Journal of Nutraceuticals and Herbal Medicine

Journal Homepage: http://journals.ums.ac.id/index.php/jnhm

Correlation of Phenolic Content of Multiflora Honey from Malang and Alastuwo to Activity Antioxidant Using DPPH (2.2-Diphenyl-1-Pickrylhydrazyl)

Marlina Septiana[1], Ahwan Abdul[2*], and Fadilah Qonitah[2]

1. Pharmacy Study Program, Sahid University Surakarta, Jl. Adi Sucipto No. 154 Laweyan 57144, Surakarta Central of Java, Indonesia

2. Department of Natural Material Chemistry, Pharmacy Study Program, Sahid University Surakarta, Jl. Jl. Adi Sucipto No. 154 Laweyan 57144, Surakarta Central of Java Indonesia

**Corresponding author: ahone.far02@gmail.com*

Abstract

Honey contains various compounds that function as antioxidants, one of which is phenolic compounds. The purpose of this study was to determine the correlation between the phenolic content of multiflora honey from Malang and Alastuwo on antioxidant activity using the DPPH method (2,2-Diphenyl-1-picrylhydrazyl). Qualitative test of phenolic compounds with $FeCl_3$ color reagent, determination of total phenolic content by UV-Vis spectrophotometry using Folin-Ciocalteu method and antioxidant activity test using DPPH method (2,2-Diphenyl-1-picrylhydrazyl). The results showed that qualitatively Malang and Alastuwo honey contained phenolic compounds. The total phenolic content of Alastuwo honey was 0.0278 ± 0.0010 mg GAE/g and Malang honey was 0.0301 ± 0.0004 mg GAE/g. The results of the antioxidant activity test showed that Alastuwo honey had weak antioxidant activity (IC_{50} of 393.37 ± 10.28 ppm), Malang honey had moderate antioxidant activity (IC_{50} of 217.20 ± 6.61 ppm) and vitamin C had strong antioxidant activity. (IC_{50} is 2.22 ± 0.19 ppm). The total phenolic content of Alastuwo and Malang honey correlates with its antioxidant activity.

Keywords: Honey multiflora, total phenolic, UV-Vis Spectrophotometry, DPPH (2,2-Diphenyl-1-picrylhydrazyl)

INTRODUCTION

Antioxidants are compounds that can eliminate, stop or break the chain reaction of free radicals. One of the mechanisms of action of antioxidant compounds is by donating hydrogen atoms or protons to free radical compounds so that radical compounds become more stable. Antioxidants are obtained in 2 ways, namely natural antioxidants and artificial (synthetic) antioxidants. Natural antioxidants can be obtained from

vegetable/fruit plants, and we can also get them from bee products such as honey, propolis, royal jelly, beeswax, and pollen (Mahantesh et al., 2012).

Free radicals are very unstable because they have one or more unpaired electrons in the outer shell. Electrons in free radicals are very reactive and can react with proteins, lipids, carbohydrates or deoxyribonucleic acid (DNA) resulting in changes in cell structure and function. If free radicals are formed in the body, a chain reaction will occur and produce new free radicals. This reaction can end if there is a molecule that provides the electrons needed by the free radical or two free radical groups that form a non-radical bond (Syaifuddin, 2015).

Antioxidants are compounds that can inhibit oxidation reactions, by binding to free radicals and highly reactive molecules, based on their source can be divided into endogenous antioxidants (enzymes obtained from the body or food) and exogenous antioxidants (enzymes obtained from outside the body or food) (Werdhasari, 2014). One natural source that can be used as an antioxidant is honey. Honey is a sweet liquid derived from plant nectar which is processed by bees into honey and stored in beehive cells (Ardiansyah, 2011).

Honey contains enzymes such as catalase, glucose oxidase, and peroxidase as well as non-enzymatic compounds such as carotenoids, amino acids, proteins, organic acids, Maillard reaction products, and more than 150 polyphenolic compounds including flavonoids, flavonols, phenolic acids, catechins, and cinnamic acid derivatives (Muawanah dan Wardhani, 2014).

The honey that we encounter is not of the same quality and characteristics, according to Nayik and Nanda (2015). Important quality indicators for consumers are color, aroma, and taste. The color, aroma, and taste of honey are influenced by the mineral content in honey. This mineral content can come from the soil where plants grow and also the influence of contaminants (Bogdanov et al., 2007).

The physical and chemical characteristics of honey vary depending on internal and external factors. Internal factors include the type of interest (Nayik and Nanda, 2015). External factors such as season (Akuru and Amadi, 2018), soil conditions or geographic location (Buba et al., 2013), processing, and storage (Babarinde et al., 2011).

Honey has an antioxidant activity which includes oxidase and catalase. Antioxidants are compounds that can counteract or reduce the negative effects of oxidizing agents. Antioxidants play an important role in maintaining immunity from free radical attacks that can cause tissue damage and dangerous diseases such as cancer in the body. Phenolic compounds and flavonoids are compounds that have high antioxidant activity, as well as the main antioxidant compounds in honey (Bintoro et al., 2015). Analysis of the total levels of phenolic compounds can be determined spectrophotometrically using the Folin-Ciocalteu method with phenolic compounds forming a blue complex that can absorb radiation so that it can be measured (Pontis et al., 2014).

Khalil et al (2010) researched tualang honey from Malaysia to get total phenolic honey of 0.22 – 0.38 mg GAE/g. Kumazawa et al (2012) analyzed multiflora honey in Japan and obtained a total phenol of 0.17 – 1.32 mg/g; Echium vulgare honey 0.29 mg/g (Nagai et al., 2012); Czech honey 0.03-0.16 mg/g (Lachman et al., 2010); Brazilian honey

0.25 – 0.54 mg/g (Pontis et al., 2014). Ratnayani et al (2012) also examined differences in phenolic levels in two types of monofloral honey and their antioxidant activity, where there were differences in the free radical activity of two types of monofloral honey, namely the average level of total phenolic compounds in randu honey was 1375.89 ± 134, 10 mg GAE/g, and the average content of total phenolic compounds in longan honey was 1136.49 ±39.63 mg GAE/g, while the average yield of antioxidant activity in 60 minutes of randu honey was 95.39 ± 8.55% while Longan honey obtained antioxidant activity of 62.00 ± 0.86% and for standard gallic acid compounds obtained antioxidant activity of 92.00%.

Based on this background, the researchers were interested in analyzing the correlation between the phenolic content of multiflora honey from Malang and Alastuwo with an anti-free radical activity using DPPH (2.2-Diphenyl-1-Pickrylhydrazyl).

METHODS

This research is an experimental study using the DPPH (2,2-Diphenyl-1-Pickrylhydrazyl) method to test the correlation between phenolic honey from Alastuwo and Malang villages on its antioxidant activity.

Materials and Tools

The tools used in this research are analytical balance (ACIS), glassware (pyrex), micropipette (Onemed), cuvette (pyrex), UV – Vis spectrophotometry (Genesys). The materials used in this study were honey (Alastuwo and Malang), filter paper (Whatman), gallic acid (Sigma Aldrich), 96% ethanol (Medika), Aqua Pro Injection (Ika Pharmindo), aluminum foil, DPPH (2-2 diphenyl 1 picrylhydrazyl) (Merck), Folin Ciocalteu (Merck), Ascorbic Acid (Merck).

Sample Preparation

Honey is obtained from farmers from the villages of Alastuwo (Magetan) and Malang who have just been harvested. Then put in a clear glass bottle, covered with a newspaper to avoid the sun. Honey is stored at room temperature until time to use.

Qualitative Analysis of Phenolic Compounds in Honey with Color Reaction

The sample was pipetted 2.0 mL, then macerated with 3.0 mL ethanol, and shaken. The extract was dripped on a drip plate and added with 3-5 drops of 5% $FeCl_3$ solution. A positive test for the presence of phenolic compounds is indicated if there is a color change to green, blue, purple, or black.

Determination of Total Phenolic Content

1. **Preparation of Gallic Acid Standard Solution**

   Gallic acid stock solution was prepared with a concentration of 100 ppm (mg/L), by dissolving 0.01 g of gallic acid in a 100 mL volumetric flask and adding distilled water to the mark. Then the concentration series is made: 2.0; 4.0; 6.0; 8.0;10 ppm

2. **Determination of Maximum Wavelength**

   The 4 ppm gallic acid stock solution was measured for absorption at a wavelength of 700 nm – 780 nm at certain intervals. The results obtained are made

in the form of a curve, as the y-axis is the absorbance and the wavelength of light is the x-axis. From this curve, the maximum wavelength can be determined.

3. Generating Gallic Acid Standard Curve

The stock solution of 100 ppm gallic acid was taken as much as 0.1; 0.2; 0.3; 0.4; 0.5 mL each was added with 0.8 mL folin reagent and put into a 10 mL volumetric flask. Then 5% Na2CO3 was added up to the limit mark. So the standard curve solution has a concentration of 2.0; 4.0; 6.0; 8.0; 10.0 ppm. Each solution was allowed to stand for 60 minutes, and the absorption was measured at the maximum wavelength. By regressing absorbance concerning concentration, a calibration curve can be obtained with the regression equation y = bx + a.

4. Determination of Total Phenolic Content

Determination of total phenolic content was carried out using the Folin-Ciocalteu method. A 0.2 g honey sample was weighed on an analytical balance and dissolved in 96 % ethanol in a 10 mL volumetric flask to the mark, the mixture was filtered, the filtrate was pipetted 1.0 mL then added with 0.8 mL Folin reagent, put in a 10 mL volumetric flask. it's a shaken mixture. then 2 mL of 5 % Na_2CO_3 was added to the limit mark, the solution was allowed to stand for 60 minutes and the absorption was measured at the maximum wavelength. Measurements were repeated 3 times. The concentration of phenolic compounds in the sample can be determined by plotting the absorbance of the sample on a calibration curve (Qonitah dan Ahwan, 2019).

Antioxidant Activity Test

1. Preparation of DPPH Solution (2.2-diphenyl-1-picrylhydrazl) 0.4 mM

DPPH powder was weighed as much as 0.0157 g then dissolved with 96% ethanol in a volumetric flask to a volume of 100 mL and then homogenized. After that, the absorbance of the DPPH solution (2.2-diphenyl-1-picrylhydrazyl) was measured using a UV-Vis spectrophotometer to obtain a maximum wavelength of 513 nm. The solution must be used immediately and kept at a low temperature and protected from light (Abdul, 2020).

2. Preparation of Test Solution / Honey Sample

A honey stock solution with a concentration of 20,000 ppm was made by weighing 200 mg of honey dissolved with 96% ethanol in a 10 mL volumetric flask to obtain a honey concentration of 20,000 ppm. Then the honey stock solution was made in 5 concentration series: 100 ppm, 200 ppm, 300 ppm, 400 ppm, and 500 ppm (Rosana et al., 2021).

3. Preparation of Comparison Solution (ascorbic acid)

Ascorbic acid standard solution with a concentration of 1000 ppm was prepared by dissolving 1 mL of 5 mg ampoule preparations, adding 96% ethanol to 100 mL. Then the solution was diluted to 6 concentrations: 1.0; 2.0; 3.0; 4.0; 5.0; 6.0 ppm (Abdul, 2020).

4. Antioxidant Activity Test Analysis of honey with DPPH

The absorbance measurement of the honey test solution was made in 5 series with concentrations of 100, 200, 300, 400, 500 ppm. A solution with a concentration of 100 ppm was prepared by pipetting 5 µL of the mother liquor. A solution with a concentration of 200 ppm was made by pipetting 10 µL of the mother liquor, a solution with a concentration of 300 ppm was made by pipetting with a pipette of

25 µL of the mother liquor. A solution with a concentration of 400 ppm was prepared by pipetting 50 µL of the mother liquor. A 500 ppm concentration solution was prepared by pipetting 100 µL of the mother liquor. The mixture is then stirred evenly using a pipette covered with aluminum foil. It was then incubated at 37 ^{o}C for 30 minutes in a dark room. The absorbance was measured at a wavelength of 513 nm. Absorbance measurements were carried out with three repetitions (Rosana et al., 2021).

Data Analysis

The antioxidant activity of the sample is calculated by the formula:

$$\%\ Reducing = \frac{(A_c - A_s)}{A_c}\ x\ 100$$

Description:

Ac : Absorbance Control

As : Absorbanse Sampel

From the value of % attenuation at various concentrations, a curve of the concentration of the test solution vs. % attenuation was made, then the regression was calculated and the IC_{50} value was determined (Qonitah dan Ahwan, 2019).

RESULTS AND DISCUSSION

Honey Organoleptic

Honey is a natural liquid, generally has a sweet taste, and has a pleasant taste, which is produced from honey bees which can scientifically be used to prevent cardiovascular disease, diabetes, inflammation, and diarrhea (Arawwawala dan Hewageegana, 2017). The sample used in this study was fresh honey derived from multiflora honey from Alastuwo and Malang villages, East Java district. From the observations in Figure. 1, Alastuwo honey is lighter in color, has a sweeter taste, and has a pleasant aroma, while Malang honey is darker in color, has a sweet taste, and has a pleasant aroma. Classification of honey can be distinguished by color. Light-colored honey tends to have more sugar than dark honey. Color can be an indicator of quality because honey becomes darker with longer storage and higher temperatures. The color of honey is also influenced by the nectar that is the source of honey, the length of storage, and processing or heating (Eleazu et al., 2013).

Figure 1. Alastuwo Honey and Malang Honey; A: Alastuwo Honey, M : Malang Honey

Qualitative Test of Phenolic Compounds in Honey with Color Reaction

In figure 2, it can be seen that there was a change in the color of the honey plates A and M with the addition of $FeCl_3$ there was a change in the color complex from yellow to blackish blue. This shows that qualitatively Alastuwo honey and Malang honey contain phenolic compounds. In Alastuwo honey the color is lighter and in Malang honey, the color is darker.

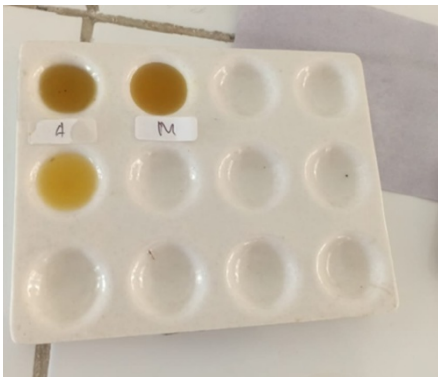

Figure 2. Honey with Color Reaction; ; A: Alastuwo Honey, M: Malang Honey

This is consistent with the results of research on Australian honey by Yaoa et al (2005), who found that the darker the color of the honey, the higher the content of phenolic compounds. This is consistent with direct visual observation, where Malang honey is darker in color than Alastuwo honey.

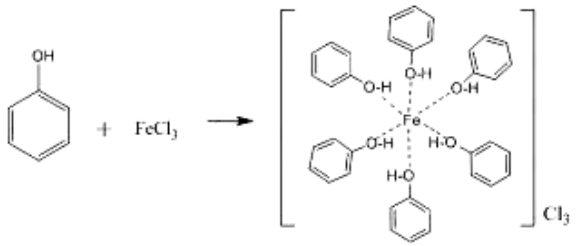

Figure 3. The reaction of Phenolates with $FeCl_3$ (Nafisah et al., 2014)

Total Phenolic Content

Analysis of the content of phenolic compounds using the Folin-Ciocalteau method. The reaction between the Folin-Ciocalteau reagent and phenolic compounds will form a complex blue color that can absorb radiation so that it can be measured (Pontis et al., 2014). The standard of phenolic compounds used in this analysis is Gallic Acid.
Absorbance data and total levels of phenolic compounds are presented in Table 1, where the results show that the total content of phenolic compounds in Malang honey is higher than in Alastuwo honey. Malang is 0.0301 ± 0.0004 (mg GAE/g) as shown in the table below:

The total content of phenolic compounds in Malang honey was higher than the total content of phenolic compounds in Alastuwo honey. This shows that different kinds of honey can produce different total levels of phenolic compounds, which is following the results of qualitative tests that have been carried out, namely Malang honey provides a sharper color intensity than Alsatuwo honey.

These results indicate that the phenolic content of honey from Alastuwo and Malang is smaller than the total phenolic content of honey from other regions. The results of previous studies in Sumbawa Besar showed that honey in several forest areas of the area produced a total phenolic compound of 0.06 – 0.38 mg/g (Saputri dan Putri, 2017). Khalil et al (2010) examined tualang honey from Malaysia to get total phenolic honey of 0.22 – 0.38 mg/g. Kumazawa et al (2012) analyzed multiflora honey in Japan and got a total phenol of 0.17 – 1.32 mg/g, Echium vulgare honey 0.29 mg/g (Nagai et al., 2012); Czech honey 0.03-0.16 mg/g (Lachman et al., 2010); Brazilian honey 0.25 – 0.54 mg/g (Pontis et al., 2014).

Table 1. Total Honey Phenolic Compounds

Sample	Weight (gr)	Replication	Absorbance	Total Rate (mg GAE/g)	Average Rate (mg GAE/g)
Alastuwo	0,2275	1	0.562	0.0288	0.0278 ± 0.0010
	0.2287	2	0.552	0.0279	
	0.2315	3	0.541	0.0268	
Malang	0,2305	1	0.590	0.0304	0.0301 ± 0.0004
	0.2345	2	0.595	0.0302	
	0.2298	3	0.582	0.0296	

The content of phenolic compounds and antioxidant activity in honey is directly proportional to the color of honey. The color of honey is related to the presence of pigments such as carotenoids and flavonoids. Darker honey has a higher total phenol and antioxidant activity (Kumazawa et al., 2012). This theory is by the results obtained from this study where Malang honey has a darker color than Alastuwo honey. The types of phenolic compounds that make up most of the honey in various countries, especially gallic acid, cinnamic acid, pinocembrin, and coumarin (Hussein et al., 2011).

Antioxidant Activity Test

From the data on the percentage of inhibition in the variation of the concentration of each sample, a curve was made by connecting the sample concentration (X axis) to the % inhibition as a parameter of antioxidant activity (Y axis), in order to obtain a linear regression equation for Alastuwo honey replication 1: y = 0.1131x + 4, 1667, replication 2: y = 0.1136x + 6.0278, replication 3: y = 0.1131x + 6.1389 and for Malang honey, a linear regression of replication 1: y = 0.0704x + 34,847, replication 2: y = 0.0611x + 36.278, replication 3: y = 0.0663x + 35.958 which can be used to determine the concentration of compounds showing the IC_{50} value in Figure 4. below:

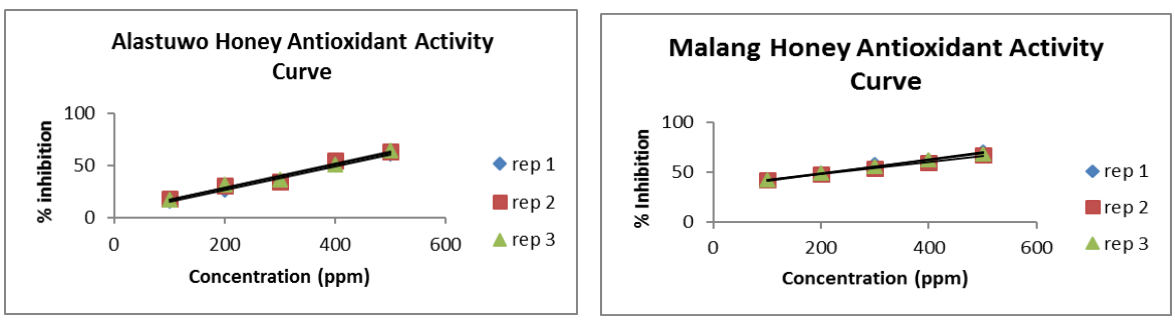

Figure 4. Alastuwo and Malang Honey Antioxidant Activity Curve

Based on the research, data on antioxidant activity were obtained from the test samples expressed in IC_{50} values as follows:

Table 2. Antioxidant activity of samples using the DPPH method

No	Sample	IC_{50} (ppm)±SD
1	Madu Alastuwo	393,37 ± 10,28
2	Madu Malang	217,20 ± 6,61
3	Vitamin C	2.22 ± 0,19

From the analysis, it was found that the IC_{50} percent of multiflora honey from Alastuwo was 393.37 ± 10.2847 ppm and for Malang honey, it was 217.20 ± 6.61 ppm. This shows that the antioxidant activity of honey samples taken from the villages of Alastuwo and Malang is smaller than that of other research honey. Muawanah dan Wardhani (2014) examined Papuan honey and got the IC_{50} result of 5453.75 ppm. Chua et al (2013) examined Malaysian Tualang honey with IC_{50} results of 96.50 ppm, Gelam honey obtained IC_{50} results of 501.69 ppm Acasia honey obtained IC_{50} results of 299.83 ppm. The antioxidant activity of a material is influenced by the content of phytochemical compounds that have antioxidant properties. These compounds include phenolic acids, flavonoids, enzymes (glucose oxidase and catalase), ascorbic acid, carotenoids, organic acids, amino acids, and proteins. The antioxidant activity of phenolic components contributes to human health (Khalil et al., 2010).

Antioxidant activity is also expressed in the IC_{50} parameter. IC_{50} states the sample concentration required to inhibit 50 % of free radicals, which in this case is DPPH. The lower the IC_{50} value, the smaller the sample concentration used to ward off 50 % of free radicals, which means the sample is stronger in counteracting free radicals (Pontis et al., 2014). The IC_{50} value of honey from Alastuwo village was 393.37 ppm and the IC_{50} value of honey from Malang was 217.21 ppm and for the ascorbic acid solution was 2.22 ppm. The results of the above study indicate that to reduce free radicals by 50% it takes 393.37 ppm Alastuwo honey, 217.21 ppm Malang honey, and 2.22 ppm ascorbic acid.

Based on the book Basic Principles of Free Radical and Antioxidant Examination by Yuslianti (2018) it is stated that the parameters of free radical scavenging activity with very strong intensity if the IC_{50} value is less than 50 ppm, the compound is said to be a strong antioxidant if the IC_{50} value is between 50-100 ppm, the compound is said to be a strong antioxidant. as a moderate antioxidant, if the IC_{50} value is between 101-250 and the compound is said to be a weak antioxidant if the IC_{50} value is more than 250-500 ppm, the compound is said to be a very weak antioxidant if the IC_{50} value is more than 500 ppm. Based on the IC_{50} value obtained through the linear regression equation, it can be concluded that ascorbic acid is a very strong antioxidant (IC_{50} 50 ppm). Followed by Malang honey with medium category (IC_{50}: 101-250 ppm). Meanwhile, Alastuwo honey is a weak antioxidant (IC_{50} 250-500 ppm). This happens because ascorbic acid is a pure compound compared to honey.

Correlation of Total Phenolic Compound Content with Free Antiradical Activity

Based on the results of the total phenolic compounds in Alastuwo honey and Malang honey, it can be seen that there is a linear relationship with their free radical activity. Where the highest levels of total phenolic compounds were found in Malang honey (0.0301 ± 0.0004) mg GAE/g which also had a high value of free radical activity indicated by the IC_{50} value of 217.20 ppm. On the other hand, Alastuwo honey which has a low total phenolic compound content (0.0278 ± 0.0010) mg GAE/g also has a low

antiradical activity value with an IC_{50} value of 393.37 ppm. As shown in the bar curve below:

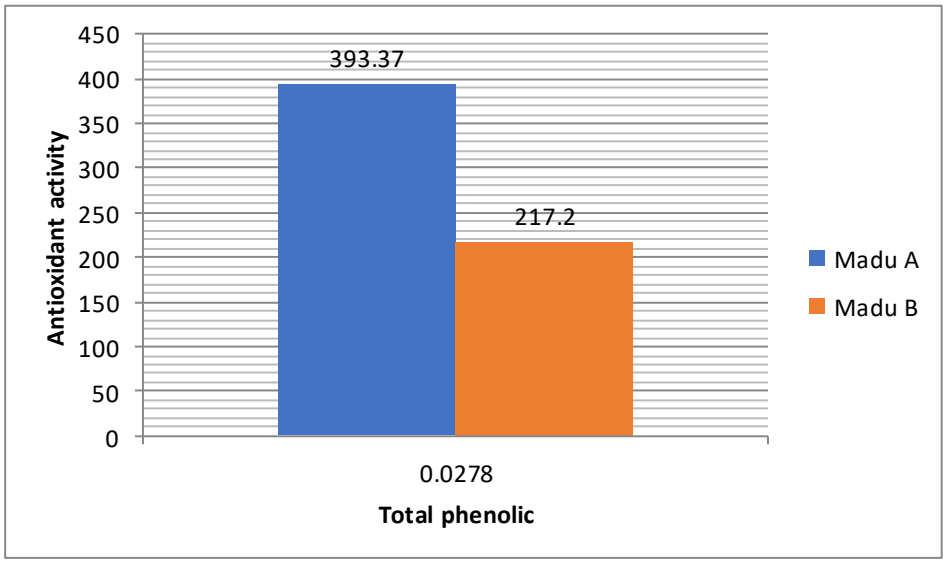

Figure 5. Graph of Honey Phenolic Correlation Curve Against Antioxidant Activity; Madu A: Alastuwo Honey and Madu B: Malang Honey

CONCLUSION

From this research, it can be concluded that alastuwo and Malang honey contain phenolic compounds from the results of qualitative and phenolic tests. The total of Malang honey is higher than that of alastuwo honey. The results of anti-oxidants using the DPPH method Malang honey has moderate antioxidant activity and alastuwo honey has weak antioxidant activity, high levels of total phenolic can increase antioxidant activity levels in poor and alastuwo honey.

ACKNOWLEDGEMENTS

Acknowledgments are conveyed to the Sahid Surakarta University Pharmacy Study Program for helping carry out this research.

REFERENCES

Abdul, A., 2020. Uji Aktivitas Antioksidan Ekstrak Etanol Daun Adas (Foeniculum Vulgare Mill) Dengan Metode DPPH Dan FRAP. Pharmed J. Pharm. Sci. Med. Res. 3, 43–54.

Akuru, U.B., Amadi, B.A., 2018. Phytochemicals and antioxidant properties of some selected medicinal plants. J. Pharmacogn. Phytochem. 7, 283–285.

Arawwawala, M., Hewageegana, S., 2017. Health benefits and traditional uses of honey: A review. J. Apitherapy 2.

Ardiansyah, 2011. Antioksidan dan Peranannya bagi Kesehatan. Artik. Iptek.

Babarinde, G.O., Babarinde, S.A., Adegbola, D.O., Ajayeoba, S.I., 2011. Effects of harvesting methods on physicochemical and microbial qualities of honey. J. Food Sci. Technol. 48, 628–634.

Bintoro, V.P., Legowo, A.M., Purnomoadi, A., Setiani, B.E., 2015. Garlic antioxidant (Allium sativum L.) to prevent meat rancidity. Procedia Food Sci. 3, 137–141.

Bogdanov, S., Haldimann, M., Luginbühl, W., Gallmann, P., 2007. Minerals in honey: environmental, geographical and botanical aspects. J. Apic. Res. 46, 269–275.

Buba, F., Gidado, A., Shugaba, A., 2013. Analysis of biochemical composition of honey samples from North-East Nigeria. Biochem Anal Biochem 2, 139.

Chua, L.S., Rahaman, N.L.A., Adnan, N.A., Eddie Tan, T.T., 2013. Antioxidant activity of three honey samples in relation with their biochemical components. J. Anal. Methods Chem. 2013.

Eleazu, C.O., Iroaganachi, M.A., Eleazu, K.C., Okoronkwo, J.O., 2013. Determination of the physico-chemical composition, microbial quality and free radical scavenging activities of some commercially sold honey samples in Aba, Nigeria:'the effect of varying colours. Int. J. Biomed. Res. 4, 32–41.

Hussein, S.Z., Yusoff, K.M., Makpol, S., Yusof, Y.A.M., 2011. Antioxidant capacities and total phenolic contents increase with gamma irradiation in two types of Malaysian honey. Molecules 16, 6378–6395.

Khalil, M.I., Sulaiman, S.A., Boukraa, L., 2010. Antioxidant properties of honey and its role in preventing health disorders. Open Nutraceuticals J. 3.

Kumazawa, S., Okuyama, Y., Murase, M., Ahn, M.-R., Nakamura, J., Tatefuji, T., 2012. Antioxidant activity in honeys of various floral origins: Isolation and identification of antioxidants in peppermint honey. Food Sci. Technol. Res. 18, 679–685.

Lachman, J., Hejtmankova, A., Sýkora, J., Karban, J., Orsak, M., Rygerova, B., 2010. Contents of major phenolic and flavonoid antioxidants in selected Czech honey. Czech J. Food Sci. 28, 412–426.

Mahantesh, S.P., Gangawane, A.K., Patil, C.S., 2012. Free radicals, antioxidants, diseases and phytomedicines in human health: Future perspects. World Res. J. Med. Aromat. Plants 1, 6–10.

Muawanah, A., Wardhani, P., 2014. Aktivitas Antikanker dan Antioksidan Madu di Pasaran Lokal Indonesia. J. Ilmu Pertan. Indones. 19, 136–144.

Nafisah, M., Tukiran, S., Hidayati, N., 2014. Uji Skrining Fitokimia Pada Ekstrak Heksan, Kloroform dan Metanol dari Tanaman Patikan Kebo (Euphorbiae hirtae), in: Prosiding seminar nasional kimia. hal. 281–282.

Nagai, T., Tanoue, Y., Kai, N., Suzuki, N., 2012. Functional property of honey from Echium vulgare. Food Nutr. Sci. 3, 614–620.

Nayik, G.A., Nanda, V., 2015. Physico-chemical, enzymatic, mineral and colour characterization of three different varieties of honeys from Kashmir valley of India with a multivariate approach. Polish J. Food Nutr. Sci. 65.

Pontis, J.A., Costa, L.A.M.A. da, Silva, S.J.R. da, Flach, A., 2014. Color, phenolic and flavonoid content, and antioxidant activity of honey from Roraima, Brazil. Food Sci. Technol. 34, 69–73.

Qonitah, F., Ahwan, A., 2019. AKTIVITAS ANTIOKSIDAN DAN KANDUNGAN FENOLIK TOTAL FRAKSI N-HEKSAN DAN KLOROFORM DAUN JERUK PURUT (Citrus hystrix). J. Ilm. As-Syifaa 11, 99–102.

Ratnayani, K., Laksmiwati, M., Septian, N.P.I., 2012. Kadar total senyawa fenolat pada madu randu dan madu kelengkeng serta uji aktivitas antiradikal bebas dengan metode DPPH (Difenilpikril Hidrazil). J. Kim. 6, 163–168.

Rosana, M., Ahwan, A., Qonitah, F., 2021. Uji Kualitatif Aktivitas Antioksidan Ekstrak Etanol Propolis. Lumbung Farm. J. Ilmu Kefarmasian 2, 154–157.

Saputri, D.S., Putri, Y.E., 2017. Aktivitas Antioksidan Madu Hutan di Beberapa Kecamatan di Kabupaten Sumbawa Besar. J. Tambora 2.

Syaifuddin, S., 2015. Uji aktivitas antioksidan bayam merah (alternanthera amoena voss.) segar dan rebus dengan metode DPPH. UIN Walisongo.

Werdhasari, A., 2014. Peran antioksidan bagi kesehatan. J. Biotek Medisiana Indones. 3, 59–68.

Yaoa, L., Jiang, Y., Singanusong, R., Datta, N., Raymont, K., 2005. Phenolic acids in Australian Melaleuca, Guioa, Lophostemon, Banksia and Helianthus honeys and their potential for floral authentication. Food Res. Int. 38, 651–658.

Yuslianti, E.R., 2018. Pengantar Radikal Bebas dan Antioksidan. Deepublish.