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Proximate Analysis and Antioxidant Activity of Kelulut (*Heterotrigona itama*) Honey from Peat Land Forest, South Kalimantan

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ABSTRACT

Honey is a source of food commonly consumed by people as a medicinal substance. This study aims to analyze the proximate composition of Kelulut (*Heterotrigona itama*) honey and its antioxidant activity. Kelulut Honey was collected from the peat land forest of subdistrict of Landasan Ulin Utara (Laura), Banjarbaru, South Kalimantan, Indonesia. The proximate analysis was done according to standard methods, while the antioxidant activity was evaluated using the hydroxyl radical scavenging, ferrous iron chelating, and hydrogen peroxide scavenging activity. The results revealed that Kelulut Honey contains proteins, carbohydrates, fats, calories, fibers, carotenoids, lycopene, and vitamin C. Kelulut Honey has antioxidant activity as shown by a high hydroxyl radical scavenging activity with percentage radical scavenging activity of $42.673 \pm 1.028\%$. The metal chelating effect and hydrogen peroxide radical scavenging activity were also remarkable, with percentage activity of $8.431 \pm 1.393\%$, and $5.508 \pm 0.744\%$, respectively.

Keywords: Kelulut Honey, Proximate analysis, Antioxidant activity, Peat Land, Banjarbaru

Introduction

Oxidation is a chemical reaction which produces free radicals that can cause a chain reaction leading to cellular damage. The human body constantly produces free radicals due to the regular use of oxygen for metabolic processes. These free radicals are implicated in a number of health challenges resulting in diseases such as cardiovascular disorders, diabetes, macular degeneration, and cancers.¹ This oxidation reaction can be mitigated by antioxidant compounds. Antioxidants are chemicals which can be found naturally in foods that prevent, or decrease the oxidative stress of the physiological system. Some of these antioxidants are free radical scavengers that incredibly help prevent and repair the damaged cells caused by these free radicals.^{1,2} Honey is a rich source of natural antioxidants, and plays a major role in health maintenance and disease prevention.³ Honey contains various kinds of nutrients required by the human body, it is used as raw material in the preparation of medicinally useful products for the treatment of wound, as anti-cancer medicine, antimicrobial agents, and as supplements used by pregnant women.⁴

The antioxidant activity of honey varies, depending on the flower source. Some influential external factors that affect the composition and medicinal effect of honey are season, environment, and the cultivation methods.^{5,6} Stingless bee honey has good nutritional content which are useful in the management of oxidative stress-related diseases.⁷ There are many studies on the chemical composition and antioxidant activity of honey from different regions of the world, such as the ones from Bangladesh,⁸ Malaysia,⁹ Sicilia,¹⁰ Brazil,¹¹ and Jordan.¹²

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Many consumers take into account the honey quality and its composition, and as a result, honey producers ensure that the quality and the composition of their honey are well-guaranteed.¹³ One of honeybee kinds is Kelulut (*Heterotrigona itama*). Its meliponiculture has begun to spread, and its being developed in many regions across Indonesia including South Kalimantan, Indonesia, since 2014, by individuals and groups.¹⁴ It is now widely practiced in almost all regions of South Kalimantan. For example, Landasan Ulin Utara (Laura) Subdistrict, Banjarbaru, which have a peat land forest serves as one of the major areas of meliponiculture in South Kalimantan.¹⁵ In some other countries, the researches on Kelulut Honey's composition and its benefits are still few.¹⁶ Therefore, this research intends to find out the composition and the antioxidant activity of Kelulut Honey produced in its meliponiculture site in Landasan Ulin Utara Subdistrict, Banjarbaru, South Kalimantan, Indonesia.

Materials and Methods

Collection of Honey Samples

Honey samples were collected in October 2021 from Peat Land Forest in Landasan Ulin Utara Subdistrict, Banjarbaru, South Kalimantan, Indonesia ($3^{\circ}24'33.07''S$ & $114^{\circ}42'51.44''E$) (Figure 1). The samples of honey collected and used for the study were those produced within one month.

Proximate Characteristics

Proteins

Following the AOAC official technique 991.20, 2005, the protein content was calculated based on the total nitrogen content using the Kjeldahl method.

Fats

The fat content was calculated using acid hydrolysis method based on the AOAC Official Method 14.019, 1984.

Fibers

Based on AOAC Official Method 991.43, 1991, the dietary fibers of the honey samples, which include the total, soluble, and insoluble fibers, were calculated.

Carbohydrates

Carbohydrates value was estimated from equation 1 below.¹⁷

$$\text{Total carbohydrate (g/100 g)} = 100 - (\text{water} + \text{ash} + \text{protein} + \text{fat} + \text{dietary fiber}) \text{----- (1)}$$

Calories

The constant energy factor is used to calculate the energy value of kelulut honey, namely 38 kJ/g (9 kcal/g) for fat, and 17 kJ/g (4 kcal/g) for both protein and carbohydrate on a dry weight basis (Equation 2).¹⁷

$$\text{Energy (kcal/g)} = 9 (\text{fat}) + 4 (\text{protein}) + 4 (\text{carbohydrate}) \text{----- (2)}$$

β -Carotene and Lycopene

β -carotene and lycopene were tested based on the method previously reported by Nagata and Yamashita (1992). The dried methanol extract (100 mg) was shaken strongly together with a 10 ml-mixture of acetone-hexane (1:1) for one minute and filtered by using a filter paper Whatman No. 4. The filtrate absorbance was measured at 453, 505, 645 and 663 nm. The contents of β -carotene and lycopene were estimated based on the following equations:

$$\text{Lycopene (mg/100 mL)} = -0.0458 A_{663} + 0.372 A_{505} - 0.0806 A_{453} \text{----- (3)}$$

$$\beta\text{-carotene (mg/100 mL)} = 0.216 A_{663} - 0.304 A_{505} + 0.452 A_{453} \text{--- (4)}$$

The test was done in triplicates, and the results were expressed as mg of carotenoid extract/g.¹⁸

Vitamin C

Ascorbic acid was estimated following the method of Klein and Perry (1982). The dried methanol extract (100 mg) was extracted with 10 mL of 1% methaphosphoric acid for 45 minutes at room temperature and used Whatman No. 4 filtering paper to sift filtrate (1 mL) was mixed with 9 mL 2,6-dichlorophenolindophenol. The mixture was incubated at room temperature for 30 minutes, after which the absorbance was measured at 515 nm. The content of ascorbic acid was estimated from a calibration curve of authentic L-ascorbic acid (0.020-0.12 mg/mL) and expressed as mg ascorbic acid/g of extract.¹⁸

Antioxidant assays

Hydroxyl Radical Scavenging Activity

The scavenging activity of hydroxyl radical was determined by using Fenton reaction.¹⁹ The absorbance of the mixture was measured at 560 nm using spectrophotometer. The scavenging was estimated from the equation below.

$$(1 - \text{sampling absorbance} / \text{controlled absorbance}) \times 100 \text{----- (5)}$$

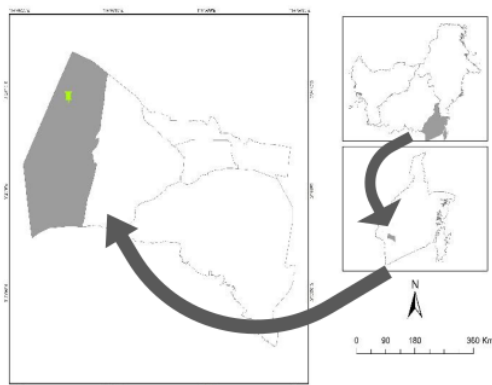


Figure 1: Location of Meliponiculture
Chelating Effect of Ferrous Iron

The method of Hung-Jo Chou *et al.* was used to determine the chelating effect of ferrous iron.²⁰ At 562 nm, the mixture's absorbance was measured. The chelating effect was calculated using the following equation:

$$(1 - \text{sampling absorbance} / \text{controlled absorbance}) \times 100 \text{----- (6)}$$

Hydrogen Peroxide Scavenging Activity

The method suggested by Ruch *et al.*²¹ was used to measure the scavenging activity of hydrogen peroxide. The absorbance of the reaction mixture was measured at 230 nm. The following equation was used to determine the scavenging activity:

$$(1 - \text{sampling absorbance} / \text{controlled absorbance}) \times 100 \text{----- (7)}$$

Statistical analysis

All experiments used 3 repetitions. The data are presented in the form of mean value with a standard deviation (SD).

Results and Discussion

Proximate parameters

Proximate analysis was aimed at obtaining the quality and nutrient contents in Kelulut Honey. The parameters assessed include proteins, carbohydrates, fats, fibers, calories, and vitamin C contents. This analysis was carried out in order to get accurate information which later can be used for listing the nutrition facts on the label of food packaging. The result of the proximate analysis of the honey produced by Laura Kelulut stingless bees is presented in Table 1.

Proteins

The result of the analysis showed that Kelulut Honey contains $0.16 \pm 0.01\%$ protein. Compared to Trigona Honey of South Sulawesi and Trigona of Malaysia which have different protein contents, Trigona of South Sulawesi contains 0.03% protein,²² while Trigona of Malaysia contains 0.26% protein.²³ On the other hand, Apis honey contains 0.5% protein consisting of free amino acids and enzymes.⁵ The percentage of protein contents may differ among different kinds of honey due to the sources of nectar consumed by the bees.

Fats

The amount of fats contained in Kelulut Honey was found to be $0.01 \pm 0.01\%$. This is smaller than the fat content in Trigona Honey of South Sulawesi which contains 0.06% fat, but higher than Apis Honey which had no fat.⁵ Many kinds of food sources in Indonesia may contain up to 10% or more of crude fat, but honey generally has lower fat content. Thus, it is rare to find any source and reference stating about fat content in honey.

Carbohydrates/Reducing Sugars

The result of the analysis showed that Kelulut Honey contain $70.7 \pm 0.65\%$ of carbohydrates. This value was consistent with the SNI (Indonesian National Standard) standardized in 2018 which required the minimum carbohydrates content of 65%. Based on the observation of Biluca *et al.*,²⁴ Kelulut Honey of Brazil contains between 48.0% - 70.5% of carbohydrates, while the one of Sulawesi contain 49.68%,²² Kelulut Honey of Malaysia has $68.53 \pm 1.39\%$ ²⁵ and East Kalimantan's Kelulut Honey contains 51.58% - 63.68% carbohydrate.²⁶ The major benefit of carbohydrates is that it acts as the energy source in human body. The findings from this study indicate that honey can be consumed as a source of energy because of its high carbohydrates content as carbohydrates or sugars is the major composition of honey.²⁷

Fiber

Fiber is a substance in certain food which has a role in facilitating food digestion. Consuming foods with high content of fiber is highly recommended by many experts. Fiber is also found in honey, in a small amount. From the result of the proximate analysis, Kelulut Honey contains $1.52 \pm 0.08\%$ of fiber. When compared with the fibre content observed in other studies, this content is low. For example, Apis

mellifera honey from Nigeria contains $1.41 \pm 0.05\%$ fiber.²⁸ Cultivated honey and wild honey of *Apis* sp. can have different fiber content. Cultivated honey has a fiber content of 1.99% whereas wild honey has a fiber content of $2.76 \pm 1.07\%$.²⁹

Calories

The energy in food sources which is derived from carbohydrate oxidation, fats, and proteins stated as heats is called calories. The total number of calories in Kelulut Honey is 283.53 ± 0.98 cal. Another research mentioned that *Apis* honey has calories of 330 kcal/100 g.³⁰ Thus, honey contains high energy, and the sugar in honey is easily digested and it has benefits like the one found in fruits. The above observations suggest that honey is a rich source of energy especially for babies and teenagers.

Lycopene

Lycopene is a carotenoid pigment, often found in fruits and vegetables. Lycopene is a substance contained in honey. The result of the analysis showed that the lycopene content in Kelulut Honey is 2.899 ± 0.185 $\mu\text{g/mL}$. This value is different from Malaysian Kelulut Honey with a value of 0.65 - 1.52 mg/kg.⁹ Lycopene is known phytochemical with strong antioxidant activity.

Carotenoids

Carotenoids are type of organic nutritional compounds found in plants or animals as pigments. The carotenoids content in peat land Kelulut Honey of Banjarbaru was found to be 0.617 ± 0.001 mg/kg. This amount is lower than that of Malaysian Kelulut Honey which had a carotenoids content of 4.61 mg/kg.⁹ Differences in season and type of bee forage might affect the carotenoids content. Dry season and varied bee forage contribute to high carotenoids content.³¹ The important benefit of carotenoids is that it act as antioxidant agent. Besides, it can be transformed into essential vitamins.⁹

Vitamin C

Vitamin C (Ascorbic acid) is provided naturally in foods including honey. Vitamin C is one of the antioxidants needed in human body, it help protect the body from the damaging effects of free radicals. The analysis result of Vitamin C contained in Kelulut Honey was 14.08 ± 0.00 mg/mL. This value differs from that of Apis Honey which contained 2.2 - 2.5 mg/100 g of vitamin C,⁵ while Kelulut Honey of Sulawesi contained 302.85 $\mu\text{g/g}$ of vitamin C.²²

Antioxidant activity

Antioxidants are compounds naturally available in many kinds of food including honey. They are able to obstruct or neutralize the breakdown caused by oxidative processes. Antioxidant is beneficial in that it protects human body from the effects of free radicals. When the body accumulates a large amount of free radicals, it may result in cellular destruction which potentially causes many kinds of diseases.³²

In this study, Kelulut Honey of Banjarbaru peat land was evaluated for its antioxidant potential *vis a viz* its free radical scavenging activity against peroxide and hydroxyl radical as well as its metal chelating ability. The results showed that Kelulut Honey had the ability to scavenge peroxide and hydroxyl radicals with scavenging activity of $5.508 \pm 0.744\%$ and $42.673 \pm 1.028\%$, respectively, and metal chelating activity of $8.431 \pm 1.393\%$. However, these values are lower than that of Kosovo Honey.³³ Plant sources affect the nutritional content of honey.⁸ For example, high antioxidant activity can be attributed to the polyphenol content in honey.³⁴ Hydroxyl radical is the most harmful radical because it has the highest rate of reactivity. Hydroxyl radical with its high reactivity is usually dangerous for living cells.³⁵ It is different from superoxide radical in that the toxic effect of superoxide radical can be neutralized by superoxide dismutase, whereas enzymatic reaction cannot eliminate the hydroxyl radical. All types of macromolecules such as amino acids, lipids, nucleic acids, and carbohydrates, are susceptible to damage by hydroxyl radicals.^{1,2} Antioxidants are known as fantastic scavengers for free radicals. Cellular damage due to free radicals can be prevented and repaired by antioxidants.² The results from the present study explained why honey has been used by traditional communities as a healthy food supplement.

Table 1: Proximate Parameter of Kelulut Honey from Peat Land Forest, Landasan Ulin Utara Village

No.	Parameter	Mean value \pm SD
1	Protein (%)	0.16 ± 0.01
2	Fats (%)	0.01 ± 0.01
3	Carbohydrate (%)	70.70 ± 0.65
4	Fiber (%)	1.52 ± 0.08
5	Calories (cal)	283.53 ± 0.98
6	Lycopene ($\mu\text{g/mL}$)	2.899 ± 0.185
7	Carotenoids (mg/kg)	0.617 ± 0.001
8	Vitamin C (mg/mL)	14.08 ± 0.00

Table 2: Antioxidants activity of Kelulut Honey from Peat Land Forest, Landasan Ulin Utara Village

No.	Parameter	Mean value \pm SD (%)
1	Hydrogen Peroxide Scavenging Activity	5.508 ± 0.744
2	Hydroxyl Radical Scavenging Activity	42.673 ± 1.028
3	Metal chelating Activity	8.431 ± 1.393

Conclusion

The results of the present research indicate that Kelulut Honey cultivated in Peat land, Landasan Ulin Utara (Laura) Subdistrict, Banjarbaru, has high proteins, carbohydrates, fibers, carotenoids, lycopene and vitamin C contents, but low fats content. This nutritional composition might have played a significant role in its antioxidants activity, in the form of hydroxyl radical scavenging activity, ferrous iron chelating effect and hydrogen peroxide scavenging activity.

3

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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