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ANTIOXIDANT ACTIVITY, TOTAL PHENOLIC CONTENT, AND FTIR ANALYSIS OF FERMENTED BADUY HONEY WITH PINEAPPLE

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ABSTRACT
Honey is one of the liquid foods known for its high nutritional value. Honey contains macronutrient and micronutrient compounds. The composition of honey is usually different from one honey to another and has different biological activities. Baduy honey is one of the forest honey sold by the Baduy tribe. Fermented Baduy honey is suspected of having better biological activity. This study aims to determine the antioxidant activity of fermented Baduy honey, the total phenolic compound, and FTIR analysis. The antioxidant activity was determined against DPPH (2,2-Diphenyl-1-picrylhydrazile) by measuring the absorbance of the sample at the wavelength of 517 nm. The Total phenolic compound was also determined using folin ciocalteu's method to measure the absorbance of the sample at the wavelength of 765nm. The FTIR analysis was also carried out to detect functional groups in Baduy honey. The antioxidant activity shows that fermented Baduy honey has the highest antioxidant capacity (IC 50 = 90,26 µg/mL) after three days of fermentation with pineapple and has better antioxidant capacity than non-fermentation Baduy honey. Total phenolic content shows that Baduy honey has 2,04 µg/mL. The FTIR analysis result indicates the presence of phenolic compounds, which can be found in flavonoids.

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1. INTRODUCTION

Honey is one of the liquid foods known for its high nutritional value and has been known in Indonesia for centuries. There are several ways to produce honey through bees, such as from flower nectars, plant secretions, and excretions of plant-sucking insects (Suarez et al., 2014). The nectar itself is a complex compound produced by nectariferous glands (Gunawan et al., 2018). In general, honey contains macronutrient and micronutrient compounds. For instance, honey generates complex sugar (fructose and sucrose) with a high concentration of around 5-7%, and protein belongs to macronutrient compounds (Bertoncelj et al. l, 2007). As for the micronutrients compounds, honey contains vitamin, mineral, and bioactive compounds, like flavonoids, phenolic acids, and alkaloids. The composition of honey is usually different from one honey to another. It depends primarily on the flower source, and other essential factors are the season and the environment (Dzugan et al., 2018). For example, honey obtained in the rainy season is usually thicker with more liquid than honey obtained from the dry season. The liquidity of honey is caused by the rainy season, which makes honey contain more water than in the dry season.

The diverse chemical compounds in honey might be responsible for its biological activities. It has been suggested that many of the medicinal properties of plants can be transmitted through honey, therefore honey could be used as a vehicle for transporting plant medicinal properties (Suarez et al. l, 2010). Consequently, depending on honey's bioactive compounds, honey has two biological activities, antioxidant and antibacterial (Dzugan et al. l, 2018). The previous study shows that the antioxidant properties in honey strongly correlated to phenolic compounds content in honey and were influence by the color of honey. The darker the color of honey being reported, the higher its total phenolic contents, consequently, higher antioxidant capacities (Suarez et al., 2014). Furthermore, the antioxidant capacity in honey is also affected by vitamin C, vitamin E, and enzymes within honey (Dzugan et al. l, 2018). Another study reported that honey has excellent antibacterial capacity

where honey can generate bacteriocidal and bacteriostatic against specific bacteria, such as Pseudomonas, Escheria coli, and Staphylococcus aureus. (Aggad et al., 2014).

Baduy honey is one of the forest honey produced in Indonesia. Furthermore, Forest honey is a honey produced by Apis dorsata, one of the forest bees in Indonesia, and belongs to one of the non-timber forest products (HHBK) (Nora et al. l, 2018). Baduy honey is forest honey sold by the Baduy tribe and has two different kinds of honey: bitter honey and sweet honey. Bitter honey has a darker color than sweet honey, whereas sweet honey has a yellow color. According to the holy Quran, honey that undergoes a fermentation process with pineapple has a better therapeutic function for human health. Fermentation itself is a natural food preservation process using certain bacteria.

Nonetheless, the study about antioxidant activity in fermented Baduy honey has not been done yet. In order to know the antioxidant activities in fermented Baduy honey, this study will be focusing on testing the antioxidant activities of fermented baduy honey with pineapple,e to get the best duration fermentation process. Also, in this study, the total phenolic content and FTIR analysis will be conducted.

2. RESEARCH METHOD

2.1 Honey Fermentation

The Baduy honey was fermented with pineapple at different times (two days, three days, seven days). The fermentation occurred inside a closed jar with the weight of pineapple was 20% of the total weight of Baduy honey.

2.2 Antioxidant Activity

The antiradical honey analysis was estimated according to the Procedure of Molyneux, (2004) using DPPH with some modifications. Three different Honey samples were dissolved in methanol at concentrations 2, 5, 10, 20, and 40 ppm, and 2 mL of each solution was mixed with 2mL DPPH (50mM). The mixtures were shaken vigorously and left for 30 minutes in the dark. Then, the mixtures were measured using UV-vis spectrophotometry at 517nm against the blank (2mL methanol and 2mL DPPH). The antioxidant activity was expressed as IC50 (the concentration of sample required to scavenge 50% of DPPH), calculated using linear regression analysis.

2.3 Total-Phenolic Content

Total Phenolic Content was determined using Folin ciocalteu's method based on Bhaskar et al., (2011). Fermented Baduy honey samples were dissolved in methanol p.a, and 0,5 mL was added to 0,5 mL folin ciocalteu 10%. Later, 2mL of Na2CO3 (2% w/v) was also added to that mixtures. The mixtures were homogenized using vortex for 15 seconds and left for two hours. Then, the absorbance was measured using UV-vi spectrophotometry at 765 nm against the blank.

The Gallic Acid was used as a standard to derive a regression linear equation showing the correlation between the concentration of gallic acid (reacted with folin ciocalteu) and absorbance. Total phenolic content was expressed as mg of gallic acid per kg of honey

2.4 FTIR Analysis

FTIR (Fourier Transform Infrared Spectroscopy) analysis was used to obtain infrared spectrum absorption, emission, and photoconductivity of solid, liquid, and gas. The honey samples were placed between two NaCl plates then measured using FTIR instruments. The Bruker alpha FTIR was used for detecting different functional groups in the sample.

3. RESULTS AND DISCUSSION

3.1 Antioxidant Activity

Honey serves as one of the natural antioxidants that play an essential role for human health for combating damage in the human body caused by oxidizing agents, which can generate some diseases (Dzugan et al. l, 2018). Three samples of Baduy Honey fermented with pineapple were analyzed for free radical scavenging activity using DPPH, a standard and straightforward method used widely to determine the antioxidant activity for many samples, including honey. The Honey samples were varied by different times used for fermenting honey with pineapple, which is two days, three days, and seven days.

Fermentation is a method used in food production and conservation (Margaoan et al. l, 2020). Each year, there is an increase in consumption of fermented foods since fermented food has excellent advantages such as antioxidants, enrichment in natural value, and therapeutic and immunological effects (Karacil et al. l, 2013). Based on that study, Baduy honey fermented with pineapple was expected to have preferable antioxidant activity.

As shown in table 1, there is a significant difference in the antioxidant activity of Baduy honey fermented with pineapple. Fermented Baduy honey in three days has the lowest antioxidant activity than fermented in two days or seven days.

The DPPH method was used in the antioxidant activity determination based on Molyneux, 2014 Procedure. In this method, 1,1- diphenyl-2-picrylhydrazyl was used as a stable organic radical to see the capacity of fermented baduy honey can scavenging free radicals and the antioxidant activity usually expressed as IC50. IC50 is the half-maximal concentration necessary to inhibit DPPH (Bertoncelj et al. l, 2007).

Table 1 Regression Equation and IC50 Value of Fermented Baduy Honey

Days	Regression Equation	R^2	IC50 (µg/mL)
2	y = 0,0303x + 8,741	0,7012	1361,683
3	y = 0,4838x + 6,3322	0,9981	90,26
7	y = 0,0813x + 19,996	0,9598	369,0529
No fermen-tation	Y= 0,0333x + 0,847	0,9531	1475,28

Based on table 1, fermented baduy honey is best consumed after three days of fermentation because it has the lowest IC50 value, 90,26 µg/mL. It means that the lower IC50 was obtained, the higher is its antioxidant activity and might be more toxic (Iqlimah et al. l,2021). A compound is said to be a powerful antioxidant if the IC50 value is less than 50 ppm, strong if its 50-100 ppm, weak if it is 101-150 ppm, and very weak if it is 151-200 ppm. According to this, it can be concluded that fermented baduy honey in three days has potent antioxidant activity. When the antioxidant activity of fermented Baduy honey is compared to Baduy honey non-fermentation (table 1), the antioxidant activity of fermented Baduy honey has a higher IC50 value than Baduy honey non-fermentation. It might happen regarding the fermentation process that gives baduy honey more nutritional value.

3.2 Total Phenolic Content

Total Phenolic content was carried out to determine phenolic compounds in fermented baduy honey. The folin ciocalteu's method was used in this analysis. In this method, oxidation-reduction occurred, where phenolic compound reduced phosphomolybdate and phosphotungstate in folin into molybdenum with blue color (Tursiman et al. l, 2012).

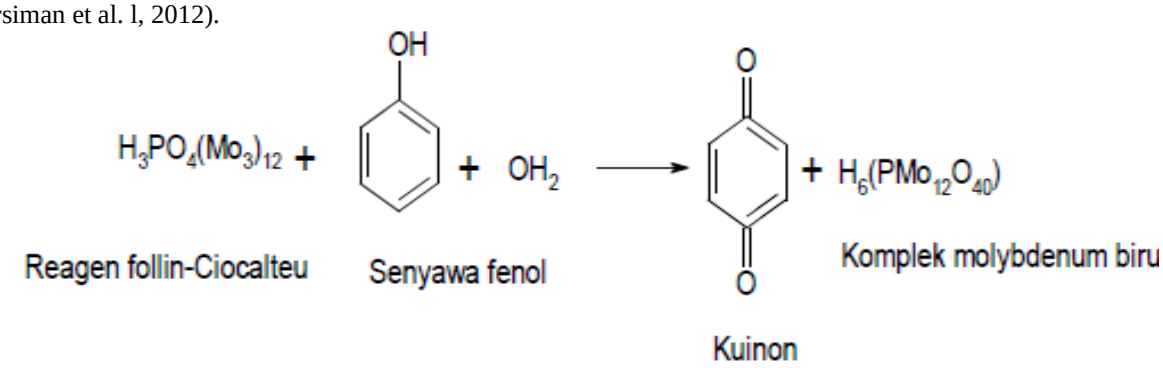

Figure 1 Reaction of Phenolic Group and Folin Reagent

Total phenolic content was determined using the gallic acid standard curve (Figure 2). From the equation y= 0,0094x + 0,0656, the total phenolic content was obtained which was 2,04 µg/mL of total phenolic from the samples.

From a previous study, phytochemical screening was carried out, and there are some phenolic compounds found, such as flavonoid and alkaloid compounds. Flavonoid and alkaloid belong to the phenolic compound. Therefore, determination of total phenolic content becomes beneficial. Commonly, phenolic compounds contribute to antioxidant activity, whereas the hydroxyl group is capable of scavenging free radicals by giving its electron to the free radical and making it stable.

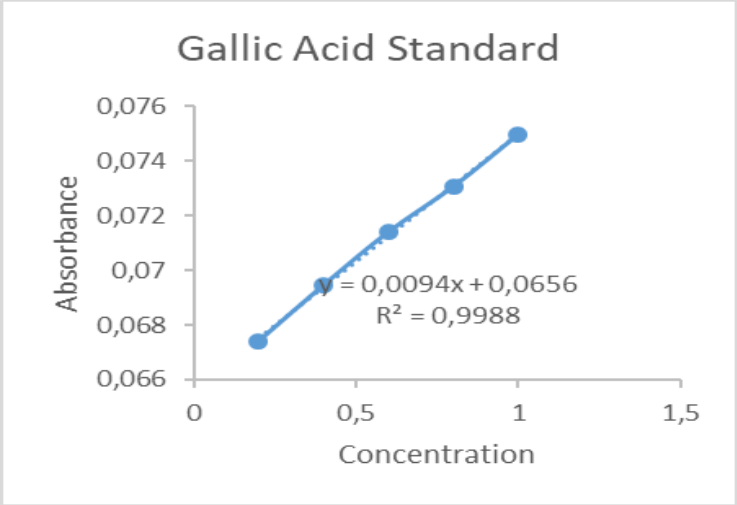

Figure 2 Gallic Acid Standard

3.3 FTIR Analysis

Fermented Baduy honey was analyzed with FTIR (Fourier Transform Infrared Spectroscopy) in order to obtain functional groups in organic compounds by measuring their absorption of infrared radiation over a range of wavelengths (Smith, 2011). The Data of FTIR analysis can be seen in Figure 3.

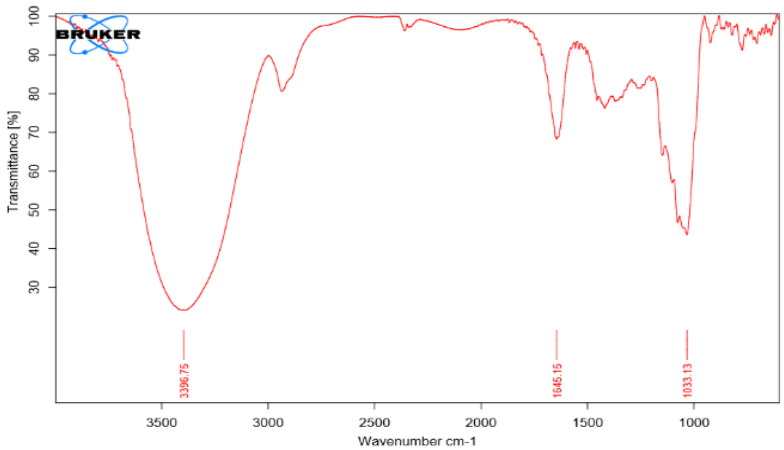

Figure 3 FTIR Analysis

The Spectrum shows that there is a broader peak in the range of wavelength at 3396,75 cm-1, which is the area of hydroxyl group vibration that can form hydrogen bonding. The Spectrum shows that there is a broader peak in the range of wavelength at 3396,75 cm-1, which is the area of hydroxyl group vibration that can form hydrogen bonding. This hydroxyl group belongs to the alcohol functional group. A small peak at 2900 cm-1 wavelength provides information that there is C-H aliphatic group.

Another peak at 1645,15 cm-1 wavelength provides information about the existence of carbonyl group (C=O) conjugated to C=C group. The C=C group can be seen in peak at 1400 cm-1 wavelength. Also, there is a strong peak at 1033,13 cm-1 wavelength, which means there is a possibility of S=O stretching. Based on the IR spectrum, it can be concluded that there is a possibility baduy honey contain phenolic compounds such as flavonoid. This finding is correlated to the phytochemical screening of baduy honey that had conducted by Nora et al. l, 2018, where flavonoid is present in Baduy honey.

4. DISCUSSION

In this present study, fermented Baduy honey has antioxidant activity better than non-fermented Baduy honey, which the best antioxidant activity was obtained from three days of fermentation. The total phenolic content in fermented Baduy honey is 2,04 µg/mL, and the FTIR shows the possibility of finding phenolic compounds in fermented Baduy honey.

5. CONCLUSSION

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