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PHENOLIC AND ANTIOXIDANT ACTIVITY OF BANGGAI SWEET POTATO ETHANOL EXTRACT (DIOSCOREA)

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ABSTRACT

The aim of this study were to determine phenolic content and antioxidant activity of three varieties of banggai sweet. Research methods used the complete random methods on banggai sweet potato varieties of purple sweet potato, yellow sweet potato and white sweet potato with 5 times replication and to obtain 15 experiment units. The influence of ethanol ratio of banggai sweet potato flour and extraction time treatment produced extract phenolic content and influence varieties of banggai sweet potato phenolic content and activity of antioxidant applied to achieve the aim. The results shown that highest phenolic content (2,63%) was white banggai sweet potato, 2,44% of purple banggai sweet potato and 1,90% of yellow banggai sweet potato at ratio 15 : 1 based volume/weight (v/w) and time extraction at 2 hours. The highest activity of antioxidant ethanol extract (74,87%) was found at white banggai sweet potato extract and lowest activity of antioxidant (37,19%) was found at yellow banggai sweet potato. Antioxidant activity had relations with phenolic content. Antioxidant activity of 200 ppm BHT as control had no significant different with antioxidant activity of white sweet potato ekstract.

Keywords: Phenolic Content, Antioxidant Activity, Ethanol Extract, Varieties Of Banggai Sweet Potato.

1. INTRODUCTION

Food and health are two important things that are closely related to each other. The development and progress of science and technology have both positive and negative impacts on the health sector. One negative impact is arising from various types of chronic diseases due to diet, such as cancer, coronary heart disease, hypertension which leads to stroke, premature aging and disruption of the immune system (Schulze et al., 2018). The results of the study stated that the emergence of chronic diseases was triggered by the presence of free radicals (Maddu, 2019). The activity of free radical molecules can cause oxidative stress which causes various diseases. Oxidative stress is an imbalance in the amount of oxidants and prooxidants in the body (Rahal et al., 2014). Chemical compounds that can inhibit aging and overcome various chronic diseases caused by free radicals are chemical compounds called antioxidants.

Banggai sweet potato plant is included into *Dioscoreaceae* family which is a vines and

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sometime has very large tuberous root (Mansur et al., 2017). There are 11 species of plants in *Dioscoreaceae* family, namely: Babanal *(Dioscorea warburgiana* Uline*)*, Ondot *(Dioscorea hispida* Dennst.*)*, Siloto *(Dioscorea cf. deltoidea* Wall.), Bakutu (*Dioscorea glabra* Roxb.), Baku makuloloang (*Dioscorea bulbifera var. celebica* Burkill), Baku pusus (*Dioscorea cf. alata*), *Dioscorea keduensis* Burkill, *Dioscorea numularia*, Ndolungun (*Dioscorea esculenta* [Lour.] Burck.), Baku butun (*Dioscorea alata* L.). One of the distinguishing characteristics of the banggai sweet potato varieties is the color of the tubers. Colored tubers indicate that the sweet potato contains a chemical component that functions as a natural antioxidant. Banggai sweet potato (Dioscorea) is one of the plant genera that cannot be separated from the Banggai Islands (Bangkep) community. This is because the banggai sweet potato is one of the staple foods of the Bangkep community, which is found in many Banggai and Lainag sub-districts. The local community processes banggai sweet potato into compote, chips, cake or boiled like a main dish. Dietary habit by consuming banggai sweet potato as the main menu is basically a healthy diet that should be maintained and developed.

The three major color groups of banggai sweet potato namely purple, yellow and white. Purple tubers are caused by anthocyanin pigments, while yellow tubers are caused by beta-carotene content (Retnati, 2009). The content of beta carotene and anthocyanin and tocopherol and phenolic compounds function as antioxidants (Husna et al., 2013). This study aimed to analyze the content of phenolic compounds, carotenoids and anthocyanins in banggai sweet potato which act as natural antioxidants

2. RESEARCH METHOD

This used experimental study which was carried out at the Chemistry Research Laboratory of the Department of Chemistry, Faculty of Mathematics and Natural Sciences, Tadulako University, Palu. The basic ingredients used were banggai sweet potato obtained from Banggai Islands farmers, consisting of 3 varieties, namely purple, white and banggai sweet potatoes. Other ingredients as extracting agents and chemicals for analysis were technical ethanol, phenol, NH_4OH 0.5 N, phosphate buffer (pH = 12), methyl orange (MO) indicator, phosphoric acid, soybean oil, tween 20, 30% ammonium thiocyanate, 0.02 M ferrochloride, 3.5% HCl, distilled water, aluminum foil and filter paper. The equipment used consists of: blender, 60 mesh sifter, aluminum gutter, analytical balance, mixer, water bath, rotary evaporator, refrigerator, UV-VIS spectrophotometer, cuvette, buchner funnel, measuring flask, measuring cup, erlenmeyer and common glassware in Chemistry laboratory.

This study was designed using a Completely Randomized Design (CRD) with the treatment of the banggai sweet potato varieties namely Dioscorea bulbifera var celebica Burkill with purple tubers, Dioscorea sp varieties with yellow tubers and Dioscorea of alata varieties with white tubers. Each treatment was repeated 5 times to obtain 15 experimental units.

This research was carried out in 2 stages, namely: production of banggai sweet potato flour, extraction and phenolic content analysis of banggai sweet potato ethanol extract.

Production of Banggai Sweet potato Flour

Banggai sweet potato (3 varieties) from farmers in Banggai Islands, were washed thoroughly and soaked in boiling water for 7 minutes, then drained and thinly sliced. Sliced banggai sweet potato were then dried in the sun to dry, then made into flour by using a blender.

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The banggai sweet potato flour produced was then stored in a plastic container before being used in further research.

Extraction of Banggai Sweet potato

Banggai sweet potato flour from three varieties were weighed then put into erlenmeyer, then ethanol was added. The ratio of ethanol to banggai sweet potato flour consists of a ratio of 5: 1 (A), 7: 1 (B), 9: 1 (C), 11: 1 (D), 13: 1 (E), 15: 1 (F) , 17; 1 (G), on the basis of volume/weight (v/w). Each treatment was repeated five times so that there were thirty-five experimental units. Extraction used agitation mixer 200 rpm for 2 hours. The mixture was then filtered to obtain a banggai sweet potato flour ethanol extract. The extract obtained was concentrated by vacuum using a rotary evaporator. The parameters observed were phenolic levels of banggai sweet potato flour by using the spectrophotometric method. The ratio of ethanol to banggai flour which produces the highest phenolic content is expressed as the ratio of ethanol to selected banggai flour and is used in the extraction time determination stage.

The extraction time applied consisted of 1 hour (A), 2 hours (B), 3 hours (C), 4 hours (D) and 5 hours (E). Each treatment was repeated five times so that there were twenty-five experimental units. The parameters observed were phenolic levels of banggai sweet potato flour by using the spectrophotometric method. The extraction time which produced the highest phenolic content of 3 varieties of banggai sweet potato was used in the antioxidant activity test.

Antioxidant Activity Test of Banggai Sweet potato Ethanol Extract

The resulting extract was determined to be 50 mL in volume each. Then the soybean oil mixture was made by mixing 0.2840 g of soybean oil, 0.2840 g of tween 20 and 50 ml of phosphate buffer pH 7.0 and was homogenized. After that, 0.8 mL was dropped from each concentration of the banggai sweet potato extract and added 1.0 mL of soybean oil mixture. The extract mixture and soybean oil mixture were incubated for 24 hours at 500C. Next, the incubated mixture was added with 10 mL of 75% ethanol, 0.2 mL of ammonium thiocyanate (30%) and 0.2 mL of ferrochloride (0.02 M in 3.5% HCl), then left for 30 minutes, and then absorbance measurement was carried out with a spectrophotometer at a wavelength of 510 nm. After that, a blank was made, which is a mixture without using extraction of banggai sweet potato consisting of 1.0 mL of soybean oil mixture and 10 mL of 75% ethanol, 0.2 mL of ammonium thiocyanate (30%) and 0.2 mL of ferrochloride (0.02 M in HCl 3.5%), and then absorbance measurement was carried out with a spectrophotometer at a wavelength of 510 nm. As a comparison, 200 ppm Butylated Hydroxy Toluent (BHT) synthetic antioxidant was used, with the same working procedure as using the proud yam extract.

Antioxidant activity was determined by using the following equation:

$$\text{Antioxidant activity (\%)} = 1 - \frac{A\,sample}{A\,blank} \times 100\%$$

Data Collection Technique

Determination of Banggai Sweet potato Phenolic Levels (SNI in Leman, 2009)

a. Make a standard curve

Pure phenol weighed as much as 1 g then put into a beaker and distilled water about 100 mL was added, then stirred until all phenols dissolved. Phenol solution was transferred quantitatively into a 1 liter measuring flask and adjusted in volume with distilled water. The phenol solution was transferred with a volume pipette, then put into a 50 mL volumetric flask

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and the volume was adjusted with distilled water. This solution was diluted to obtain a standard solution of phenol with a concentration of 5 ppm, 10 ppm, 15 ppm, 20 ppm and 25 ppm, in a 50 mL volumetric flask. Each solution was transferred to a 100 mL erlenmeyer then heated in a water bath for 5 minutes, then 1-2 drops of the methyl orange (MO) indicator was added to form a yellow color. The solution was then added with 2-3 drops of 1: 9 phosphoric acid to form an orange red color. The cooled solution was then added 1.2 mL NH_4OH 0.5 N, then the pH of the solution was adjusted to 7.9 ± 0.1 with a phosphate buffer (pH = 12). The absorbance of each solution was measured at the maximum wavelength (460 nm) based on the measurement results of the absorption spectrum of 10 ppm phenol standard solution. The measurement results of absorption were plotted in the relationship curve between absorbance and phenol concentration to produce a standard curve.

b. Sample Analysis

Banggai sweet potato flour extract obtained from the previous stage, then put into a 100 mL erlenmeyer then heated in a water bath for 5 minutes, then 1-2 drops of methyl orange (MO) indicator was added to form a yellow color. The solution was then added 2-3 drops of 1: 9 phosphoric acid to form an orange red color. The cooled solution was then added 1.2 ml of NH_4OH 0.5 N, then the pH of the solution was adjusted to 7.9 ± 0.1 with a phosphate buffer (pH = 12). The sample was then measured at the maximum wavelength (460 nm). The total phenolics of the sample were determined by the formula:

$$\text{Phenolic levels (\%)} = \frac{\text{X (mg/1000 mL)x volume of sample (ml)}}{\text{Sample weight (mg)}} \times 100\%$$

Description : X = Phenolic concentration (mg/1000 ml)

Antioxidant Activity Test

Antioxidant activity was determined by using the following equation:

$$\text{Antioxidant activity (\%)} = 1 - \frac{A\,sample}{A\,blank} \times 100\%$$

Data Analysis Technique

Data obtained in the study were analyzed with variance, if there was influence between treatments, HSD test was performed at a significant level α = 5%.

3. RESULTS AND DISCUSSION

Phenol Standard Curve

Total phenolic content in banggai sweet potato extract from various varieties was obtained by using spectrophotometric method. The abosorption of the phenol standard solution between 5 ppm and 25 ppm concentrations with a 5 ppm concentration interval are presented in Figure 1. The relationship between absorbance of phenol concentration followed a linear curve with the equation Y = 0.017x + 0.111, and the regression coefficient (R^2) was close to 1, that is 0.999 (Figure 1). This shows the linearity of absorption and concentration was quite high.

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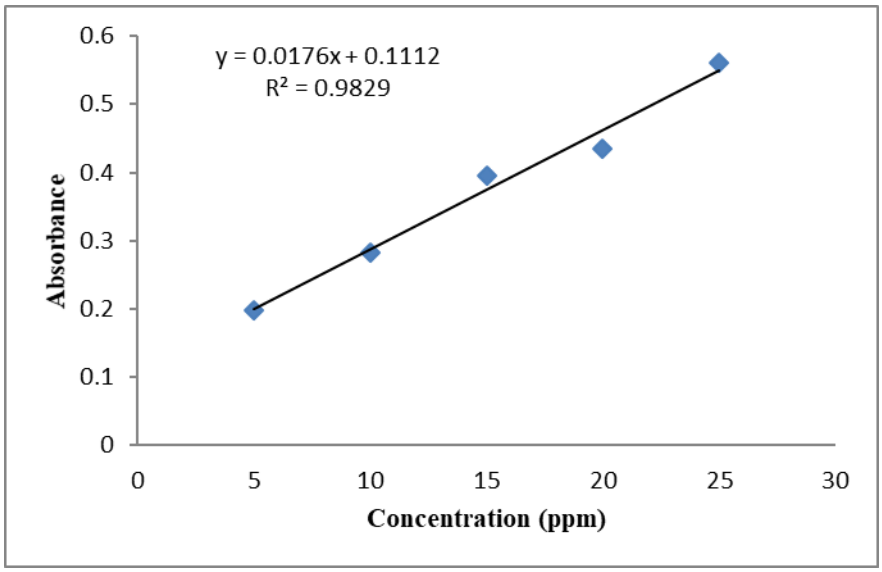

Figure 1. Phenol standard curve at 460 nm wavelength (Total Phenolic Levels in Various Banggai Sweet potato Flour Ratios/Ethanol Extractors).

One factor that affects the extract yield in the extraction process is the relative concentration of extractor to the amount extracted or the ratio between the extractor to the extracted material. Based on that, phenolic compound extraction of the banggai sweet potato by extracting ethanol at various ratios of ethanol extraction from the banggai sweet potato flour. The highest total phenolic levels for the three varieties of proud banggai sweet potato (purple banggai sweet potato, yellow banggai sweet potato, and white banggai sweet potato) were found at a ratio of 15: 1 on the basis of volume/weight (v / b), but total phenolic levels differed between varieties of banggai sweet potato (Figure 2). White banggai sweet potato produced ethanol extract with the highest phenolic content of 2.63% then purple banggai sweet potato with 2.44% phenolic content and finally the lowest phenolic content was found on banggai sweet potato of 1.90%

The results of the study by Fitrya et al (2010) stated that the ethyl acetate fraction of Gandaria plant bark (Bouea macrophylla Griff), 10 mg of white crystalline compounds were isolated (Fitrya et al., 2010). Based on spectroscopic analysis and phytochemical tests by reacting FeCl3 color compounds it was suspected that these compounds are phenolic compounds substituted by aliphatic and carbonyl groups. The results of the quantitative analysis showed that p-hydroxybenzoic acid had the highest percentage among the four types of phenolic acids that had been identified.

The difference in phenolic levels between the banggai sweet potato varieties is thought to be caused by differences in the variety related to the chemical composition of the tubers due to genetic factors such as species. Yellow banggai sweet potato expected to have relatively high carotenoids compared to other banggai sweet potato. For comparison the yellow banggai sweet potato (Ipomoea batatas L.) contains beta-carotene of 245.30 ug - 533.80 ug (Soplanit, 2007). Purple banggai sweet potato contains relatively higher content of flavonoid that yellow banggai sweet potato. The largest group of phenolic compounds are flavonoids, which are compounds

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that are generally found in all types of plants and anthocyanins in purple tubers which is flavonoid compound. With high flavonoid content, the phenolic content is relatively higher compared to yellow banggai sweet potato, but relatively lower than white banggai sweet potato.

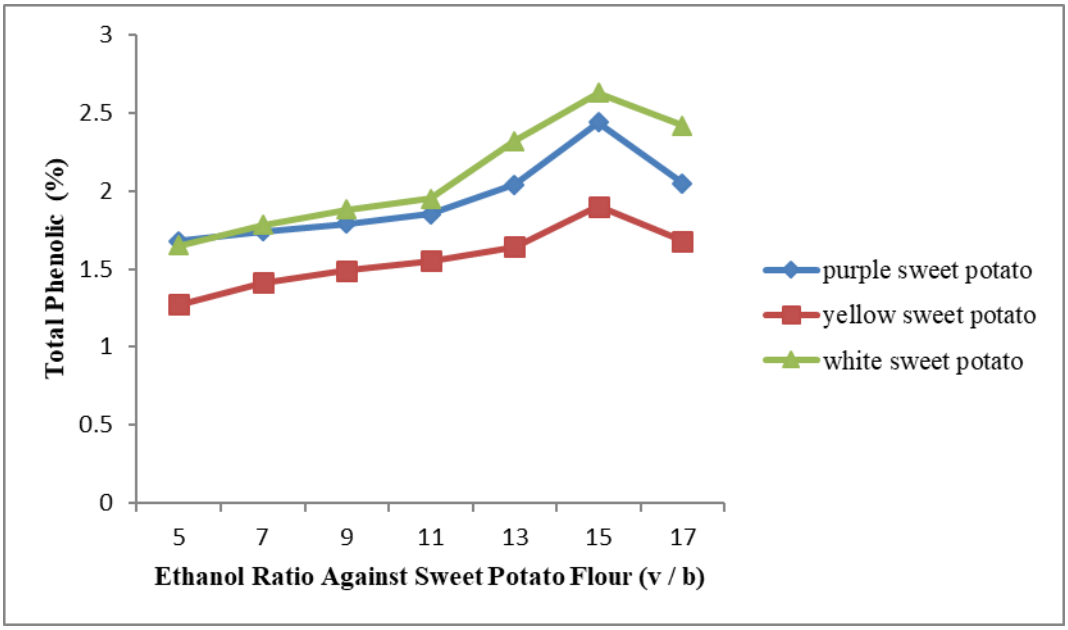

Figure 2. Curve of Total phenolic on Ethanol Ratio Against Sweet Potato Flour

The pattern of changes in phenolic levels at various ratios for all varieties of banggai sweet potato followed a parabolic curve line that showed an increased phenolic levels and decreased phenolic levels (Figure 2). Ethanol extraction at a ratio of 5: 1, 7: 1, 9: 1 and 11: 1 were not optimal to penetrate in sweet potato flour, as a result, the phenolic content dissolved was also not optimal. In the 13: 1 ratio, an increase in the level of dissolved phenolics had not yet reached its optimum level. The highest total phenolic content for all types of sweet potato was found in the ethanol ratio of 15: 1 because in this ratio the sweet potato flour reached optimum levels of phenolics.

The ratio of ethanol/sweet potato flour had a very significant effect on phenolic levels. The results of further analysis with a 5% level HSD test showed the ratio of ethanol/banggai sweet potato 15: 1 (v / b) was different from the ratio of ethanol/banggai sweet potato for all types of banggai sweet potato.

Total Phenolic Levels at Various Extraction Times

Another factor that also affects the phenolic content of the extraction is the extraction time. To find out the extraction time that produces extracts with high phenolic content, the effect of extraction time on the phenolic content was applied. The highest phenolic levels for all types of sweet potato were found at extraction time of 2 hours. White sweet potato with the highest phenolic content (2.62%) was found at extraction time of 2 hours, and the lowest phenolic content (1.55%) was found at extraction time of 1 hour. Purple sweet potato with the highest

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phenolic content (2.39%) was found in the use of extract time of 2 hours, and the lowest phenolic content (1.15%) was found at the extraction time of 5 hours. Yellow sweet potato with the highest phenolic content (1.90%) was found at extraction time of 2 hours, and the lowest phenolic content (1.13%) was found at extraction time of 5 hours.

The lowest phenolic content of white banggai sweet potato at 1 hour extraction time was suspected because white banggai sweet potato requires longer extraction time to dissolve the phenolic compounds. While the lowest phenolic content of purple and yellow sweet potato was suspected to be caused by extraction time of more than 2 hours resulting in phenolic compounds which lead to decomposition. Polyphenolic compounds such as phenolics and flavonoids and tannins will be easily oxidized in the presence of oxygen to form ortho-quinone radical compounds.

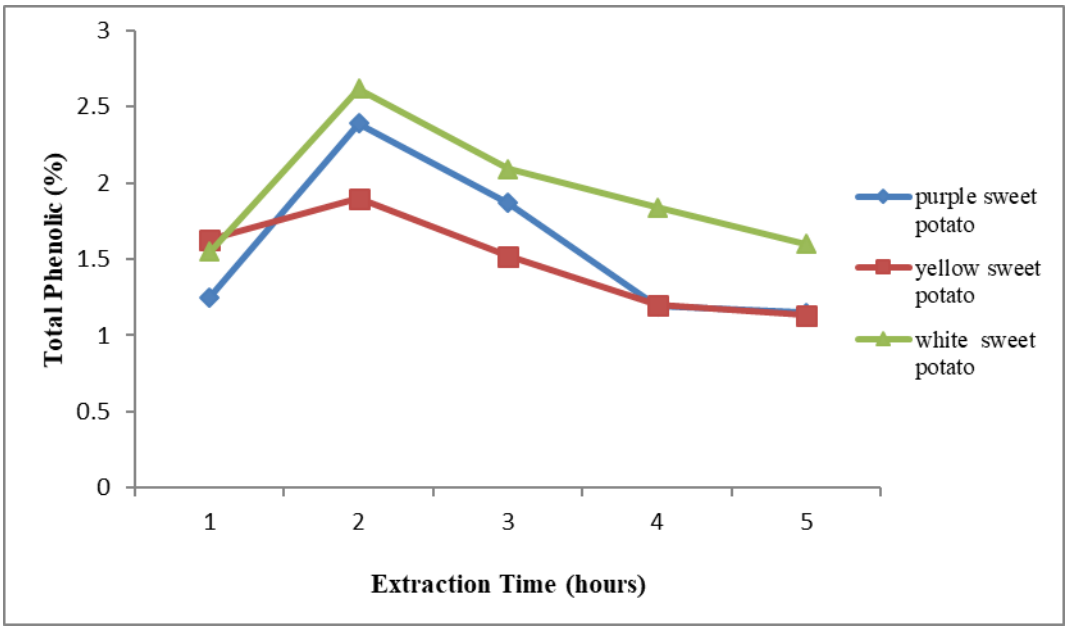

Figure 3. Curve of Total Phenolic on Extraction Time

Changes in total phenolic levels to reaction time followed the pattern of changes in parabolic curve lines where the optimum extraction time occurs at the reaction time of 2 hours (Figure 3). The pattern of changes in total phenolic levels to the extraction time showed that the longer the extraction, the more phenolic obtained and reached its optimum point at the extraction time of 2 hours where the phenolic extraction process reached its optimum point and then decreased until the extract was saturated with ethanol.

The pattern of changes in phenolic levels to the reaction time that followed the parabolic curve indicated that phenolic compounds are easily oxidized during the extraction process. Phenolics in all three tuber varieties had undergone oxidation after extraction time of 2 hours. Factors causing phenolic damage during extraction are thought to be caused by light factors which cause phenol compounds to be oxidized by certain enzymes resulting in phenolic decomposition. Besides that, ethanol as an extractor also evaporates, thus affecting the phenolic content of the extract produced.

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Analysis of variance showed that extraction time had a very significant effect on phenolic levels. Further analysis with a 5% HSD test showed phenolic levels differed between extraction times. This reinforces the assumption that the pattern of changes in phenolic levels to the extraction time follows a parabolic curve.

Antioxidant Activity of Banggai Sweet Potato Ethanol Extract

Phenolic compounds derived from the flavonoid group or from the polyphenol group act as antioxidants. However, its antioxidant activity is largely determined by the type of phenolic compound. Phenol compounds are compounds that are found in all types of plants (Anwariyah, 2011).

The extraction results from 3 varieties of banggai sweet potato showed that the three varieties contained phenolic compounds with the highest content in white sweet potato then followed by purple sweet potato and yellow sweet potato. White sweet potato was suspected to contain natural phenol compounds which are known to have more than a thousand structures with flavonoids as the largest class. Most of the flavonoid compounds are found in the form of glycosides, which are a combination of a sugar and alcohol that bind to each other through glycoside bonds (Anwariyah, 2011).

The activity test of the banggai sweet potato extract with the highest phenolic content was performed which was then compared with the synthetic antioxidant activity commonly used in food processing, namely 200 ppm Butylated Hydroxy Toluent (BHT). The results obtained showed that the highest antioxidant activity (75.75%) was found in BHT, and the lowest antioxidant activity (37.19%) was found in yellow sweet potato.

Antioxidant activity is in line with phenolic content, because the highest antioxidant activity (74.87%) was found in white sweet potato, followed by purple sweet potato (61.17%) and finally yellow sweet potato with antioxidant activity at 37.19% (Figure 5). Phenol compounds that have antioxidant activity usually have the -OH and -OR groups such as flavonoids and phenolic acids. Phenol compounds can function as antioxidants because of their ability to eliminate free radicals and peroxide radicals so that they effectively inhibit lipid oxide. Besides that, it is thought that there are other phenolic compounds found in white sweet potatoe so that they can increase their antioxidant activity.

Purple sweet potato and yellow sweet potato have relatively lower antioxidant activity because the phenolic content of the two sweet potato varieties is also relatively lower than white sweet potato. The extraction of phenolic compounds with ethanol extractor influenced their antioxidant activity because the use of extracters that are not in accordance with the characteristic of the extracted material can influence the measurement of the amount of antioxidant compounds and their activity.

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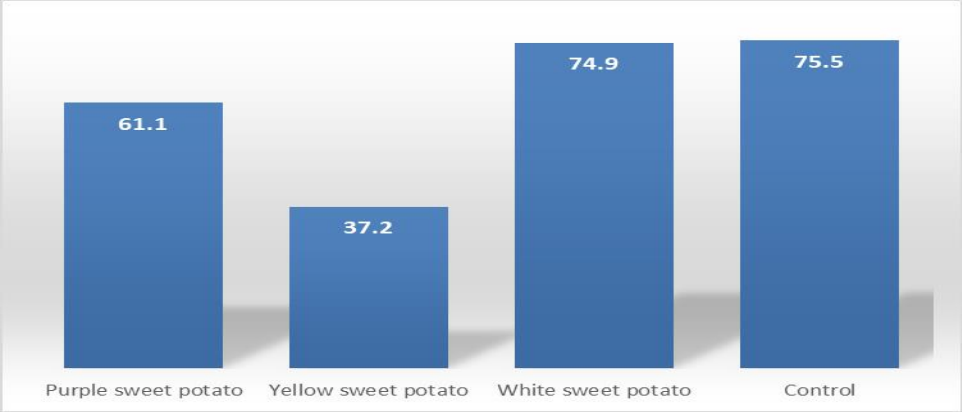

Figure 5. Histogram of Banggai Sweet Potato Antioxidant Activity and BHT

Banggai sweet potato varieties had a significant effect on antioxidant activity. The results of further analysis with a 5% HSD test showed different antioxidant activities between sweet potato varieties. This indicated that the phenolic level of sweet potatoes is in line with their antioxidant activity.

The antioxidant activity of white sweet potato extract iwa not different from the antioxidant activity of BHT. Thus, white sweet potato is a relatively high producer of natural antioxidant compounds. Natural antioxidant compounds in plants are generally phenolic compounds that can be in the form of flavonoids, cinnamic acid derivatives, coumarin, tocopherols, and polyfunctional organic acids. Natural antioxidant compounds namely polyphenol and flavonoid are relatively polar and play an active role as antioxidants. As an antioxidant, flavonoids have the ability to capture free radicals and as metal ion complexing (Cherrak et al., 2016).

The antioxidant activity of sweet potatoes is determined by the content of flavonoids, while flavonoids is included into phenolic compounds. Total phenolic content of sweet potato extraction is highly correlated with antioxidant activity (Everette & Islam, 2012). Antioxidant activity varies between sweet potato varieties and the color intensity of sweet potatoes tends to be in line with their antioxidant activity (Teow et al., 2007).

4. CONCLUSION

The ratio of ethanol to sweet potato flour from various varieties that produce the highest phenolic content was at a ratio of 15: 1 (v/w). The extraction time of sweet potato flour from various varieties that produce the highest phenolic content was at the extraction time of 2 hours. White sweet potato contained highest phenolic (2.63%) followed by purple sweet potato (2.44%) and yellow sweet potato (1.90%). White sweet potato had the highest antioxidant activity (74.87%) followed by purple sweet potato (61.17%) and yellow sweet potato (37.19%).

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