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Physicochemical Properties and Antioxidant Activity of Three Types of Monofloral Honey from Indonesia

Sulistyianingsih[1], Achmad Toto Poernomo[2], Riesta Primaharinastiti[2]*
[1]Master Program of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia
[2]Department of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia

*Corresponding author: r.nastiti@gmail.com

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Abstract

Background*: In addition to minerals, honey contains carbohydrates (glucose and fructose), protein, amino acids, water, enzymes, ash, vitamins, and other substances. Compounds of honey can affect the chemical properties of honey. Knowing the physicochemical properties of honey is very important because physicochemical properties affect the quality of honey. One of the biological activities of honey is an antioxidant. Antioxidants can interfere with oxidative processes, prevent disease, and play an important role in the body's defence system.* ***Objective****: to determine and compare physicochemical properties (color, viscosity, ash content, water content, reducing sugar (glucose), total phenolic compound, HMF) and antioxidant activities of monofloral honey samples from Indonesia.* ***Methods****: The color of honey are categorized using the Pfund scale. Viscosity measurement is carried out using a Brookfield viscometer. The water content is carried out using a refractometer. Phenolic content and antioxidant activities analysis were carried out by UV-VIS spectrophotometer.* ***Results****: The results show that rambutan honey from Malang has the highest physicochemical properties and antioxidant activity, which had an amber color, water content of 21.7% b/b, acidity 20.7 mL NaOH/Kg, viscosity of 33.08 poise, ash content of 0.17% b/b, reducing sugar 69.38%b/b, total phenolics content 533.7 mg GAE/Kg sample and IC_{50} 0.111 µg/mL.* ***Conclusion****: The quality of honey varies from region to region. The best honey (according to SNI) is rambutan honey from Malang.*

Keywords*: honey, physicochemical properties, antioxidant activity, total phenolics*

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INTRODUCTION

Honey is a natural liquid, that is generally sweet tasting and produced by bees (*Apis* sp.) from floranectars or other parts of the plant (SNI, 2013). Honey is widely circulated and produced in Indonesia, and can be divided into monofloral and multifloral. Monofloral honey is obtained from *Apis cerana* or *Apis mellifera* bees with feed derived from one type of nectar source. This honey is commonly named based on the source of nectar, such as kelengkeng honey, rambutan honey, randu honey, and other. Monofloral honey has a specific aroma, flavor and color based on the source of honey (Suranto, 2007). Results from several studies showed that monofloral honey, which is randu honey, kelengkeng honey, and rambutan honey had different physicochemical properties (Chayati, 2008).

Honey contains not only minerals but also carbohydrates, protein, water, ash, small of vitamins, enzymes, amino acids, and other substances (Buba et al., 2013). The mineral contents in honey are Cr, Na, Al, Ni, Ca, K, Mg, Zn, Co, Cu, and Fe (Conti et al., 2014. The compounds in honey will affect physicochemical properties of honey. Knowing the physicochemical properties of honey is very important because physicochemical properties affect the quality of honey. Several areas in Indonesia are known to produce monofloral honey are Kediri, Malang and Bogor. *Ceiba pentandra* (randu) honey, *Dimocarpus longan* (kelengkeng) honey, and *Nephelium lappaceum* (rambutan) honey are types of honey that are produced continuously in Indonesia.

Empirically, honey has long been used as a component in traditional medicine in various countries. One of the biological activities of honey is an antioxidant. Natural antioxidants protect the body from the attack of free radicals, and can delay the onset of chronic diseases (Wahdaningsih *et al.*, 2011). Based on RISKESDAS (2007), the prevalence of degenerative diseases such as heart disease, hypertension, stroke, tumors, and diabetes were 16.1; 53.7; 20.2; 8.8; and 3.7%. Several studies have been done to determine the antioxidant activity of honey against chronic diseases. Research by Saputra & Wulan (2015) proves that honey with antioxidant activity can reduce the risk of chronic obstructive pulmonary disease and lung cancer. The antioxidant potential of manuka honey, a type of honey that has been registered as a wound care product, contain methyl syringate (a type of phenolic compound), which is considered to be quite potential to interfere with the process of amplification of inflammation by ROS (Molan, 2011). Manuka honey is also commonly used for cosmetic treatments (Suranto, 2004). Vallianoul *et al.*, (2014), stated that antioxidants in manuka honey play a role in cleaning the skin, eliminating skin discoloration, and increasing skin elasticity. Research (Chayati & Isnatin, 2015) shows differences in the antioxidant activity of monofloral honey originating from Central Java, which is rambutan, kelengkeng, coffee, randu, and calliandra honey, respectively, 11.9; 8.73; 5.56; 13.1 and 48.0%.

Given the many benefits of honey to human health, the purpose of this study is to identify and compare physicochemical parameters (viscosity, color, ash content, water content, reducing sugar (glucose), acidity, total phenolic content, Hydroxymethylfurfural (HMF), and antioxidant activities of monofloral honey from Indonesia.

MATERIALS AND METHODS

Materials

Randu honey from Kediri, kelengkeng honey from the cultivation of National Beekeeping Center (Pusbahnas) Bogor, and rambutan honey from Malang, DPPH (2,2-difenil-1-pikrilhidrazil) p.a (Sigma-Aldrich.), aquadest, gallic acid p.a (Sigma. Co.), Folin-Ciocalteau (E. Merck), ethanol p.a (E. Merck), hydrochloric acid 37% (E. Merck), dan $AlCl_3$ 10% (E. Merck).

Instruments

Spektrofotometer UV-Vis Shimadzu UV 260 and Brookfield DV3TLV digital viscometer.

Method

Color analysis

One hundred milliliter of honey was placed in a clear glass jar with a bright enough light and compared with the standard. The colors of honey are categorized with the Pfund scale.The color scale are divided into seven levels, which are dark amber, amber, light amber, extra light amber, white, extra white, and water white (White, 1984).

Viscosity

Viscosity measurements were carried out using the Brookfield DV3TLV Digital Viscometer. The honey sample was put into a special container on the viscometer. The rotor is dipped in the honey sample.

Ash content

The cup that has been heated in the oven at 105°C for 24 hours is cooled in a desiccator and weighed. Two grams of honey were weighed and placed into the cup and weighed, then burned on a hot plate at 400°C until smokeless and turn to ashes. Then put into the furnace at a temperature of 550°C for 6 hours, then removed and

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cooled in a desiccator. The cup was weighed, and the weight was recorded. Ash content analysis was replicated twice (Sudarmadji, 2007).

Water content

The water content measurement was carried out by a refractometer to read the index of refraction of a honey sample at a temperature of 20°C. The water content analysis was replicated twice. The water content of honey is determined by comparing the refractive index values of honey and water (SNI, 2018).

Reducing sugar (glucose)

Anhydrous Na_2CO_3 was weighed and dissolved in ± 300 mL of distilled water to make Luff's solution. Fifty grams of citric acid was added in 50 mL of distilled water while stirring. Twenty-five grams of $CuSO_4.5H_2O$ was added and dissolved in 100 mL of distilled water. The solution was transferred to a 1000 mL volumetric flask, and squeezed to the mark with distilled water, stored for 24 hours. Reducing sugar is determined by weighing 1.5 grams of honey and then putting it into a 500 mL erlenmeyer. 100 mL of 3% HCl was added and heated for 3 hours in an upright cooler. Then cooled and neutralized using 30% NaOH solution and a little 3% CH_3COOH. The solution was transferred to 500 mL volumetric flask to volume and filtered. 10 mL of the filter results in a 500 mL Erlenmeyer, then added 25 mL of luff solution and 15 mL of distilled water. Heated for 3 minutes, then cooled. 15 mL of 20% KI solution and 25 mL of 25% H_2SO_4 were added. Titrated with 0.1 N sodium thiosulfate solution and added a small amount of 0.5% starch solution. The same treatment was carried out for the blanks. Glucose level analysis was replicated twice. (SNI, 1992).

HMF Content

The honey sample was weighed as much as 5 g and put into a 50 mL volumetric flask, then dissolved with distilled water until the volume of the solution was 50 mL. The Carrez I solution and the Carrez II solution were added as much as 0.50 mL, shaken and diluted with aquadest to the line mark. Added a drop of alcohol to remove the foam on the solution's surface. The solution was filtered and the first 10 mL of the filter was discarded. The filter results were pipetted as much as 5 mL and put into 18 mL x 150 mL test tube. 5 mL of aquadest was pipetted and put into a tube for the sample solution and as a comparison, solution 5 mL of 0.20% sodium bisulfate was added, then homogenized until completely mixed, and the absorbance of the sample against the comparison was determined at a wavelength of 284 nm and 336 nm. HMF level analysis was replicated twice (SNI, 2018). The results were expressed in mg HMF / 100 g honey using the following formula:

$$\text{HMF} = \left(\frac{mg}{100}\ g\ madu\right) = \frac{A284 - A336\ x\ 14{,}97\ x\ 5}{bobot\ sampel\ (g)}$$

Acidity

Acidity analysis was carried out by weighing 10 grams of honey, then dissolving with 75 mL of CO2-free water in a 250 mL beaker, stirring with a stirring rod and inserting a pH meter to record the pH, titrated with 0.05 M NaOH at a rate of 5.0 mL/min. The titration was stopped when the pH reached 8.50. 0.05 M NaOH was taken with a 10 mL pipette and immediately titrated with 0.05 M HCl to pH 8.30. In the blank test, 75 mL of CO_2-free water was titrated with NaOH to pH 8.5. Acidity analysis was replicated twice (SNI, 2018).

Total phenolics content

Procedure for preparing a calibration curve: A standard solution of gallic acid (H_2O solvent) was prepared with a concentration of 5 to 25 ppm. One mL of each concentration was pipetted into a test tube. 0.5 mL of Folin-Ciocalteu was added and left for 5 minutes, and then 2 mL of 10% sodium carbonate solution was added.

Sample preparation: 0.5 g honey was weighed into a 25 mL volumetric flask and added water to the measuring line. 1.0 mL aliquot was pipetted into the vial and 0.5 mL Folin-Ciocalteu was added. The mixture was incubated for 5 minutes followed by addition of 2 mL 10% sodium carbonate. The mixture was further incubated for 10 minutes followed by absorbance measurement of 770 nm. The total content of phenolic compounds was expressed as gallic acid equivalents (mg GAE/Kg honey) using the standard curve for gallic acid. Total phenolics content analysis was replicated twice (Alfian & Susanti, 2012).

Antioxidant activity

One milliliter of the aqueous honey solution was mixed with 1 mL DPPH ethanol. The mixture was left in the dark for 30 minutes, and the absorbance was spectrophotometrically read at 519 nm. Ascorbic acid was used for calibration, and the results were expressed as mmol of ascorbic acid per Kg of honey. Antioxidant activity analysis was replicated twice. The antioxidant activity of honey can be read from the IC_{50} value. The IC_{50} value is the sample concentration value to measure the ability of the sample to consider its antioxidant activity to be 50% free radical. Antioxidant activity analysis was replicated twice (Chayati & Isnatin, 2015).

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RESULTS AND DISCUSSION

Honey color

Using the Pfund scale, honey can be categorized into dark amber, amber, light amber, extra light amber, white, extra white, and water white (Pontis *et al.*, 2014). Analysis of the test results for honey color parameters in Table 1 and Figure 1 shows that monofloral honey (randu, kelengkeng and rambutan) has a color from amber to extra light amber. The color of honey consists of water-soluble and fat-soluble fractions. Brightly colored honey has less water soluble than fat-soluble honey. The existence of an oxidation process causes a color change in honey (Adriani, 2011). The dye's persistence is caused by a mixture of several amino acids with iron from packaging or processing equipment (Sihombing, 2005). In this research, rambutan honey was darker than randu and kelengkeng honey. Indonesia has a very diverse vegetation that blooms regularly. This enables beekeepers to gather various single or multi-flowered honey in different colors. Previous studies have shown that transition metals react with organic compounds in honey to form colorful complexes. The darker honey color indicates higher total phenolic content and antioxidant activity (Harris, 2014).

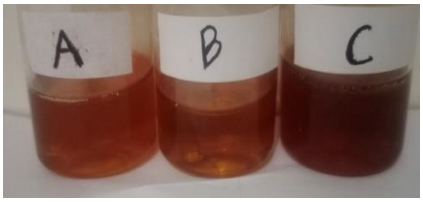

Figure 1. Color of (A) randu; (B) kelengkeng and (C) rambutan honey

Viscosity

The viscosity requirement based on SNI (Indonesian National Standard) is minimum 10 poise. The value of test results for viscosity parameter (Table 1) randu honey, kelengkeng and rambutan was 4.85, 75, and 3.31 poise. The viscosity of rambutan and kelengkeng honey the label has a viscosity value according to SNI standards. The viscosity of honey can be affected by temperature and water content. The higher the water content, the higher the liquid content of honey, and the lower the water content, the higher the density of honey. The viscosity of randu honey from Kediri is lower than the SNI standard. . This is because the water content in randu honey from Kediri is higher than kelengkeng and rambutan honey. Honey with high water content is very susceptible to fermentation because water can stimulate the growth and development of yeast cells. The condition of honey that has a higher water content or is more dilute (lower viscosity) can also cause a faster fermentation process which can change the taste of honey to become sour (Apriani, 2013).

Ash Content

Ash content is a mixture of inorganic or mineral components in a food ingredient. If the ash content is high, the mineral content is also high. Testing the ash content in honey needs to be done to determine the total mineral content of honey because each honey has a different mineral content. It depends on the source of soil and nectar around the bees (Sihombing, 2005). The honey quality requirement for ash content based on SNI 8664:2018 is a maximum 0.5% w/w. The higher the ash content of the sample, the higher the mineral content of the honey (Qadar *et al.*, 2015).

As a result of ash analysis, Randu honey from Kediri, kelengkeng honey from the cultivation of the National Beekeeping Center (Pusbahnas) Bogor, and rambutan honey from Malang, have the appropriate ash content with the standard set by SNI 8664:2018 which are 0.17; 0.10; 0.17 %w/w. This matter indicates that the mineral content in those areas is still quite good because still in accordance with the standards that have been set.

Water content

The results of the water content analysis show that each type of honey from different regions has different water content. The results show that kelengkeng honey from Bogor has moisture of 19% w/w. The reason is that the environmental temperature in the Bogor area is higher by 31.9°C (Badan Pusat Statistik, 2021), so honey in that area has low hygroscopic properties. Honey in this area is following with the water content standard in SNI 8664:2018. Therefore, honey in the Bogor area has good quality. Good quality honey is honey that contains water, about 17 - 21% (Sihombing, 2005).

The water content of randu honey from Kediri is 27.30%. These results indicate that the water content in this area is slightly higher than the honey quality requirements in SNI 8664:2018. This can be influenced by the environmental temperature in Kediri which is around 22.5°C (Badan Pusat Statistik, 2020). The water content of rambutan honey from Malang has a water content of 21.7%. The results of the water content in this area are in accordance with the water content standard in SNI 8664:2018. This is because Malang has an altitude of 498.48 above sea level and an environmental temperature of around 22.38°C (Badan Pusat Statistik, 2021). Low temperatures cause honey to absorb more water (Evahelda *et al.*, 2017). Therefore, the lower the

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ambient temperature, the higher the moisture content in honey.

In addition, the level of maturity of honey that has not been perfect also affects the water content (Savitri, *et al.,* 2017). This is in accordance with the taking of honey in Kediri, which is not in accordance with the harvest time so that the maturity is not perfect. Generally, the honey harvest time that has been determined is at 11-12 days marked by a nest covered with beeswax (Fatma, *et al.,* 2017).

Reducing sugar (glucose)

Reducing sugar is an important parameter to determine the quality of honey. Honey has two important components, namely sugar and water. However, two types of sugar are more dominant, glucose and fructose as much as 70 - 80% and water 10 - 20% (Evahelda *et al*., 2017). The results for the analyzed level of reducing sugar (glucose) honey can be seen in Table 1. Requirements for reducing sugar content based on SNI are at least 65% w/w. The reducing sugar content produced in this study from randu honey, kelengkeng, and rambutan were 63.74; 72.36 and 69.53%b/w. These results stated that kelengkeng and rambutan honey complied with the requirements for glucose-reducing sugar levels. Randu honey with a value of 63.74% w/w does not meet the requirements for reducing sugar content because it is less than 65%/w. Alcohols reacting with oxygen can form reactions with acetic acid. The formation of acetic acid can cause an increase in acidity in honey (Kuntadi, 2013). The different types of plants that are a food source for bees to produce honey will affect the characteristics of honey, such as taste, aroma, color, quality, and sugar content in honey (Mulu *et al.,* 2004).

Acidity

Based on SNI 8664:2018 the maximum acidity value is 50 mL NaOH/Kg. The results of the acidity test on some honey samples Table 1 showed that kelengkeng and rambutan honey have acidity levels of 11.63 and 20.67; mL NaOH/Kg that had the acidity value according to SNI standards. Kelengkeng and rambutan honey have good quality because it indicates that microbes will not grow in the honey. This can be seen in the slightly thick honey texture (Savitri, *et al.,* 2017). Meanwhile, randu honey, has high acidity values, 70.0 mL NaOH/Kg. These results are not following SNI 8664:2018. High acidity values can be affected by the water content of honey. Honey is acidic and has a high water content which will increase fermentation. Increasing the fermentation process can produce an increasingly sour taste of honey and decrease the value of reducing sugar (glucose) (Prica & Balos, 2014). Storage at high humidity also affects the acidity of honey (Savitri, *et al.,* 2017). In addition, high acidity in honey can also be caused by unhygienic post-harvest processing, which can lead to honey being easily contaminated. It is very important to pay attention to the acidity level of honey to keep honey hygienic and safe for consumption (Karnia *et al.,* 2019).

HMF Content

HMF content analysis has been performed as a measure of honey quality and has served as a reference for some studies to determine the authenticity of honey. The HMF value is an indicator for measuring the freshness of honey, the heating process, and the shelf life. If the honey is stored for too long, HMF levels will increase (Suranto, 2004). This is because the C atoms of glucose, fructose, and monosaccharides decompose when heated and undergo levulinic oxidation to formic acid (Anjana, 2014). The permissible HMF level for honey is 40 mg/Kg according to Indonesian National Standard (SNI 01-8664-2018). Based on the presented results Table 1 HMF content of kelengkeng honey (27.86 mg/Kg) and rambutan honey (36.59 mg/Kg) were in accordance with the mentioned standards. Higher levels of HMF (76.75 mg/Kg) have been found in randu honey, and this significant increase can be attributed to inferior products due to overheating, improper storage, or the addition of inverted sugar syrup.

Excessive heating can cause HMF levels to increase (Minarti *et al.,* 2016). High levels of HMF in honey will reduce honey quality because the HMF content is related to several other chemical characteristics of honey, such as water content, pH, free acid content, reducing sugar content, and enzymatic activity in honey (Kowalski *et al.,* 2013). HMF levels increased during the heating process, with a decrease in water content, reducing sugar, diastase enzyme activity, and increased free acid levels.

Total phenolics content

Total phenol content was determined using the Folin-Ciocalteu method and standard gallic acid by UV-Vis spectrophotometry. Gallic acid plays an important role, so we use it as a comparative substance because it has a heteropolymer with three hydroxy phenol groups. Phenolic hydroxy groups will be oxidized by the Folin-Ciocalteu reagent under alkaline conditions. Folin-Ciocalteu reagent will oxidize gallic acid in its phenolic hydroxy group to form a molybdenum-tungsten complex with a blue color (Alfian & Susanti, 2012).

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Table 1. Analysis results of physicochemical properties and antioxidant activity

Honey Samples	Physicochemical properties								Antioxidant Activity
	Color	Viscosity	Ash content	Water	Glucose	Acidity	HMF	Phenolic content	
Randu	Light amber	4.85	0.17 ± 5.6	27.3 ± 2.1	63.74 ± 4.5	70.0 ± 4.8	76.75 ± 1.6	465.9 ± 7.3	0.096 ± 2.1
Kelengkeng	Light amber	72.2	0.10 ± 0.14	19.0 ± 2.1	72.35 ± 3.7	11.6 ± 0.2	27.86 ± 3.8	272.4 ± 45.7	0.251 ± 8.8
Rambutan	Amber	33.08	0.17 ± 1.0	21.7 ± 0.9	69.38 ± 1.2	20.7 ± 5.4	36.59 ± 0.2	533.7 ± 14.0	0.111 ± 2.7

Note: The data represent the mean ± RPD (Relative Percent Different) data of the two replications

Total phenolic content is influenced by environmental factors such as light, rainfall, soil nutrients, altitude, and humidity. Besides that, total phenolic content is also affected by the cultivation process, such as fertilization, irrigation, and post-harvest treatment (Malinikova *et al.,* 2013). The results in this study showed that total phenolic compounds in rambutan honey (533.7 mg GAE/Kg honey) were higher than in randu honey and kelengkeng honey were 465.9 and 272.4 mg GAE/Kg honey (Table 1). According to Ferreira *et al.* (2009), dark honey was richer in phenolic compounds, and this was also confirmed in our study. Phenolic compounds are associated with antioxidant activity. Plants that have high phenolic compounds also have high antioxidant activity. Phenolic compounds protect antioxidants because phenolic compounds can scavenge the action of free radicals and react with reactive oxygen species (ROS) so that they no longer damage cells in the human body.

Antioxidant Activity

Antioxidant activity was determined by the DPPH method with a UV-Vis spectrophotometer. DPPH is a molecule containing unstable nitrogenous radicals that can bind with hydrogen ions, so it was used to test antioxidant activity. The presence of antioxidant compounds in the sample caused a color change of the methanol DPPH solution, which was initially dark purple to pale yellow. This color change occurs because DPPH is reduced, leading to electrons becoming paired (Zuraida *et al*., 2010).

In testing the antioxidant activity Table 1, IC_{50} values of randu, kelengkeng and rambutan were 0.095; 0.240 and 0.109 ppm. Kelengkeng honey from Bogor showed the highest DPPH radical scavenger activity compared with randu honey from Kediri and rambutan honey from Malang. Pointis *et al,* (2014) demonstrated a positive relationship between phenol concentration, antioxidant capacity and color of monoflora honey. The higher the concentration of phenolic compounds, the greater the antioxidant activity (Shahwar *et al.,* 2010). Capacity of phenolic antioxidants is affected by several factors, one of which is the functional group associated with the main structure. The study by Mohsen & Ammar (2009), showed that the radical scavenging activity tested on Phenolics is related to the number and position of hydroxyl group bonds (OH) in the molecule. The more hydroxyl groups are substituted in the molecule, the stronger the antioxidant capacity becomes because more hydrogen atoms can be generated (Yu Lin et al., 2009).

CONCLUSION

The quality of honey varies from region to region. The best honey (according to SNI) is rambutan honey from Malang has the highest physicochemical properties and antioxidant activity and has an amber color, water content of 21.7% b/b, acidity 20.7 mL NaOH/Kg, viscosity 33.08 poise, ash content 0.17 %b/b, reducing sugar 69.38 % b/b, total phenolics content 533.7 (mg/Kg GAE) and IC_{50} 0.111 ppm.

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AUTHOR CONTRIBUTIONS

Conceptualization, R. P.; Methodology, S., R. P., A. T. P.; Sotfware, A. T. P.; Validation, S.; Formal Analysis, S.; Investigation, S.; Resources, S.; Data Curation, S., R. P., A. T. P.; Writing - Original Draft, S.; Writing - Review & Editing, R. P., A. T. P.; Visualization, R. P.; Supervision, R. P.; Project Administration, R. P.; Funding acquisition, R. P.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

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