every day. The following data on the temperature and humidity is obtained (Table 1).
Table 1. Temperature and humidity of place of Trigona spp. at Lombok.

| Number | Parameters | Location |
|--------|------------|----------|
|        | LN         | SR       | GG       | KB       |
| 1.     | Temperature| 27.8°C   | 28°C     | 29°C     | 27°C     |
| 2.     | Humidity   | 60.5%    | 71%      | 70%      | 76%      |

Table 1 showed the measured temperature is still too high for Tetragonula sp. to indulge. This impacts the quality of propolis is more dry and chewy due to lack of moisture. Humidity is measured relative to normal because it is still included in the normal range of humidity like Trigona spp. According to Finstrom and Spivak, honey bees use propolis to help them keep the environment around the nest remains stable by reducing the growth of microbes in the nest wall, keeping the air circulation into the nest and the nest walls are waterproof to resist moisture out of the nest, as well as hold attacks from outside intruders [12]. The data indicate that propolis is neutralizing the ambient temperature to the temperature in the hive. If high humidity outside the nest, it will retain moisture propolis in the hive so that the resulting propolis will be mushy. On the other side, if the dry environmental conditions, the outer portion of propolis in the nest will dry and harden, the softened into the nest. This statement is supported by Ghassan et al. [13] which states that propolis samples found to form chewy, soft, and dry.

3.2. Content of raw propolis in the hive
Propolis has been described previously as sap or glue produced by bees from plants in the vicinity. Propolis in the hive that has harvested an average of brownish-black, it calls raw propolis. Raw propolis consists of approximately 50% resin compounds (flavonoids and phenolic acids), 30% wax, 10% aromatic oils, 5% pollen, and 5% organic compounds [14]. Raw propolis present in some parts of the hive. In this study, the position of raw propolis in the nest is divided into four parts: entrances, hive coverings, honey pot, and egg holder. The fourth section is shown in Figure 3.

Figure 3. Parts of Tetragonula’s hive.

In Table 2, it can be seen that there is some raw propolis in each position in the two types of bees in the hive. Some of the factors that might impact this area include nests, nest conditions, and the number of colonies. A large hive does not always result in large numbers of propolis when the nest is in good condition, tightly closed, and small colonies. But the small hive also does not always produce a small amount of propolis when the condition of nest cavities and many large colonies. According to Krell has estimated that 200,000 honey bees produce propolis 20 grams each year [3].
Table 2. Weight (gram) of raw propolis from two species of bee in each location on a hive.

| Location | Entrance | Hive cover | Honey pot | Egg pot | Total |
|----------|----------|------------|-----------|---------|-------|
| Tetragonula clypearis |
| GG B     | 16.56    | 29.95      | 32.93     | 1.19    | 80.63 |
| Sira B   | 8.59     | 19.13      | 20.81     | 7.04    | 55.57 |
| KB A     | 12.58    | 14.32      | 28.54     | 12.92   | 68.36 |
| KB C     | 25.03    | 17.98      | 81.35     | 21.73   | 146.09 |
| Tetragonula sapiens |
| LN A     | 32.46    | 73.41      | 16.66     | 8.59    | 131.12 |
| LN B     | 7.18     | 34.78      | 44.55     | 15.10   | 101.61 |
| LN C     | 7.08     | 31.26      | 26.23     | 0.99    | 65.56 |
| KB B     | 5.64     | 17.30      | 40.16     | 5.22    | 68.32 |

Description: GG= Gangga; Sira= Pantai Sira; KB= Karang Bayan; LN= Lendang Nangka

The tables can also be seen that propolis in a honey pot which is the most widely produced. At the same time, the other positions are relatively few. But unlike most of the hive, honey pot at Lendang Nangka (LN) A and C contain less propolis than it in hive cover. This could be due to the condition of the nest of LN A and C were damaged. As a result, a lot of bees produce propolis to seal the nest. Bee resin used to coat the inside of the nest, repairing broken strokes, patching holes, and minimize drive way cells to escape the cold [15].

Propolis is ready to be consumed, usually obtained by extraction. The extraction of propolis has been done to date is to use a water solvent, ethanol, or glycol on the research activities of propolis extraction using only water. Propolis extraction results conducted for 7 days resulted in a yield of data presented in Table 3.

Table 3. The yield of AEP.

| Stingless bee species | Hive code | Raw propolis weight (B0) | Propolis weight (B1) | Yield (%) | Mean of yield (%) |
|----------------------|-----------|--------------------------|----------------------|-----------|------------------|
| Trigona clypearis    |
| GG B                 | 9.60      | 3.32                     | 34.59                | 41.80     |
| Sira B               | 9.64      | 5.35                     | 55.56                |           |
| KB A                 | 9.11      | 3.18                     | 34.90                |           |
| KB C                 | 9.99      | 4.21                     | 42.16                |           |
| Trigona sapiens      |
| LN A                 | 9.63      | 0.37                     | 3.93                 | 16.34     |
| LN B                 | 9.02      | 2.18                     | 24.17                |           |
| LN C                 | 9.06      | 1.61                     | 17.87                |           |
| KB B                 | 9.15      | 1.77                     | 19.37                |           |

Description: GG= Gangga; KB= Karang Bayan; LN= Lendang Nangka

Table 3 showed that the yield of propolis produced by T. clypearis is at an interval of 34-55%, while the yield of propolis produced by T. sapiens species is at an interval of 3-24%. The average yield of these two species is also quite different. T. clypearis produce propolis yield greater than T. sapiens. T-test analysis showed that each species produced a significantly different yield.
In the visible part of propolis obtained from each breeding site, Trigona sp almost the same greenish-brown or blackish brown. But there is also a yellow-green. At room temperature, the raw propolis is very sticky, so it isn't easy to obtain the melting point of propolis trigona. Propolis is generally soft, pliable, and sticky at temperatures 25-45°C, but at temperatures below 15°C, propolis will be a hard and brittle texture. At a temperature of 60-70°C would propolis liquid form. Some types of propolis have a boiling point above 100°C [3].

A compound of propolis that makes it valuable is the flavonoid. In this research, an analysis of the flavonoid content equivalent to quercetin was obtained. Flavonoid content based on the analysis that has been done can be seen in Table 4.

Table 4. Flavonoid equivalent with quercetin in propolis extract.

| Stingless bee species | Hive code | Flavonoid (ppm) 7 days extraction | Flavonoid (ppm) 14 days extraction |
|-----------------------|-----------|-----------------------------------|-----------------------------------|
|                       |           | Honey pot | Hive cover | Honey pot | Hive cover |
| **T. clypearis**      | GG B      | 1.47      | 2.57       | 2.64      | 2.20       |
|                       | SIRA B    | 11.01     | 1.10       | 0.88      | 8.08       |
|                       | KB A      | 4.03      | 3.67       | 2.19      | 1.10       |
|                       | KB C      | 9.99      | 0.73       | 7.64      | 3.31       |
| **T. sapiens**        | LN A      | 1.95      | 2.93       | 2.57      | 3.67       |
|                       | LN B      | 5.87      | 2.92       | 5.88      | 4.40       |
|                       | LN C      | 5.14      | 5.14       | 2.64      | 1.84       |
|                       | KB B      | 5.88      | 6.84       | 5.58      | 4.04       |

Comparing with the levels of flavonoids 7 days extraction time, 50% of the honey pot contains higher levels of flavonoids than the hive cover. A different thing happened on the propolis extraction time of 14 days. More than 50% of the honey pot contains a higher level of propolis. It was suspected that compounds in the honey storage area are more required to support the content of the honey itself. While closing the nest has more physical functions, such as keeping the temperature and humidity of the nest so that the compound is abundant in these parts.

Table 4 also showed that the propolis extraction originating from the type T. clypearis well done for 7 or 14 days looks diverse. There is a high flavonoid-containing extraction at 7 days, but some are 14 days. Something similar occurs in propolis originating from T. sapiens. Alleged that the condition of each beehive diverse flavonoids affects the placement of the nest. Nest in good condition will produce propolis with flavonoid smaller. But the damaged hive would produce propolis with a higher content to maintain the nest’s condition and contents.

This table explains that the levels of flavonoids contained in the honey storage area on each type with different extraction times showed a relatively uniform difference. Propolis extracted for 7 days generally contains higher levels of flavonoids. But ANOVA analysis showed no significant difference between species, duration of extraction, and propolis hive part in flavonoid content. Allegedly a longer extraction in propolis honey storage area that can result in fermentation affects the levels of flavonoids which are carbon chains.

4. Conclusion

There are two species of stingless bees found in three Lombok districts which are T. sapiens and T. clypearis. Yield propolis produced by T. clypearis is at 34-55%, while the yield interval propolis produced by T. sapiens is at an interval of 3-24%. In general, the flavonoid content of propolis extracts is very diverse both in term of bee species, duration of extraction, and propolis hive part.
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