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Pollen Diversity and Propolis's Bioactive Compounds of Stingless Bees *(Tetragonula laeviceps,* Smith 1857) From Kedungpoh Meliponiculture, Gunungkidul, Yogyakarta.

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ABSTRACT
The progression of many diseases due to viruses and bacteria makes for an increase in natural alternative medicine. One source of natural medicine is honey and propolis of stingless bees. Efficacy physical and chemical properties of honey are influenced by the type of pollen and the environment, while geological factors influence the composition of propolis. Bee and stingless bee colonies have a behavior called feed preference. This preference is influenced by several aspects, such as food source location and the selected plant species that produce pollen, nectar, and resins. Therefore, this research was conducted to study the diversity of pollen and the composition of propolis bioactive compounds of stingless bees from Kedungpoh Meliponiculture in Gunungkidul, Yogyakarta. Identification of bees was carried out using a *Digital Microscope Super eye*. Pollen preparation was made using a modified acetolysis method and observed under a light microscope. The competition of bioactive compounds of propolis was investigated by GC-MS, Gas Chromatograph-Mass Spectrometry. The results showed the diversity of pollen of stingless bees (*T. laeviceps)* from Kedungpoh Meliponiculture in Gunungkidul, Yogyakarta was from various plants belonging to 27 families consisting of herbs, shrubs, and trees. The dominated pollen family was from Arecaceae, 26,00 % of the total amounts of pollen. GC-MS result showed that the propolis was composed of 42 types of the bioactive compound and grouped into four categories: terpenoid (62,50%), phenolic (29,22%), steroid (7.08%), fatty acid (1,20%). The most dominant bioactive compound was (Z)-3-(pentadec-8-en-1- yl) phenol ($C_{21}H_{34}O$) (23.32%) from the phenolic group.

Keywords: *Bioactive Compounds, Pollen Diversity, Propolis, T. laeviceps.*

1. INTRODUCTION

Currently, many developing diseases are caused by viruses or bacteria that attack the body's immune system. One way to prevent this disease is to take alternative medicine. Indonesia has many natural resources that can be used as a source of alternative medicine, including the *T. laeviceps* bee's product. *T. laeviceps* bee is classified as a stingless bee that can live in tropical areas. These bees produce honey, bee pollen, propolis, royal jelly, and beeswax [1].

Pollen is the male gametophyte of seed plants produced in the anthers of Angiosperms. The pollen eaten by bees depends on the types of available plants. This difference affects the diversity of pollen produced and stored by bees and directly affects the composition and quality of honey. Pollen morphology can be used to

identify taxon and plant species widely used by bees to produce honey [2].

Propolis is a complex resin compound collected by bees from plant exudates and strengthens the hive's structure. The chemical compounds in propolis can be used as antibacterial, antiviral, and antitumor [3]. The characteristics of propolis vary due to differences in the composition of its chemical compounds. Geological factors and selected plant species that produce resin are the main factors that cause differences in the composition of chemical compounds inpropolis [4].

Bee and stingless bee colonies also have feed preference behavior. This preference is influenced by several aspects, such as selected plant species that produce pollen, nectar, propolis, and food source location. Kedungpoh Meliponiculture located in Gunungkidul. This area includes mountainous, hills, and lowlands areas. This area also has karst hills with relatively dry and barren soil teak, mahogany, and pine as dominant vegetation [5].

Therefore, it is deemed necessary to conduct this study to determine the diversity of pollen as a preference for flowering plants as a food source for *T. laeviceps* bees to produce honey as well as the composition of *T. laeviceps* bee propolis bioactive compounds, which can be used as a medicine in these locations.

2. MATERIALS AND METHODS

The primary materials used in this research are *T.laeviceps* bee, pollen, and propolis collected from *T. laeviceps* beehives taken from the locations.

2.1. Collecting pollen and propolis

Pollen and propolis collected from *T. laeviceps* beehives taken from Kedungpoh Meliponiculture, Gunungkidul, Yogyakarta. Pollen was collected using a spoon and taken into 2.5 ml flacon bottles, while propolis was collected using a spoon and taken into bottles glass.

2.2. Pollen preparation and identification

Pollen preparation was made by using the modified acetolysis method [6]. About 5 grams of pollen were weighed using an analytical balance and then put into a 20 ml beaker glass filled with distilled water. Beaker glass containing pollen was heated on IKA C hotplate. - MAG HS 7 at 200°C for 15 min while stirring. The mixture of pollen and debris in the beaker glass was transferred into a centrifuge tube. The sample was centrifuged at 1700 rpm at room temperature for 5 min using the PLC series. After that, the supernatant was discarded. After that, 7 ml of glacial acetic acid was added into the sample and vortexed used vortex Thermolyne Type 37600 Mixer. Then the sample was centrifuged at a speed of 1700 rpm for 5 min, and the supernatant was discarded. 7 ml mixture solution of glacial acetic acid and sulfuric acid (CH_3COOH: H_2SO_4) (9:1) was added. Then the sample was heated in a water bath for 3 min at a temperature of 200°C. The sample was cooled for 15 min. After that sample was centrifuged at a speed of 1700 rpm for 5 min, and the supernatant was discarded. 10 ml of distilled water was added to the sample, vortexed, and centrifuged again. The supernatant was discarded. The pellet was added with 1% safranin in 2-3 drops of distilled water using dropper pipettes, vortexed, and centrifuged again. Next, safranin was replaced with glycerin jelly and heated with bunsen. Samples were taken with a glass rod placed in an object-glass and then covered with a cover glass. Furthermore, the morphological character of pollen was observed using a binocular light microscope with 40 × 10 and 100 × 10 magnification which was connected to the Optilab 3.0 software on a laptop. The observed pollenwas measured using the Image Raster 3 application.

2.3. Propolis's bioactive compounds analysis

Samples were extracted by the maceration method, and the solution was investigated by GC-MS, Gas Chromatograph-Mass Spectrometry [7]. First, the sample was heated using distilled water to remove the remaininghoney. Then the sample was sliced into small pieces and was aired. After dried, the sample was grounded until small and weighed as much as 0.5 grams. Then the sample was extracted with MeOH solution with ratio (1:1) and centrifuged at 900 rpm at room temperature for 5 min. About 3 liters of the solution was injected into Shimadzu GCMS-QP2010S gas chromatograph, with an initial temperature of 50°C and a final temperature of 240°C. The type of column used is Agilent HP-5MS UI, the typeof detector is FID with a temperature of 300 °C, and the carrier gas is helium. The result is a compound chromatogram profile.

2.4. Data Analysis

Pollen identification was carried out using references from journals, the Australian Pollen and Spore Atlas website (http://apsa.anu.edu.au), Paldat (www.paldat.org), globalpollen.org, and textbooks. The spectra of the GC-MS results were compared with the spectral databases of WILEY229.LIB and NIST62 libraries to see the possible compounds contained in the sample. The potential compounds were searched through Pubchem.NCBI database and journal.

3. RESULTS AND DISCUSSION

The *T. laeviceps* bee is one of the stingless bees widely cultivated by the community because of its

propolis and bee pollen which are beneficial for health and have high nutritional value. The morphology of the *T. C* worker bee is generally characterized by a glossy black dominant body color, as shown in Figure 1. The head and thorax are black, while the abdomen is blackish brown.

Figure 1. *T. laeviceps* bee Morphology

Based on the study results, the diversity of pollen as feed source of T. laeviceps bees from Kedungpoh Meliponiculture in Gunungkidul, Yogyakarta, is shown in Figures 2 3. Based on Figure 2, pollen from various plants belonging to 27 families and five have not been identified in Kedungpoh Meliponiculture, Nglipar, Gunung Kidul, Yogyakarta. Pollen that has not been identified maybe because it has not been found in the literature and similar characters in several plantfamilies.

The dominated pollen family was from Arecaceae, 26,00 % of the total amounts of pollen. Plant from the Arecaceae family can grow in the highlands and lowlands [11]. Arecaceae were classified as monocot plants and generally dominated by perennial plants, which could continue growth for more than two years with the habitus of trees and shrubs [12]. The dominance of food sources from Arecaceae pollen was also caused by the time of pollen collection of T. laeviceps bees and the flowering period of Arecaceae plants around the hive. This was supported by Arecaceae flowers which were generally relatively small, with colored crowns and very rich in nectar that attracted pollinating insects [13].

Other dominant pollen came from the Araceae family,12.80%, and the Amaranthaceae family, 8.60% of the total amounts of pollen. The Araceae family included terrestrial herbs, aquatic, and epiphytes [14]. Herbaceous plants could flower any time with a small size, so the T. laeviceps bee was taken nectar quickly. While Amaranthaceae family generally came from annual and perennial plants and was dominated by herbs and shrubs. The habitus of the Amaranthaceae family in the form of shrubs or wild plants can increase the spread of this plant.

There are three ocelli eyes in the head, a pair of compound eyes that are blackish-brown in color, and two brown antennae. The flagellum on the antenna is composed of 11 segments that characterized the individual worker bee as female [8]. On the thorax, there are forewing, hindwing, prothorax legs, mesothorax legs, and metathorax legs. The scutum on the thorax has hair bands [9]. The femur, tibia, and tarsus are black. The tibia has a few hairs. The hind basitarsus is slightly oval and covered with hairs. On the wings, some hamuli hooks function to connect the forewing and hindwing when the T. laeviceps bee flies [10].

Based on Figure 3, the pollen of the Arecaceae family was oval in shape, monad, monocolpate aperture type, psilate to echinate ornamentation, and rectangular. This was related to the study that the pollen of Arecaceae was monad with reticulate ornamentation [13]. Araceae pollen has a tentative-collumelate pollen character, monosulcate aperture type, circular, ornament generally foveolate or psilate [15]. Amaranthaceae has round pollen grains, spheroidal monad, pastorate aperture, and perforate ornamentation.

Based on Table 1, the most dominant components of bioactive compounds in the locations were terpenoids and phenolic groups. The percentage of terpenoids compounds was 62.50%. This large number is due to the extensive vegetation in the areas that produce terpenoids as secondary metabolites. In different environments, plants adapted to produce other compounds [16]. Differences in plant metabolites affected propolis's character and chemical content [4]. Also, this large number is due to various terpenoids groups with multiple functions in plants. Monoterpenoids gave a distinctive aroma to fruit and flowers as an attractant for insects. Diterpenoids were used as a resin for the physical protection of plants—triterpenoids as a defense against fungi, insects, and certain bacteria and microbes [17]. The compound from the terpenoid group with the most significant percentage

Table 1. Bioactive Compounds of *T. laeviceps* Propolis in Kedungpoh Meliponiculture, Nglipar, Gunung Kidul, Yogyakarta

Location	Bioactive Compounds				
	Terpenoids	Steroids	Phenolic	Fatty Acid	Alkaloids
Gunungkidul	62,50 %	7,08 %	29,22 %	1,20%	-

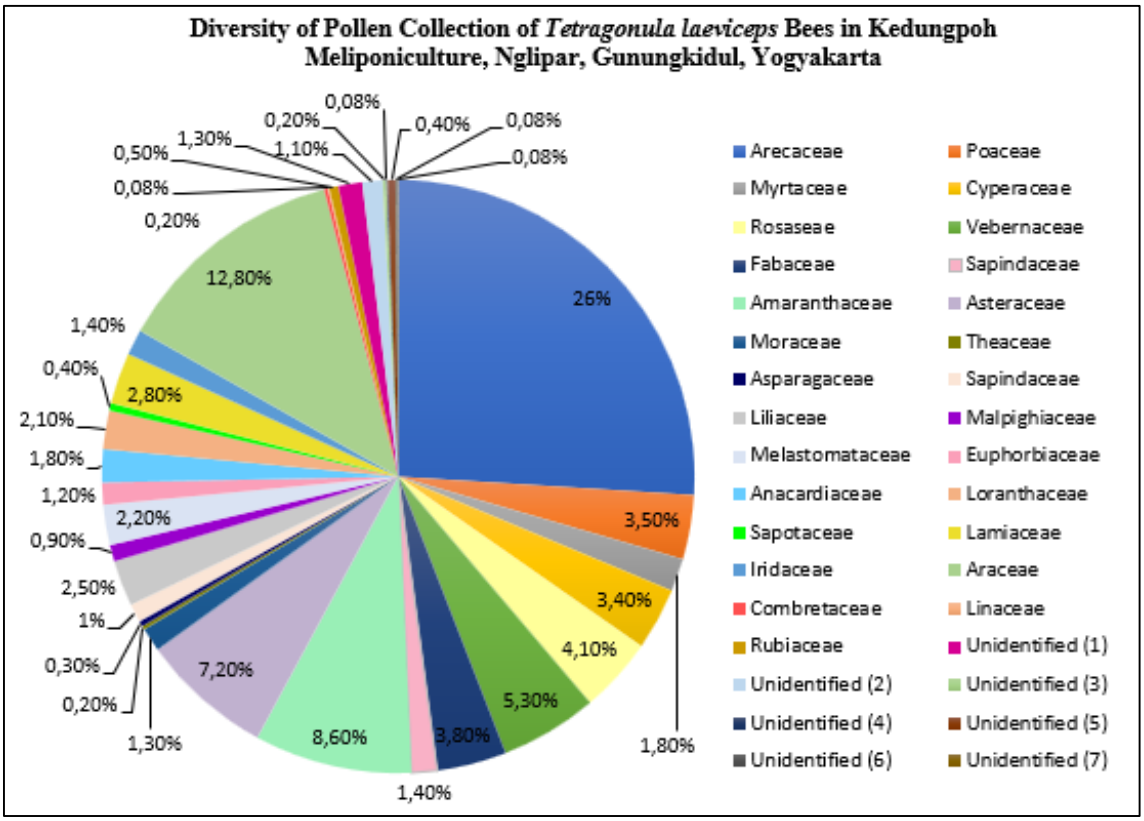


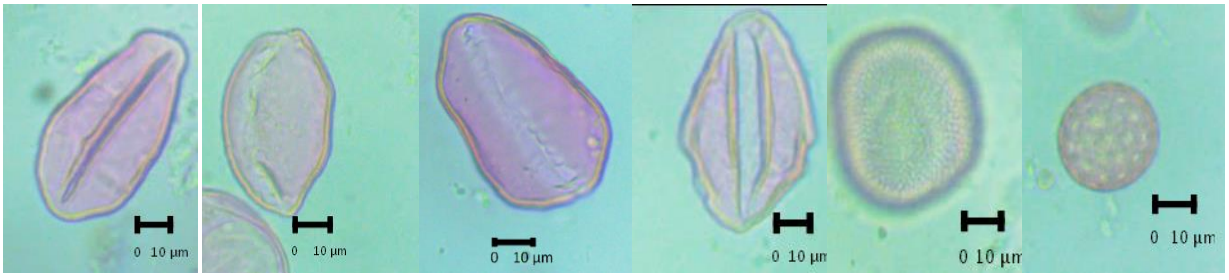
Figure 2. Diversity of pollen collection of *T.laeviceps* in Kedungpoh Meliponiculture, Nglipar, Gunung Kidul, Yogyakarta.

was Abietic acid (C20H30O2) 14.18%. A diterpenoid that used for cardiovascular and cerebrovascular diseases [18].

The percentage of phenolic compounds was 29.22%. The large portion of phenolic compounds found could be due to the largest group of these compounds, which plants produce as natural antioxidants [19]. The combination from the terpenoid group with the most significant percentage was (Z)-3-(pentadec-8- en-1-yl) phenol ($C_{21}H_{34}O$) 23.32%. This compound has another name Cardanol monoene. Besides being an antioxidant, it also functions as an antitumor and antimicrobial [20].

Steroids were found at 7.08% in the locations, with the most significant percentage being Diazoprogesterone ($C_{21}H_{30}N_4$) 3.19%. This compound is used as a contraceptive drug. In addition, there were fatty acids 1.20%, with the most significant percentage of these compounds was Octadecynoic acid ($C_{18}H_{32}O_2$) (0.50%). These compounds are used as lipid synthesis material. Besides, these compounds function as bioorthogonal probes for labeling palmitoylated proteins in human cells [21].

From the results, no alkaloids compounds were found. This can be caused by a few plants that produce alkaloid compounds in the area visited by *T. laeviceps* bees. In different environments, plants will adapt to produce other compounds. So, the plant's location could influence the content of bioactive compounds present in plants, and plant bioactive compounds taken by bees will affect the content of propolis [16]. Different



(1) (2) (3) (4) (5) (6)

Figure 3. *T.laeviceps* bee pollen in Kedungpoh Meliponiculture, Nglipar, Gunungkidul, Yogyakarta :(1-4)Arecacear; (5) Araceae; (6) Asteraceae

geographical conditions, time of day, plant species, and location could affect propolis's compound composition, color, and aroma [22].

Based on the research, it can be concluded that pollen diversity as feed source of stingless bees from Kedungpoh Meliponiculture in Gunungkidul, Yogyakarta was from various plants belonging to 27 families dominated by pollen from Arecaceae (26.00%), Araceae (12.80%), Amaranthaceae (8.60%) and (Z)-3-(pentadec-8-en-1-yl) phenol ($C_{21}H_{34}O$) from the phenolic group was the most dominant bioactive compound in propolis product.

AUTHORS' CONTRIBUTION

All authors have the same contribution to this research and publication. S.S. and I.S designed the study and supervised all the processes; F.O. collected and analyzed the data. F.O and I.S. wrote the manuscript.

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