OPEN ACCESS International Journal of Applied Biology

ISSN : 2580-2410
eISSN : 2580-2119

Analysis of Antioxidant Content in Pollen and Honey Produced by Bees Trigona spp At Several Locations In South Sulawesi

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Abstract

Trigona bees spp has a potential utilization of this bee is not inferior to Apis sp. The use of stingless honey bees in South Sulawesi has been carried out in several districts such as Selayar, Maros and Gowa. Trigona bees spp produce pollen and honey which contain antioxidants, antioxidants are compounds that can neutralize or destroy free radicals, so as to prevent various diseases. This research was conducted to analyze the antioxidant content of pollen and honey produced by the three districts and compare them with each other. Antioxidant levels was carried out using the DPPH method (2,2-diphenyl-1-picrylhydrazyl) with vitamin C was used as positive control. The results of the determinining the wavelength used using a UV Vis spectrophotometer obtained a wavelength of 513 nm used with the test results of antioxidant levels of Pollen extract from the Selayar location were 1666.33 µg/g, Pollen Maros 431.70 µg/g and Pollen Gowa 259.56 µg/g and the antioxidant content of honey from Selayar was 43.62 µg/g, Maros honey 28.08 µg/g and Gowa honey 31.80 µg/g. Pollen and Honey from Selayar had the highest antioxidant levels from the three locations.

Article History
Received November 30, 2021
Accepted June 14, 2022

Keyword
Trigona spp;
Honey;
Pollen;
DPPH

Introduction

Indonesia has the most rich species of honey bees from the Apis clan in the world and has about 40 types of stingless bees or commonly called kelulut/klanceng/teuweul. Sulawesi is one of the islands in Indonesia which is famous for its endemicity. More than nine species of stingless honey bees found in Indonesia (*Trigona apicalis, T. melina, T. itama, T. leviceps, T. drescheri, T. terminate and T. fuscibasis*), there is one species endemic to Sulawesi, namely T. insica. most of them can be developed as honey-producing bees (Harjanto et al., 2020). According to Rasmussen 2008, Indonesia has at least 40 species of stingless bees, divided into several genera, including: *Geniotrigona, Heterotrigona, Lepidotrigona, and Tetragonula.*

The use of stingless honey bees in South Sulawesi has been carried out in several districts, namely Jeneponto (11 farmer groups), Palopo (12 farmer groups), Luwu (2-5 farmer groups), North Luwu (100 people) and East Luwu (50 people). and continue to be developed in various potential areas in South Sulawesi (Hasan et al., 2020). One of the districts producing honey and other products is Selayar.

Honey that contains pollen also has anti-radiation properties, which can protect the body from health damage due to exposure to free radicals. This means that honey and pollen can be expected as free radical scavengers (Hartanto, 2017). Free radicals tend to have a chain reaction which, if they occur in the body, can cause ongoing and continuous damage. The

number of free radicals can increase due to stress factors, radiation, cigarette smoke and environmental pollution causing the body's existing defense system to be inadequate, so the body requires additional antioxidants from the outside that can protect against free radical attacks (Wahdaningsih et al., 2011).

Observations were made in the KHDTK (Special Purpose Forest Area) area of Kepau Jaya that there are plants that have the potential to be a source of food for Trigona spp. Types of potential nectar-producing species found in KHDTK were temon, coconut, Israeli grass, and durian and potential sources of pollen found were coconut, oil palm, sugar palm, and putri malu. The types of plants from the palm family are the best sources of pollen because palm plants are able to produce flowers in large quantities and throughout the year so that the pollen produced is abundant (Wiratmoko *et al*., 2018).

Knowledge of the potential antioxidants contained in pollen and honey is expected to be one of the drivers in increasing the cultivation of *Trigona* spp bees, which can become plant pollinating agents and can increase awareness of the surrounding community to conserve feed plants and increase forest protection as a place to live for feed plants. as an important factor in the development of beekeeping in the region.

Materials and Methods

Research Site

The study was carried out in January-July 2021. Sampling was carried out in the districts of Selayar, Gowa and Maros, South Sulawesi which were tested. The materials used in this study were beehive colonies of Trigona spp. which contains pollen and honey, aquadest, 70% alcohol, 95% ethanol, concentrated H2SO4 solution, Mayer and Dragendorff reagents, chloroform, ammonia, concentrated HCl, HCl IN, 1% FeCl3, Mg powder, Acetic Acid Anhydride, 2,2- diphenyl-1-picrylhydrazyl (DPPH).

Data Collection

In manually taking bee pollen by selecting a beehive box with lots of beehives, then pure pollen is taken in the form of colored solids wrapped in propolis. The collected samples were then air-dried in a shady place to avoid direct sunlight to dry for ± 24 hours. After that the sample is ground and mashed, the refining of the sample aims to maximize the interaction of ethanol (solvent) with the bee pollen sample so that it is expected that all secondary metabolites can be extracted, after the sample is mashed then the sample that has been refined is weighed.

The sample used in this study was honey taken directly from the beehive of Trigona spp. at 3 bee farms in Selayar, Maros and Gowa. by taking a bag of honey. The honey is then removed from the bag using a syringe and a spoon. Honey is then filtered using a filter to clean it from dirt.

Antioxidant Test with DPPH . Radical Attenuation Method

To determine the percentage of DPPH radical reduction (% inhibition) of pollen and honey from bees Trigona spp. the antioxidant activity test was carried out using the DPPH radical reduction method (Kumalaningsih, 2006).

The DPPH radical is soluble in ethanol and has a strong absorbance at a wavelength of 517 nm with a characteristic purple color. After reacting with antioxidant compounds, the DPPH will be reduced and the color will turn yellow.

Pollen and honey samples from 3 beekeeping locations in Selayar, Maros and Gowa were weighed as much as 25 grams and then put in a maceration vessel. Extraction liquid in the form of ethanol was added as much as 75 ml to 100 ml, put into a vessel stored for 3 days in a closed room, the solution was then concentrated at room temperature (370C) to 50 ml, added 4 ml of 40 g/ml DPPH solution in a vial. The solution was incubated for 30 minutes in a dark place.

Weighed 100 mg standard vitamin C of 1000 g/ml and dissolved with 96% ethanol to 100 mL, added 4 mL of 40 g/ml DPPH solution in the vial. The solution was incubated for 30 minutes in the dark.

Preparation of the Standard Curve, as much as 4 mL of 40 µg/ml DPPH solution was added with 1 mL of 96% ethanol into the vial, then the absorbance was measured in the wavelength range of 400 nm - 800 nm. The maximum wavelength and absorbance value of the DPPH standard solution will be obtained (Sinala and Dewi, 2019).

Determination of Antioxidant Level

Pollen and honey extract solutions were measured for absorbance at the maximum wavelength. The same treatment was also carried out on the DPPH solution (which did not contain the test material) and vitamin C as a positive control.

The data from the analysis of antioxidant levels in the sample per gram is calculated using a formula to calculate the levels of a substance in solution, as follows :

$$Antioxidant\ levels \frac{Sample\ consebtration\ x\ Final\ volume}{Sample\ Weight} \text{ x } Dilution\ factor$$

Data Analysis

Pollen research data are presented in the form of figures and tables. The value of the determination of antioxidant levels obtained is presented in the form of a bar graph to see the comparison.

Results and Discussion

Antioxidant Content of Pollen and Honey *Trigona* spp From Selayar, Maros and Gowa.

Extraction of pollen and honey using maceration method using 96% ethanol liquid. Ethanol is an organic solvent that can attract most of the bioactive compounds found in pollen and honey because it has a high polarity, also based on the extraction of phenolic compounds such as flavonoids and tannins from plant tissues using ethanol as a solvent at room temperature by maceration.

Wavelength Measurement

Ultraviolet-Visible (UV-Vis) spectrophotometer is an analytical instrument that belongs to absorption spectroscopy. Radiation or light is passed through a colored solution, then radiation with a certain wavelength will be absorbed selectively and other radiation will be passed on (Syaifuddin, 2015). The results of the wavelength measurement are presented in Figure 1 below:

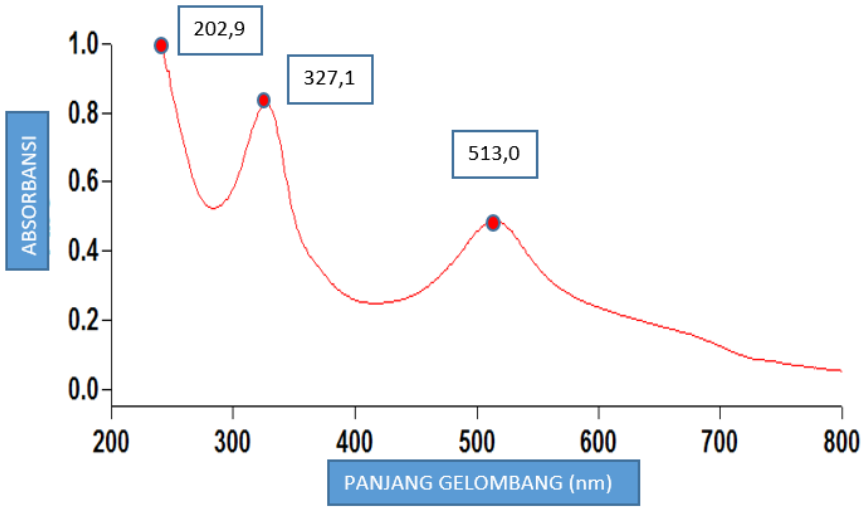

Figure 1. Determination of the Maximum Wavelength of DPPH Solution using a Spectromotometer.

Spectrophotometer (UV-Vis) is based on the absorption of visible light by a colored solution. This method is also known as the colorimetric method, because only the colored solution can be determined by this method. Colorless compounds can be made colored by reacting them with reagents that produce colored compounds (Syaifuddin, 2015). The light in question is in the form of visible light, UV and infrared while the material is in the form of atoms and molecules but the more important role is the valence electrons (Chalid, 2012).

Determination of the maximum wavelength aims to determine the wavelength that has maximum absorption, that is, when the colored compound formed has been optimum so that maximum sensitivity is obtained. The wavelength of the sample was carried out using a solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) with a solution of 100 l and the data from the analysis of the wavelength used was 513 nm.

Determination of Antioxidant Vitamin C

The mechanism of ascorbic acid as an antioxidant is by reducing DPPH free radicals by donating hydrogen atoms to produce L-ascorbate radicals. The L-ascorbic acid radical will immediately turn into L-ascorbyl radical and dehydro L-ascorbyl acid. The radicals formed are stable. This is due to the ability of radicals to stabilize themselves by resonating (Latifah, 2015).

Charkraborty (2011) said, vitamin C is one of the important non-enzymatic antioxidants, which react with free radicals to form their own radicals which are less reactive than these radicals. They break radical chain reactions by trapping peroxyl and other reactive radicals. Vitamin C is essential for the biosynthesis of collagen, carnitine and neurotransmitters. It is possible that this vitamin is consumed in the process of lipid peroxidation caused by oxygen radicals in reperfusion injury by ischemia to prevent tissue damage. Vitamin C can be found in most fruits, especially citrus fruits and vegetables. Vitamin C is found in tomatoes, eggplant, potatoes, chilies and red peppers. The fennel tribe (Apiaceae), the pumpkin tribe (Curcubitaceae), Brussels sprouts and broccoli are also rich in vitamin C.

Vitamin C has a free hydroxy group that acts as a free radical scavenger and if it has a polyhydroxy group it will increase antioxidant activity. Samples of vitamin C or ascorbic acid

are positive controls that are used as comparisons and standards for samples tested to determine the potential for antioxidant activity in a sample (Julizan et al., 2019) because they function as secondary antioxidants that counteract extracellular free radicals. The results of the analysis of antioxidant levels from the spectrophotometer are shown in Figure 2, as follows:

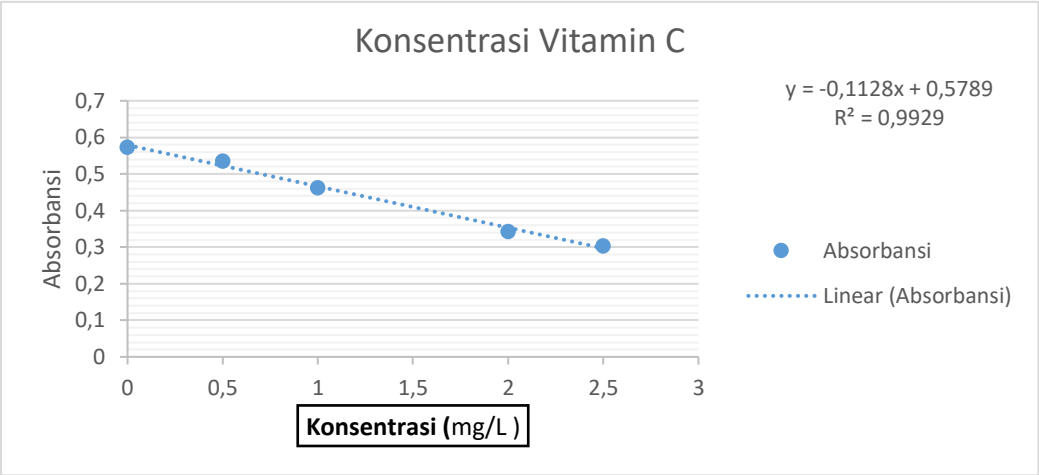

Figure 2. Concentration of Vitamin C

Figure 2 shows the concentration of vitamin C tested using a UV Vis spectrophotometer. The concentration value of vitamin C was 86.565 g/g. The use of positive control in testing this antioxidant activity is to determine how strong the antioxidant potential is in the pollen and honey bee extracts of *Trigona* spp from 3 locations Selayar, Maros and Gowa when compared with vitamin C.

Determination of Pollen and Honey Antioxidant Levels

The value of the antioxidant content of the sample was calculated by taking pollen and honey samples from three different locations, namely Selayar, Maros and Gowa. The method used is the DPPH method because it is simple, easy, fast and sensitive and only requires a small sample. The mechanism of action of the DPPH method is that antioxidant compounds will react with DPPH radicals through the mechanism of hydrogen atom donation and cause DPPH color decay from purple to yellow which is then measured at the maximum wavelength. In this study, a wavelength of 513 nm was used to see the antioxidant levels of pollen and honey from 3 different locations, the results of antioxidant analysis using a UV Vis spectrophotometer are shown in table 1, as follows:

Table 1. Scan Analysis Report Results of Pollen and Honey Samples from UV Vis Spectromotometer

Sample	Concentration mg/L	F Readings
Pollen Gowa	0.8000	0.4886
Pollen Maros	0.7028	0.4996
Pollen Selayar	0.7296	0.4965
Madu Gowa	1.3018	0.4320
Madu Maros	0.8649	0.4813
Madu Selayar	0.9662	0.4698

The reduced color intensity of the DPPH solution can indicate that there is a reaction between the hydrogen atoms released by the test material and the DPPH radical molecule to form a yellow compound 2,2-diphenyl-1-picrylhydrazine. According to Widyaningsih (2010), the greater the concentration of the test material, the stronger the yellow color produced.

The absorbance test of free radical immersion was carried out on honey and pollen extracts as a result of the absorbance obtained from the UV Vis spectrophotometer in Table 4 and then the antioxidant levels were calculated in grams. The level of antioxidants contained in the sample extract shows its antioxidant ability, the more antioxidants contained in a sample, the higher the sample's ability to capture free radicals. Free radicals actively bind electrons so that they can cause a lot of damage to cells so that they can cause many degenerative diseases. The process of making ethanolic extracts of pollen and honey samples was carried out without heating this was done to avoid damage to antioxidant compounds so that they could not function to inhibit DPPH free radicals optimally. The level of antioxidant activity is influenced by various factors, including its easily damaged when exposed to oxygen, light, high temperatures, and drying (Putri and Nurul, 2015).

Comparison of Pollen Antioxidant Levels

After the DPPH test using a UV-Vis spectrophotometer on honey and pollen samples was obtained, the absorbance value of the sample was obtained, this absorbance value was then used to determine the levels of antioxidants contained in each gram of the sample tested. The results of the antioxidant levels obtained are shown in figure 3 below:

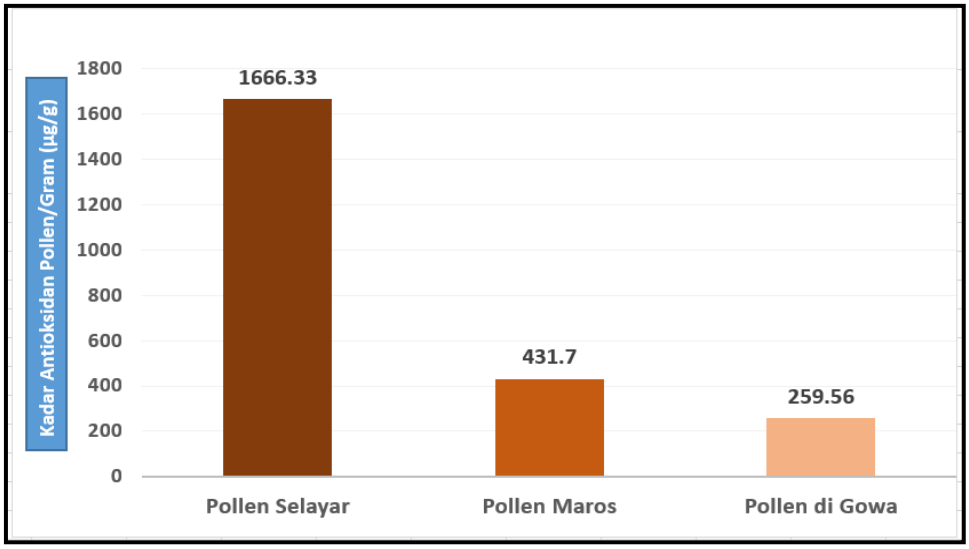

Figure 3. Comparison of Pollen Antioxidant Levels from Several Locations.

Comparison of pollen from these three locations can be seen in figure 3. The antioxidant levels in pollen samples from the Selayar location were 1666.33 µ/g, Maros pollen 431.70 µ/g and Gowa pollen 259.56 µ/g. These three samples had higher antioxidant values than vitamin C, which was 86.565 µ/g. This shows that the pollen sample has a higher anti-radiation ability than vitamin C. From figure 3 it can also be seen that the pollen from the Selayar location has higher antioxidant levels than the other two locations, namely pollen from the Gowa and Maros locations.

Comparison of Honey's Antioxidant Levels.

Honey from the three locations of Selayar, Maros and Gowa was tested for DPPH using a UV-Vis spectrophotometer and the absorbance value of the sample was obtained which was then used to determine the level of antioxidants contained in each gram of the sample tested. The results of the antioxidant levels obtained are shown in figure 4 below:

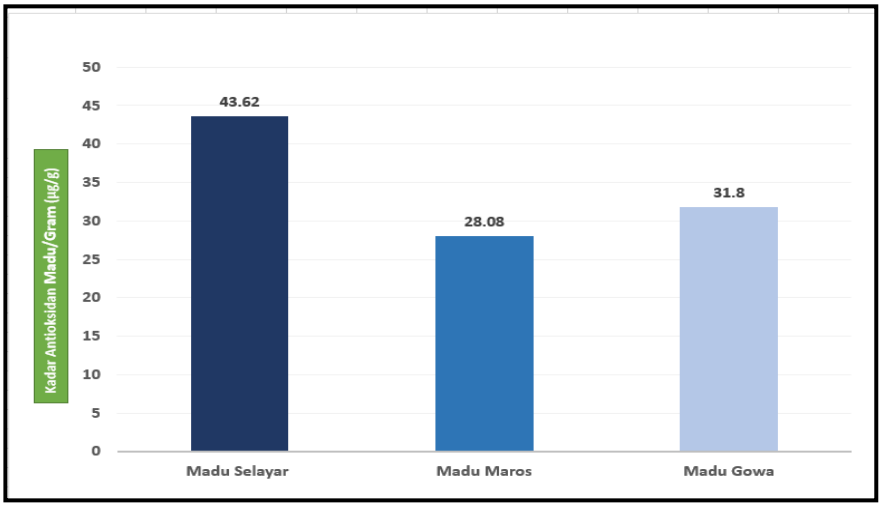

Figure 4 . Comparison of Antioxidant Levels of Honey from Several Locations

Figure 4 shows the antioxidant content of honey from Selayar, Gowa and Maros locations. Antioxidant levels of Selayar Honey are 43.62 µ/g, Gowa honey 28.08 µ/g and Maros honey 31.80 µ/g. From the results obtained, it can be seen that the highest antioxidant content is found in honey from the location in Selayar. The results of the antioxidant values of the three samples obtained showed that all samples had antioxidant values that were below the antioxidant value of vitamin C of 86.565 µ/g. This shows that the ability to scavenge free radicals in honey samples is lower than vitamin C.

Determination of Locations that Produce the Highest Pollen and Honey from Selayar, Maros and Gowa Locations.

From the test of antioxidant levels of pollen and honey from three locations, namely Selayar, Gowa and Maros, it can be seen that there are differences in the antioxidant content of pollen and honey produced by Trigona spp bees. of these three locations.

Table 2. Calculation of Pollen and Honey Antioxidant Levels from Several Locations in South Sulawesi.

No.	Sample Type	Selayar	Maros	Gowa	Average
1.	Polen	1666,33 µg/g	431,70 µg/g	259,56 µg/g	785,86 µg/g
2.	Madu	43,62 µg/g	28,08 µg/g	31,80 µg/g	51,75 µg/g

Discussion

The results of the average antioxidant value obtained when compared to all honey samples from the three locations had a honey antioxidant content value of 51.75 g/g, much lower than the pollen sample from the three sampling locations which was 785.86 g/g as shown in table 5. This shows that the antioxidant content of pollen may vary at each location depending on the type of plant feed consumed by Trigona spp bees. The chemical composition of feed plants affects the content of pollen and honey produced. The chemical content of its complex and diverse composition makes bee pollen has various properties, one of which is as an antioxidant (Fiergiyanti, 2015).

This is in accordance with previous research conducted by Idris (2017) where from 5 parts of beehives tested for their antioxidant levels, the highest to lowest antioxidant content were honey bag extract, egg bag extract, propolis extract, pollen bag extract and activity The lowest antioxidant was found in honey extract. Although low, honey still contains antioxidants that can be beneficial if consumed.

Conducted a study and claimed that in bee pollen there are polyphenols (flavonoids and folic acid) with a large capacity that have the potential as antioxidants, while in a study conducted by Cahyaningrum (2019) the value of free antiradical activity comes from several antioxidant compounds. such as flavonoids, vitamin E, vitamin C, beta carotene, phenolic acids and so on.

The distance of the farm from available feed also affects the frequency of harvest. Nunes *et al.* (2010) also found that the larger the size of the bee, the greater its flight ability. The body size of the bee Trigona spp. which reach 5 mm can cover a distance of up to 600 m in search of food sources. So that the quality of the antioxidant value of the surrounding feed which is in the flying radius of the Trigona spp bees. greatly affects the content of the collected feed in the form of honey and pollen which can be consumed directly by humans.

Sources of honey bee feed are plants which include fruit crops, vegetable crops, ornamental plants, food crops, forest plants, and plantation crops. These plants contain nectar and pollen which are very influential in the production of honey that will be produced by honey bees. In this case the forest becomes a home and nesting place and a source of food for honey bees, while bees help in the process of pollinating forest plants, and produce honey for the community (Marhiyanto, 2013).

The highest yield of antioxidant pollen and bee honey Trigona sp. obtained from the Selayar location. Where the data on potential feed plants collected around the farm from 3 locations shows that the Selayar location is dominated by food sources originating from large trees which generally only live in the forest, because the location of the farms is in the forest. In contrast to the other 2 locations, which are located in plantations and rice fields, the location is not too far from residential areas, with plants that have the potential as a source of food from both Maros and Gowa locations which are almost the same, namely in the form of fruit trees.

The difference in altitude where the Trigona bees live causes differences in several different types of pollen collected due to differences in plant species that can grow at certain heights (Pratama *et al.* 2013). The location of Selayar in Kalepadang village is in the Bontoharu sub-district with an altitude of 350-600 meters above sea level, which is a plateau in the form of undulating hills. Pollen and honey samples from Selayar were taken from Kalepadang village, where this village is located near a forest that has various types of flowering tree species whose forests are still maintained due to the absence of logging activities. There are many sugar palm and coconut trees which are the preferred food for

Trigona spp *bees*. which blooms all year round. Almost all types of flowering plants can be a source of food for bees, but there are several types of flowering plants that produce toxic compounds, so they are not visited by bees and insects in general (Adler, 2000). In addition, plants that are sprayed with disinfectants and other chemicals cannot be visited or can be used as a source of food by bees.

Honey is taken from plant fluids, namely nectar and pollen collected by bees from pollen or pollen produced by flowering plants. Soluble substances in nectar, namely water, ions, carbohydrates, amino acids, and nectar also contain fragrant compounds to attract pollinators' attention and nectar enzymes and antioxidants (Carter and Thornburg, 2004).
The antioxidant content of pollen and bee honey is influenced by the feed consumed. In a study conducted by Cahyaningrum (2019), honey with different types of flowers, namely farm honey and longan honey, had different anti-free radical activities. Where the anti-free radical activity in longan honey was greater, namely 82.10% compared to animal honey, which was 69.37%.

In a study conducted by Rahmi (2017), it can be seen that the antioxidant activity of different fruits is due to the presence of different secondary metabolites. Antioxidant activity shows that avocado has tannin which is an active compound with antioxidant properties, strawberries and dragon fruit have antioxidant activity because they contain anthocyanin compounds. Jamblang fruit contains flavonoids, also contains several other polyphenolic compounds such as tannins.

The antioxidant capacity of a material is influenced by the components in the material that are able to act to inhibit oxidation (Ariviani and Parnanto, 2013). The content of these antioxidants has differences due to geographical differences or the origin of the bees and the time of harvesting. The antioxidant activity is influenced by the value of flavonoids contained in honey and pollen. Flavonoids are one of the components of phenolic compounds which are natural antioxidants derived from plants.

The content of flavonoids contained in the product produced by bees is influenced by the origin of the plant as raw material. The chemical composition of propolis depends on the specificity of the local flora at the collection site (Jaya, 2017). The most abundant plants in the Selayar location are coconut (Cocos nucifera) types of coconut fruit which are known to be rich in antioxidants in the flesh, coconut water, coir, to the processed product, namely coconut milk. Coconut fruit can be used for treatment and beauty because it contains chemical compounds such as polyphenols, flavonoids, tannins, steroids, triterpenoids. These compounds, especially polyphenols and flavonoids, can be used as an alternative source of natural antioxidants (Lima, *et al*., 2015). From the research conducted by Jauziyah et al., (2019), it was found that coir extract and coconut pulp have potential as antioxidants with strong antioxidant activity seen from the IC50 value. The IC50 value of coconut coir extract is 63.95 ppm and for coconut pulp extract is 95.44 ppm, it is known that coconut coir extract has a higher antioxidant potential than coconut pulp extract.

Conclusion

The antioxidant content of pollen extract from Selayar is 1666.33 µ/g, Maros pollen is 431.70 µ/g and Gowa pollen is 259.56 µ/g and antioxidant content of honey from Selayar is 43.62 µ/g, Maros honey is 28,08 µ/g and Gowa honey 31.80 µ/g. *Trigona* spp pollen and honey. from Selayar had the highest antioxidant content of the three sampling locations.

Acknowledgments

The author expresses his deepest gratitude for the financial support from the Hasanuddin University campus.

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