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

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ABSTRACT
Honey is food source which is consumed by many people as a health agent. This research aims to analyze the proximate composition of kelulut honey and its antioxidant activity. Kelulut honey cultivated in Landasan Ulin Utara (Laura) village, Banjarbaru city is typically peat land meliponiculture. The parameter of proximate includes the analysis of some particular contents; they are protein, carbohydrate, fats, calories, fibers, β-carotenes, lycopene, and vitamin C. The antioxidants activity on the other hands uses the parameter of hydroxyl radical scavenging, ferrous iron chelating effect and hydrogen peroxide scavenging activity. From the research it can conclude that the result shows honey has potentially worthy antioxidants source. Kelulut honey contains protein, carbohydrate, fats, calories, fibers, β-carotenes, lycopenes and vitamin C.

Keywords: Kelulut Honey, Proximate, Antioxidants, Peat Lands, Banjarbaru

Introduction

Oxidation is a chemical reaction resulting in free radical that causes chain reaction to damage the organism's cells. Human's body constantly can produce a free radical because the use of regular oxygen. This free radical is responsible for the body cells damage and gives some disadvantageous effect for any health problems, such as heart disease, diabetes, macular degeneration, and cancer[1]. This oxidation reaction can be avoided by antioxidant compounds. Antioxidants are chemical substance class which can be found naturally in our foods that prevent us from or decrease oxidative stress of physiologic system. Antioxidants might be the free radical scavengers that incredibly help prevent and repair the damaged cells caused by this radical[1,2].
Natural antioxidant sources can be obtained in honey. The honey's roles in the health world have been stated by many experts. Honey is wellknown as an appropriate antioxidant source which effectively reduces any sorts of diseases[3]. Honey contains vary of nutrition required by humans' body. Hence, honey is used at most as a raw material in health, some of which are scientifically proven for wound treatment, anti- cancer medicine, antimicrobials, and health treatment of pregnant women[4].
The antioxidants activity of honey varies, which depends on the flower source. Some external factors that influence are season, environment, and the cultivation methods[5,6]. Stingless bee honey has good nutritional content and can be used for oxidative stress-related disease therapy[7]. There have been many researches accomplished to talk about the honey's composition and its antioxidants activity such as research from Bangladesh[8], Malaysia[9],

Sicilia[10], Brazil[11], and Jordan[12]. Many consumers take into consideration on the honey's quality and composition. By doing so, honey producers are required to ensure that its quality and composition are well guaranteed[13].
One of bees producing honey is kelulut (*Heterotrigona itama*). Its meliponiculture has begun to spread out being developed, including in South Kalimantan, Indonesia, since around 2014, by individuals and groups[14]. It is widely in almost all regions in South Kalimantan. Landasan Ulin Utara Village, Banjarbaru City, which is one of peat land forest in South Kalimantan, is one of many places becoming a sample area of meliponiculture[15]. At first, it was still focused to how to develop the honey production continuously. In some other countries, there are still a few researches about kelulut honey composition and its benefits[16]. This research intends to find out the composition and antioxidants activity of kelulut honey produced in its meliponiculture site in Landasan Ulin Utara Village, City of Banjarbaru, South Kalimantan, Indonesia.

Materials and Methods

The Collection Method of Honey Samples
The samples are taken from Peat Land Forest in Landasan Ulin Utara (Laura) Village, Banjarbaru City, Indonesia. The samples of honey examined are those that are currently produced with the period of one month storage.

Proximate Characteristics
Protein
The protein content was determined by Kjeldahl method based on the total nitrogen content from the AOAC Official Method 991.20, 2005.

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

Carbohydrate
Carbohydrate value was estimated from Equation 1[17].

Fiber
The dietary fiber consisted of the total, soluble and insoluble fiber of honey samples were determined based on AOAC Official Method 991.43, 1991.

Calories
The energy values for the honey samples were calculated by using constant energy factors, 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].

Total carbohydrate (g/100 g) = 100 – (water + ash + protein + fat + dietary fiber) (1)
Energy (kcal/g) = 9 (fat) + 4 (protein) + 4 (carbohydrate) (2)

β-carotene and Lycopene
β-carotene and lycopene were tested based on the method which was ever used by Nagata and Yamashita (1992). The dried methanol extract (100mg) was shaken strongly together with a-10 ml-mixture of acetone-hexane (4:6) for one minute and filtered by using a filtering paper Whatman No. 4. The filtrate absorbance was measured at 453, 505, 645 and 663 nm. The contents of β-carotene and lycopene were counted based on the following equation:

lycopene (mg/100 ml) = -0,0458 A663 + 0,372 A505 - 0,0806 A453; β-carotene (mg/100 ml) = 0,216 A663 - 0,304 A505 + 0,452 A453. The test was held on a-three-ply examination; the result showed that the average values ± standard deviations (SD) was stated as mg of carotenoid extract/g[18].

Vitamin C

Ascorbic acid was specified in accordance with Klein and Perry's Method (1982). The dried methanol extract (100) mg would be extracted with a 10 ml of 1 % methaphosphate acid for 45 minutes in the room temperature and filtered through Whatman No. 4 filtering paper. Filtrate (1 ml) was mixed with 9 ml 2,6 dichlorophenolindophenol then the absorbance was measured in 30 minutes at 515 nm toward the form. The content of ascorbic acid is counted according to calibration curve of authentic L-ascorbate acid (0,020-0,12 mg/ml). The test was done in triplicate; the result was that the average value ± SD and stated as mg ascorbic acid/g of extract[18].

Antioxidant assays

Hydroxyl Radical Scavenging Activity

The scavenging activity of hydroxyl radical was measured by using Fenton reaction[19]. The mixture absorbance at 560 nm was gauged using spectrophotometer. The scavenging was counted with the equation (1-sampling absorbance / controlled absorbance) × 100. Each experiment was made in triplicate and it resulted in the average amount stated as average ± SD.

Chelating Effect of Ferrous Iron

Chelating effect of ferrous iron was estimated by using the method of Hung-Jo Chou *et al.*[20]. The absorbance mixture was measured at 562nm. The chelating effect was calculated using the equation formula as the following: (1-sampling absorbance/controlled absorbance) x 100. Each of experiment was held triplicate and the average amount was stated as mean ± SD.

Hydrogen Peroxide Scavenging Activity

Hydrogen flusher was specified according to a method of Ruch *et al.*[21]. The mixture reaction of absorbance was recorded on 230nm. The flushing activity was counted with an equation as the following: (1-sampling absorbance/controlled absorbance) x 100. Every experiment was accomplished in triplicate and the mean result was stated as ± SD.

Result and Discussion

Proximate assays

Proximate assays aims to obtain the fact of quality and nutrient containing in honey analyzed to be consumed. The observation parameter used in the test is the contents of protein, carbohydrate, fats, fiber, calories, and vitamin C. This is necessary to get accurate information that is usable to list the nutrition fact labeled in food packaging. The result of proximate assays on honey resulted by Laura Idaman kelulut stingless bees is presented in Table 1.

Table 1. Proximate of kelulut honey from Peat Land Forest, Landasan Ulin Utara Village

No.	Parameter	Value±SD
1	Protein (%)	0.16±0.01
2	Fats (%)	0.01±0.01
3	Carbohydrate (%)	70.7±0.65

4	Fiber (%)	1.52±0.08
5	Calories (cal)	283.53±0.98
6	Lycopene((µg/ml)	2.899 ± 0.185
7	Carotenoids (mg/kg)	0.617 ± 0.001
8	Vitamin C (mg/ml)	14.08 ± 0.00

Protein

The result of the analysis shows that kelulut honey contains of 0.16±0.01% protein. Compared with trigona honey from South Sulawesi, it has different contents of protein where it contains 0.03%[22] and Malaysia 0.26% [23], while *Apis* honey contains 0.5% consisting of free amino acid and enzyme[5]. The difference shown can be caused by the nectar consumed by the bees.

Fats

The analysis of fat contained in kelulut honey is 0.01±0.01%. It is smaller than the fat value in trigone honey from South Sulawesi which contains 0.06%, but higher than Apis honey with 0%[5]. Many kinds of food sources in Indonesia may contain 10% or more of ether extract (fat). The storage can influence the rancidity which may decrease its value. Honey has lower fat rate. Thus, it is rare to find any sources and references stating about fat content in honey.

Carbohydrate / Reducing Sugar

The analysis test results a findings that Kelulut honey contain 70.7±0.65% of carbohydrate observed by using refractometer. This value has been appropriate with the SNI (Indonesian National Standard) standardized in 2018 which required the minimum value of 65%. Based on the observation of Biluca, *et al.*[24], kelulut honey from Brazil contains between 48% - 70.5% of carbohydrate, while one from Sulawesi produces 49.68%[22], Malaysia 68.53±1.39%[25] and East Kalimantan 51.58%-63.68%[26]

The major benefit of carbohydrate is as the energy sources in humans' body. It indicates that honey can be consumed as energy supplier because of its high carbohydrate content. Carbohydrate or sugar is the most composition in honey[27].

Fiber

Fiber is a substance in a certain food whose role is to help digesting process. Consuming food sources with high content of fiber is highly recommended by many experts in the world. Fiber can boost human body's health entirely because it will help the organs working normally. Fiber is also contained in honey, even though in a few amounts. According to the analysis result, Kelulut honey contains 1.52+0,08% of fiber. Compared with the other studies, this content is less. Fiber of *Apis* honey obtained directly from the farmers contains 5.43+0.22% while from the seller is 9.08+0.06%[28]. Farmed honey and natural honey from *Apis* sp. can provide a difference in fiber content. Farmed honey has a fiber value of 1.99% and natural honey 2.76±1.07%[29]

Calories

The energy included in food sources which derive from carbohydrate oxidation, fats, and protein stated with heats is called calories. The total amount of calories in kelulut honey is 283.53±0.98 cal. Honey contains high energy and sugar in honey is easily digested in humans' body as its benefits we often find in fruits. The explanation above indicates why

honey is deeply suggested as a consumption source for babies and teenagers. Another research mentioned that *Apis* honey has calories by 330kcal/100g[30].

Lycopene

Lycopene is carotenoids pigment, which is a phytochemist availabled in fruits and vegetables. Lycopene is a substance contained in honey. The result of analysis shows that lycopene in *kelulut* honey is 2.899±0.185 µg/ml. This value is different from Malaysian kelulut honey (0.65-1.52 mg/kg)[9]. Lycopene is known to be a herb medicine with strong antioxidants. Antioxidant is beneficial to keep body tissue system and organ since it functions as an antidote of free radicals in humans' body. The free radicals substance with large amounts in our body results in cells' destruction which potentially causes many kinds of diseases and many health problems[31].

Carotenoid

Carotenoid is a type of nutrient organic chemical compound that is available in plants' or animals' natural pigment. In Banjarbaru peat land kelulut honey, there found 0.617± 0.001 mg/kg carotenoids. This amount is lower than Malaysian kelulut honey with 4.61 mg/kg[9]. Differences in season and type of beeforage can affect carotenoid content. Summer and varied beeforage resulted in high carotenoid values [32]. The important benefit of carotenoids is as the agent of antioxidants. Besides that, it can be transformed to be essential vitamin[9].

Vitamin C

Vitamin C (Ascorbate acid) is provided naturally in foods consisting of honey. Vitamin C is one of antioxidants needed in humans' body to help protect our body cells from the bad effects of free radicals.

The analysis result of Vitamin C contained in kelulut honey is 14.08±0.00 mg/ml. It is different from Apis honey contained 2.2-2.5 mg/100gr of vitamin C[5] and kelulut honey from Sulawesi contained vitamin C with the amount of 302.85 µg/g[22].

Antioxidants Analysis

Antioxidant is a compound or chemical component that in a particular amounts or contents is able to obstruct or decelerate the breakdowns caused by oxidation process. Antioxidant is beneficial to protect humans' body from the attacks of free radicals. It is a compound that is naturally available in many kinds of food including honey. Free radical is a reactive oxygen compound, known as a compound having unpaired electron[2].

In this research, Banjarbaru peat land kelulut honey can be experimented as an antioxidant at radical peroxide, hydroxyl and metal chelating (Table 2).

Table 2. Antioxidants activity of Kelulut honey from Peat Land Forest, Landasan Ulin Utara Village

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

The results showed that kelulut honey had the ability to scavenging peroxide at 5.508±0.744%, 42.673±1.028% hydroxyl scavenging activity and 8.431±1.393% metal chelating. This value is lower than Kosovo honey[3]. Plant sources affect the nutritional

content of honey[8]. High antioxidant activity can be caused by the polyphenol content in the honey[34]. Hydroxyl radical is the most harmful one because it has the highest rate of reactivity. Hydroxyl radical with its high reactivity is usually dangerous for livings[35]. It is different from superoxide that its toxics can be neutralized by superoxide dismutase. In contrary, radical hydroxyl cannot be eliminated by enzymatic reaction. It is caused by the slower diffusion of active enzyme than its part time. Radical hydroxyl can damage all types of macromolecules such as carbohydrate, nucleate acid, lipid and amino acid[1,2].
Antioxidants being fantastic free radical scavengers help in preventing and repairing the cell damage caused by these radicals[2]. The results also explain why honey has been used by traditional communities as a health supplement daily.

Conclusion

The research result indicates that kelulut honey cultivated in Peat lands, Landasan Ulin Utara Village, Banjarbaru City has a potency of proper proximate which contains high protein, carbohydrate, fibers, β-carotene, lycopene and vitamin C but low fats. This composition then plays a worthy role as beneficial antioxidants, in the form of hydroxyl radical scavenging, ferrous iron chelating effect and hydrogen peroxide scavenging activity.

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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3. Bukti konfirmasi artikel accepted (email dan surat) (31 Mei 2023)

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Trisnu Satriadi <trisnu.satriadi@ulm.ac.id>

TJNPR Editorial Decision

Editor-in-Chief Tjnpr <editor.tjnpr@gmail.com> 31 Mei 2023 pukul 15.59
Kepada: Trisnu Satriadi <trisnu.satriadi@ulm.ac.id>

Dear Dr. Satriadi,

The manuscript submitted to the Tropical Journal of Natural Product Research www.tjnpr.org Q4 https://www.scopus.com/sourceid/21100933230 has been carefully reviewed by competent experts.

Find attached the details of the decision.

Please send your response urgently to the editor-in-Chief, to enable us to process your manuscript for the next issue **Vol 7 issue 6, 2023**.
Kindly acknowledge the receipt of the mail.

Title: Proximate and Antioxidant Activity Analysis of Kelulut (*Heterotrigona itama)* Honey from Peat Land Forest, South Kalimantan
Authors: Trisnu Satriadi*, Mahrus Aryadi, Susilawati, Adistina Fitriani, Badaruddin

TJNPR Editorial Decision: accepts with moderate corrections

Congratulations

Best regards

Abiodun

Professor Abiodun Falodun, PhD; FAAS, FISPON

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Laboratory of Forest Product Technology, Faculty of Forestry, Lambung Mangkurat University, Banjarbaru, South Kalimantan, 70714, Indonesia

Dear Dr Satriadi,

Provisional Acceptance letter for Article Manuscript Number TJNPR MY124ARN

Title: Proximate and Antioxidant Activity Analysis of Kelulut (*Heterotrigona itama)* Honey from Peat Land Forest, South Kalimantan
Authors: Trisnu Satriadi*, Mahrus Aryadi, Susilawati, Adistina Fitriani, Badaruddin

I am pleased to inform you that your manuscript sent to the Tropical Journal of Natural Product Research has been reviewed and recommended for publication as a research article.
However, before the issues raised by the Reviewers are forwarded, to enable you revise your manuscript accordingly, please pay a publication charge of **$ USD270**. The actual publication of the paper will be in the upcoming issue (**Vol 7 issue 6, 2023**).

Please, the manuscript number (**TJNPR MY124ARN**) should be included in the bank transfer.

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Sincerely,

Professor Abiodun Falodun
Editor-in-Chief

4. Bukti konfirmasi review dan hasil review (10 Juni 2023)

Editorial and Reviewer comments

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Editor-in-Chief Tjnpr <editor.tjnpr@gmail.com> 10 Juni 2023 pukul 03.13
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Please see the editorial comments (below) and attached copies of the reviewer comments for manuscript title **" Proximate and Antioxidant Activity Analysis of Kelulut (*Heterotrigona itama)* Honey from Peat Land Forest, South Kalimantan."**

Editorial comments to authors

Title: Names (First and Last name in full, middle name as initials) and affiliations of authors should be written correctly. Correspondence authors' contact address (email and telephone number) should also be stated.

Include date (Month and Year) of sample collection

Materials and Methods: Include section for statistical analysis.

Move all tables under the reference section.

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Adhere strictly to the Journal's style for listing references. Abbreviate all journal names;
Falodun A, Siraj R, Choudhary MI. GC-MS Insecticidal Leaf essential oil of *P. staudtii* Hutch and Dalz (Icacinaceae). Trop J. Pharm Res. 2009; 82:139-143.

Okolie NP, Falodun A, Oluseyi D. Evaluation of the antioxidant activity of root extract of pepper fruit (*Dennetia tripetala*), and its potential for the inhibition of Lipid peroxidation. Afr J. Trad Compl and Altern Med. 2014; 11(3):221-227. Doi: 10.4314/ajtcam. v11i3.31

Grammatical presentation Is poor. Please, manuscript should be submitted for English Language editing.Dr Erharuyi osayemwenre.erharuyi@uniben.edu **assist you.**

All comments/corrections made by reviewers should be completely addressed, point by point, and make appropriate changes in the manuscript, or provide a suitable rebuttal to any specific request for change that has not been made.
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corrections have been made.

Strictly adhere to the author guidelines. Make sure that all the facts and information provided in the manuscript are correct. Check grammar, spelling, spacing, other information and facts including scientific names, formulae, symbols, equations, etc.

Best regards

Abiodun

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Trisnu Satriadi <trisnu.satriadi@ulm.ac.id> 20 Juni 2023 pukul 20.39
Kepada: Editor-in-Chief Tjnpr <editor.tjnpr@gmail.com>

Dear Prof Abiodun,
Please accept our sincere apology that our article is still in the process of being checked. The substantial part has been edited as suggested by reviewers. However, we are still waiting for Dr. Erharuyi's response. We have emailed him asking for his assistance on proofreading and language editing.
We are really sorry for this inconvenience, and we do still expect that our article is going to be accepted if sent after June 20th, 2023.
Once our article is sent back by Dr. Erharuyi, We are to send it to you as soon as possible.

Regards,
Trisnu Satriadi
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Editor-in-Chief Tjnpr <editor.tjnpr@gmail.com> 20 Juni 2023 pukul 21.11
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It will be resolved.

Best regards

Abiodun

Professor Abiodun Falodun, PhD; FAAS, FISPON

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Trisnu Satriadi <trisnu.satriadi@ulm.ac.id> 4 Juli 2023 pukul 15.39
Kepada: Editor-in-Chief Tjnpr <editor.tjnpr@gmail.com>

Dear Prof Abiodun,

I hereby submit the revised manuscript that has been completed based on the guidance of the reviewers and editors. English editing has also been carried out, assisted by Dr Erharuyi (Certificate no: EO/EES/Cert/M002/06/23). The manuscript has also passed the plagiarism check (Certificate no: 885/UN8.1.24/PP/JTAM/2023).
I hope this revised manuscript has answered the requests of the reviewers and editors. Thank you for your understanding.

Regards,
Trisnu Satriadi

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Kepada: Trisnu Satriadi <trisnu.satriadi@ulm.ac.id>

Many thanks for the revised manuscript

Best regards

Abiodun

Professor Abiodun Falodun, PhD; FAAS, FISPON

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

ABSTRACT

Honey is food source which is consumed by many people as a health agent. This research aims to analyze the proximate composition of kelulut honey and its antioxidant activity. Kelulut honey cultivated in Landasan Ulin Utara (Laura) village, Banjarbaru city is typically peat land meliponiculture. The parameter of proximate includes the analysis of some particular contents; they are protein, carbohydrate, fats, calories, fibers, β-carotenes, lycopene, and vitamin C. The antioxidants activity on the other hands uses the parameter of hydroxyl radical scavenging, ferrous iron chelating effect and hydrogen peroxide scavenging activity. From the research it can conclude that the result shows honey has potentially worthy antioxidants source. Kelulut honey contains protein, carbohydrate, fats, calories, fibers, β-carotenes, lycopenes and vitamin C.

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Keywords: Kelulut Honey, Proximate, Antioxidants, Peat Lands, Banjarbaru

Introduction

Oxidation is a chemical reaction resulting in free radical that causes chain reaction to damage the organism's cells. Human's body constantly can produce a free radical because the use of regular oxygen. This free radical is responsible for the body cells damage and gives some disadvantageous effect for any health problems, such as heart disease, diabetes, macular degeneration, and cancer[1]. This oxidation reaction can be avoided by antioxidant compounds. Antioxidants are chemical substance class which can be found naturally in our foods that prevent us from or decrease oxidative stress of physiologic system. Antioxidants might be the free radical scavengers that incredibly help prevent and repair the damaged cells caused by this radical[1,2].

Natural antioxidant sources can be obtained in honey. The honey's roles in the health world have been stated by many experts. Honey is wellknown as an appropriate antioxidant source which effectively reduces any sorts of diseases[3]. Honey contains vary of nutrition required by humans' body. Hence, honey is used at most as a raw material in health, some of which are scientifically proven for wound treatment, anti- cancer medicine, antimicrobials, and health treatment of pregnant women[4].

The antioxidants activity of honey varies, which depends on the flower source. Some external factors that influence are season, environment, and the cultivation methods[5,6]. Stingless bee honey has good nutritional content and can be used for oxidative stress-related disease therapy[7]. There have been many researches accomplished to talk about the honey's composition and its antioxidants activity such as research from Bangladesh[8], Malaysia[9], Sicilia[10], Brazil[11], and Jordan[12]. Many consumers take into consideration on the honey's quality and composition. By doing so, honey producers are required to ensure that its quality and composition are well guaranteed[13].

One of bees producing honey is kelulut (*Heterotrigona itama*). Its meliponiculture has begun to spread out being developed, including in South Kalimantan, Indonesia, since around 2014, by individuals and groups[14]. It is widely in almost all regions in South Kalimantan. Landasan Ulin Utara Village, Banjarbaru City, which is one of peat land forest in South Kalimantan, is one of many places becoming a sample area of meliponiculture[15]. At first, it was still focused to how to develop the honey production continuously. In some other countries, there are still a few researches about kelulut honey composition and its benefits[16]. This research intends to find out the composition and antioxidants activity of kelulut honey produced in its

meliponiculture site in Landasan Ulin Utara Village, City of Banjarbaru, South Kalimantan, Indonesia.

Materials and Methods

The Collection Method of Honey Samples

The samples are taken from Peat Land Forest in Landasan Ulin Utara (Laura) Village, Banjarbaru City, Indonesia. The samples of honey examined are those that are currently produced with the period of one month storage.

Comment [EF3]: State the date of collection
Provide the coordinates of location

The name and affiliation of the scientist who identified the honey

Proximate Characteristics

Protein

The protein content was determined by Kjeldahl method based on the total nitrogen content from the AOAC Official Method 991.20, 2005.

Fats

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

Carbohydrate

Carbohydrate value was estimated from Equation 1[17].

Fiber

The dietary fiber consisted of the total, soluble and insoluble fiber of honey samples were determined based on AOAC Official Method 991.43, 1991.

Calories

The energy values for the honey samples were calculated by using constant energy factors, 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].

Total carbohydrate (g/100 g) = 100 – (water + ash + protein + fat + dietary fiber) (1)
Energy (kcal/g) = 9 (fat) + 4 (protein) + 4 (carbohydrate) (2)

β-carotene and Lycopene

β-carotene and lycopene were tested based on the method which was ever used by Nagata and Yamashita (1992). The dried methanol extract (100mg) was shaken strongly together with a-10 ml-mixture of acetone-hexane (4:6) for one minute and filtered by using a filtering paper Whatman No. 4. The filtrate absorbance was measured at 453, 505, 645 and 663 nm. The contents of β-carotene and lycopene were counted based on the following equation: lycopene (mg/100 ml) = -0,0458 A663 + 0,372 A505 - 0,0806 A453; β-carotene (mg/100 ml) = 0,216 A663 - 0,304 A505 + 0,452 A453. The test was held on a-three-ply examination; the result showed that the average values $\pm$ standard deviations (SD) was stated as mg of carotenoid extract/g[18].

Vitamin C

Ascorbic acid was specified in accordance with Klein and Perry's Method (1982). The dried methanol extract (100) mg would be extracted with a 10 ml of 1 % methaphosphate acid for 45 minutes in the room temperature and filtered through Whatman No. 4 filtering paper. Filtrate (1 ml) was mixed with 9 ml 2,6 dichlorophenolindophenol then the absorbance was measured in 30 minutes at 515 nm toward the form. The content of ascorbic acid is counted

according to calibration curve of authentic L-ascorbate acid (0,020-0,12 mg/ml). The test was done in triplicate; the result was that the average value ± SD and stated as mg ascorbic acid/g of extract[18].

Antioxidant assays

Hydroxyl Radical Scavenging Activity

The scavenging activity of hydroxyl radical was measured by using Fenton reaction[19]. The mixture absorbance at 560 nm was gauged using spectrophotometer. The scavenging was counted with the equation (1-sampling absorbance / controlled absorbance) × 100. Each experiment was made in triplicate and it resulted in the average amount stated as average ± SD.

Chelating Effect of Ferrous Iron

Chelating effect of ferrous iron was estimated by using the method of Hung-Jo Chou *et al.*[20]. The absorbance mixture was measured at 562nm. The chelating effect was calculated using the equation formula as the following: (1-sampling absorbance/controlled absorbance) x 100. Each of experiment was held triplicate and the average amount was stated as mean ± SD.

Hydrogen Peroxide Scavenging Activity

Hydrogen flusher was specified according to a method of Ruch *et al.*[21]. The mixture reaction of absorbance was recorded on 230nm. The flushing activity was counted with an equation as the following: (1-sampling absorbance/controlled absorbance) x 100. Every experiment was accomplished in triplicate and the mean result was stated as ± SD.

Result and Discussion

Proximate assays

Proximate assays aims to obtain the fact of quality and nutrient containing in honey analyzed to be consumed. The observation parameter used in the test is the contents of protein, carbohydrate, fats, fiber, calories, and vitamin C. This is necessary to get accurate information that is usable to list the nutrition fact labeled in food packaging. The result of proximate assays on honey resulted by Laura Idaman kelulut stingless bees is presented in Table 1.

Table 1. Proximate of kelulut honey from Peat Land Forest, Landasan Ulin Utara Village

No.	Parameter	Value±SD
1	Protein (%)	0.16±0.01
2	Fats (%)	0.01±0.01
3	Carbohydrate (%)	70.7±0.65
4	Fiber (%)	1.52±0.08
5	Calories (cal)	283.53±0.98
6	Lycopene((µg/ml)	2.899 ± 0.185
7	Carotenoids (mg/kg)	0.617 ± 0.001
8	Vitamin C (mg/ml)	14.08 ± 0.00

Protein

The result of the analysis shows that kelulut honey contains of 0.16±0.01% protein. Compared with trigona honey from South Sulawesi, it has different contents of protein where it contains 0.03%[22] and Malaysia 0.26% [23], while *Apis* honey contains 0.5% consisting of free

amino acid and enzyme[5]. The difference shown can be caused by the nectar consumed by the bees.

Fats

The analysis of fat contained in kelulut honey is 0.01±0.01%. It is smaller than the fat value in trigone honey from South Sulawesi which contains 0.06%, but higher than Apis honey with 0%[5]. Many kinds of food sources in Indonesia may contain 10% or more of ether extract (fat). The storage can influence the rancidity which may decrease its value. Honey has lower fat rate. Thus, it is rare to find any sources and references stating about fat content in honey.

Carbohydrate / Reducing Sugar

The analysis test results a findings that Kelulut honey contain 70.7±0.65% of carbohydrate observed by using refractometer. This value has been appropriate with the SNI (Indonesian National Standard) standardized in 2018 which required the minimum value of 65%. Based on the observation of Biluca, *et al.*[24], kelulut honey from Brazil contains between 48% - 70.5% of carbohydrate, while one from Sulawesi produces 49.68%[22], Malaysia 68.53±1.39%[25] and East Kalimantan 51.58%-63.68%[26]

The major benefit of carbohydrate is as the energy sources in humans' body. It indicates that honey can be consumed as energy supplier because of its high carbohydrate content. Carbohydrate or sugar is the most composition in honey[27].

Fiber

Fiber is a substance in a certain food whose role is to help digesting process. Consuming food sources with high content of fiber is highly recommended by many experts in the world. Fiber can boost human body's health entirely because it will help the organs working normally. Fiber is also contained in honey, even though in a few amounts. According to the analysis result, Kelulut honey contains 1.52+0,08% of fiber. Compared with the other studies, this content is less. Fiber of *Apis* honey obtained directly from the farmers contains 5.43+0.22% while from the seller is 9.08+0.06%[28]. Farmed honey and natural honey from *Apis* sp. can provide a difference in fiber content. Farmed honey has a fiber value of 1.99% and natural honey 2.76±1.07%[29]

Calories

The energy included in food sources which derive from carbohydrate oxidation, fats, and protein stated with heats is called calories. The total amount of calories in kelulut honey is 283.53±0.98 cal. Honey contains high energy and sugar in honey is easily digested in humans' body as its benefits we often find in fruits. The explanation above indicates why honey is deeply suggested as a consumption source for babies and teenagers. Another research mentioned that *Apis* honey has calories by 330kcal/100g[30].

Lycopene

Lycopene is carotenoids pigment, which is a phytochemist availabled in fruits and vegetables. Lycopene is a substance contained in honey. The result of analysis shows that lycopene in *kelulut* honey is 2.899±0.185 µg/ml. This value is different from Malaysian kelulut honey (0.65-1.52 mg/kg)[9]. Lycopene is known to be a herb medicine with strong antioxidants. Antioxidant is beneficial to keep body tissue system and organ since it functions as an antidote of free radicals in humans' body. The free radicals substance with large amounts in our body results in cells' destruction which potentially causes many kinds of diseases and many health problems[31].

Carotenoid

Carotenoid is a type of nutrient organic chemical compound that is available in plants' or animals' natural pigment. In Banjarbaru peat land kelulut honey, there found 0.617± 0.001 mg/kg carotenoids. This amount is lower than Malaysian kelulut honey with 4.61 mg/kg[9]. Differences in season and type of beeforage can affect carotenoid content. Summer and varied beeforage resulted in high carotenoid values [32]. The important benefit of carotenoids is as the agent of antioxidants. Besides that, it can be transformed to be essential vitamin[9].

Vitamin C

Vitamin C (Ascorbate acid) is provided naturally in foods consisting of honey. Vitamin C is one of antioxidants needed in humans' body to help protect our body cells from the bad effects of free radicals.

The analysis result of Vitamin C contained in kelulut honey is 14.08±0.00 mg/ml. It is different from Apis honey contained 2.2-2.5 mg/100gr of vitamin C[5] and kelulut honey from Sulawesi contained vitamin C with the amount of 302.85 µg/g[22].

Antioxidants Analysis

Antioxidant is a compound or chemical component that in a particular amounts or contents is able to obstruct or decelerate the breakdowns caused by oxidation process. Antioxidant is beneficial to protect humans' body from the attacks of free radicals. It is a compound that is naturally available in many kinds of food including honey. Free radical is a reactive oxygen compound, known as a compound having unpaired electron[2].

In this research, Banjarbaru peat land kelulut honey can be experimented as an antioxidant at radical peroxide, hydroxyl and metal chelating (Table 2).

Table 2. Antioxidants activity of Kelulut honey from Peat Land Forest, Landasan Ulin Utara Village

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

The results showed that kelulut honey had the ability to scavenging peroxide at 5.508±0.744%, 42.673±1.028% hydroxyl scavenging activity and 8.431±1.393% metal chelating. This value is lower than Kosovo honey[3]. Plant sources affect the nutritional content of honey[8]. High antioxidant activity can be caused by the polyphenol content in the honey[34]. Hydroxyl radical is the most harmful one because it has the highest rate of reactivity. Hydroxyl radical with its high reactivity is usually dangerous for livings[35]. It is different from superoxide that its toxics can be neutralized by superoxide dismutase. In contrary, radical hydroxyl cannot be eliminated by enzymatic reaction. It is caused by the slower diffusion of active enzyme than its part time. Radical hydroxyl can damage all types of macromolecules such as carbohydrate, nucleate acid, lipid and amino acid[1,2].

Antioxidants being fantastic free radical scavengers help in preventing and repairing the cell damage caused by these radicals[2]. The results also explain why honey has been used by traditional communities as a health supplement daily.

Conclusion

The research result indicates that kelulut honey cultivated in Peat lands, Landasan Ulin Utara Village, Banjarbaru City has a potency of proper proximate which contains high protein, carbohydrate, fibers, β-carotene, lycopene and vitamin C but low fats. This composition then plays a worthy role as beneficial antioxidants, in the form of hydroxyl radical scavenging, ferrous iron chelating effect and hydrogen peroxide scavenging activity.

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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5. Respon Kepada Reviewer dan Hasil Revisi Artikel (4 Juli 2023)

Matrix of Responses to reviewers' comments

manuscript number: TJNPR MY124AR (Satriadi et al)

	Reviewer / editor	Responses
Abstract	Findings are not explicitly stated. "…..The antioxidants activity on the other hands uses the parameter of……"	Rewrite and English editing by Dr. Erharuyi
Introduction	Poor grammatical construction. Improper use of tenses	English editing by Dr. Erharuyi
Methodology	Should be in reported speech	Rewrite Include the date (Month and Year) of sample collection and map Materials and Methods: Include a section for statistical analysis.
Results	The presentation is very poor	All experiments were performed in triplicates but The sample is only taken at one period and one place.
Discussion	The data obtained from the analysis are all muddled up	Rewrite and English editing by Dr. Erharuyi
Conclusion	A bunch of disjointed sentences "….kelulut honey cultivated in Peat lands, Landasan Ulin Utara Village, Banjarbaru City has a potency of proper proximate which contains high protein….."	Rewrite and English editing by Dr. Erharuyi
References	Cite relevant and related references from the published articles of TJNPR www.tjnpr.org	1. Seder N, Rayyan WA, Dayyih WA, Al-Natour MA, Hilmi ABM. Phytochemical Investigation, Comparison and Characterization Study of Malaysian Stingless Bee Honey versus Jordanian Honey by LC-MS/MS. Trop J Nat Prod Res. 2021; 5(9):1597-1605 2. Khleifat KM, Qaralleh H, Al-limoun MO, Al-khlifeh EM, Aladaileh SA, Tawarah N, Almajali IS. Antibacterial and antioxidant activities of local honey from Jordan. Trop J Nat Prod Res. 2021; 5(3):470-477 3. Bouacha M, Besnaci S, Boudiar I, Al-kafaween MA. Impact of storage on honey antibacterial and antioxidant activities and their correlation with polyphenolic content. Trop J Nat Prod Res. 2022; 6(1):34-39
References	50% of the references cited should be between 2016 - 2022	1. ≥ 2016 = 68,5% 2. most of the references before 2016 are methods
Figures, Tables	Not placed the proper section and wrongly captioned	Figure and all tables were removed under the reference section
Plagiarism	17% allowed in TJNPR	16%
English language editing	The manuscript should be submitted for English Language editing.	Assist by Dr. Erharuyi (Ref No.:EO/EES/Cert/M002/06/23)

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 ± 1.028%. The metal chelating effect and hydrogen peroxide radical scavenging activity were also remarkable, with percentage activity of 8.431 ± 1.393%, and 5.508 ± 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] 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 ($3^0$24'33.07"S & $114^0$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]

Total carbohydrate (g/100 g) = 100 – (water + ash + protein + fat + dietary fiber) -------- (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]

Energy (kcal/g) = 9 (fat) + 4 (protein) + 4 (carbohydrate) ---------- (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 (4:6) 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:

Lycopene (mg/100 mL) = -0.0458 A663 + 0.372 A505 – 0.0806 A453 ---------- (3)

β-carotene (mg/100 mL) = 0.216 A663 - 0.304 A505 + 0.452 A453 ---------- (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-sampling absorbance / controlled absorbance) × 100 ---------- (5)

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-sampling absorbance/controlled absorbance) x 100 ---------- (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-sampling absorbance/controlled absorbance) x 100 ---------- (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 ± 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 ± 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 ± 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±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 ± 0.08% of fiber. When compared with the fibre content observed in other studies, this content is low. For example, *Apis melllifera* honey from Nigeria contains 1.41 ± 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 ± 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 µg/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 µg/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 breakdowns 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 *viz 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 ± 0.744% and 42.673 ± 1.028%, respectively, and metal chelating activity of 8.431 ± 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.

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.

Conflict of interest

The authors would like to say that there is no conflict of interest in relation to this study.

Authors' Declaration

The authors hereby confirm and verify that the data given here in article are true, and we are fully responsible for the authenticity of its content as well as its accuracy.

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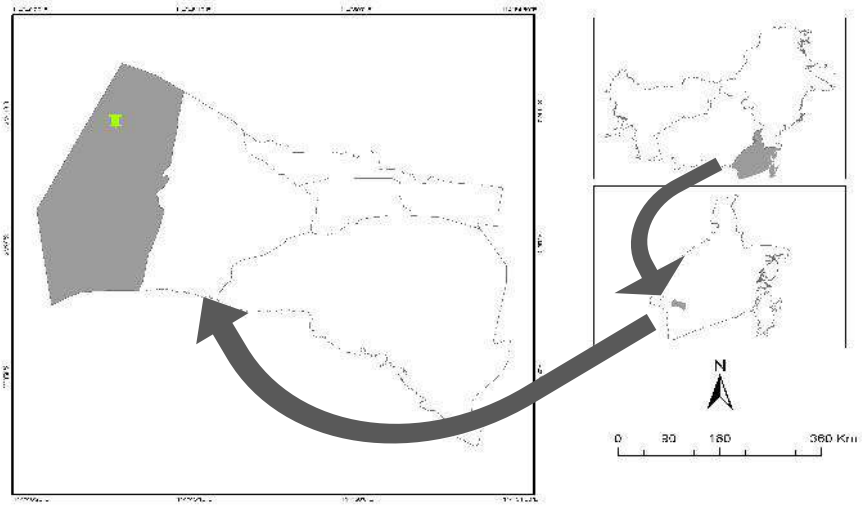

Figure 1: Location of Meliponiculture

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

No.	Parameter	Mean value ± 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 (µg/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 ± 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

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Original Research Article

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 ± 1.028%. The metal chelating effect and hydrogen peroxide radical scavenging activity were also remarkable, with percentage activity of 8.431 ± 1.393%, and 5.508 ± 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 ($3^0$24'33.07"S & $114^0$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.

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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]

Total carbohydrate (g/100 g) = 100 – (water + ash + protein + fat + dietary fiber) -------- (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]

Energy (kcal/g) = 9 (fat) + 4 (protein) + 4 (carbohydrate) ---------- (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 (4:6) 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:

Lycopene (mg/100 mL) = -0.0458 A663 + 0.372 A505 – 0.0806 A453 ---------- (3)
β-carotene (mg/100 mL) = 0.216 A663 - 0.304 A505 + 0.452 A453 ---------- (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-sampling absorbance / controlled absorbance) × 100 ---------- (5)

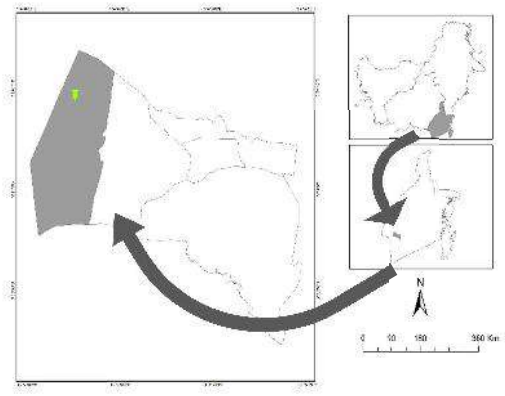

Figure 1: Location of Meliponiculture

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-sampling absorbance/controlled absorbance) x 100 ---------- (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-sampling absorbance/controlled absorbance) x 100 ---------- (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 ± 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 ± 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 ± 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±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 ± 0.08% of fiber. When compared with the fibre content observed in other studies, this content is low. For example, *Apis melllifera* honey from Nigeria contains 1.41 ± 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 ± 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 µg/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 µg/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 breakdowns 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 *viz 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 ± 0.744% and 42.673 ± 1.028%, respectively, and metal chelating activity of 8.431 ± 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 ± 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 (µg/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 ± 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.

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

by Trisnu Satriadi

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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 ± 1.028%. The metal chelating effect and hydrogen peroxide radical scavenging activity were also remarkable, with percentage activity of 8.431 ± 1.393%, and 5.508 ± 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] 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 ($3^0$24'33.07"S & $114^0$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]

Total carbohydrate (g/100 g) = 100 – (water + ash + protein + fat + dietary fiber) -------- (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]

Energy (kcal/g) = 9 (fat) + 4 (protein) + 4 (carbohydrate) ---------- (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 (4:6) 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:

Lycopene (mg/100 mL) = -0.0458 A663 + 0.372 A505 – 0.0806 A453 ---------- (3)

β-carotene (mg/100 mL) = 0.216 A663 - 0.304 A505 + 0.452 A453 ---------- (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-sampling absorbance / controlled absorbance) × 100 ---------- (5)

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-sampling absorbance/controlled absorbance) x 100 ---------- (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-sampling absorbance/controlled absorbance) x 100 ---------- (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 ± 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 ± 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 ± 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±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 ± 0.08% of fiber. When compared with the fibre content observed in other studies, this content is low. For example, *Apis melllifera* honey from Nigeria contains 1.41 ± 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 ± 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 μg/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 μg/g of vitamin C.[22]

Antioxidant activity

Antioxidants are compounds naturally available in many kinds of food including honey. They are able to obstruct 11 neutralize the breakdowns 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 *viz 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 ± 0.744% and 42.673 ± 1.028%, respectively, and metal chelating activity of 8.431 ± 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.

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.

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Conflict of interest

The authors would like to say that there is no conflict of interest in relation to this study.

Authors' Declaration

The authors hereby confirm and verify that the data given here in article are true, and we are fully responsible for the authenticity of its content as well as its accuracy.

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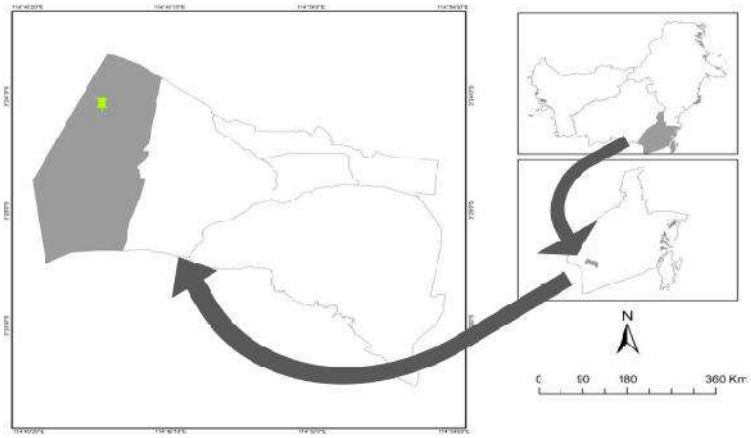

Figure 1: Location of Meliponiculture

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

No.	Parameter	Mean value ± 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 (μg/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 ± 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

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

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