Analysis of phenolic content and antioxidant activity of cocoa pod husk (*theobroma cacao* l.)

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Abstract. This research has been conducted to measure the total of phenolic content and the test of antioxidant activity in cocoa pod husk. Cocoa pod husk extract was obtained by maceration using 2 kinds of solvents, they are 70% ethanol and acetone: water (7: 3). Folin Ciocalteu method was used to measure the total of phenolic compounds and DPPH (1,1-diphenyl-2-pikrilhidrazil) method was used to measure the antioxidant activity. The results obtained, the total phenolic content of 70% ethanol extract and acetone extract: water (7: 3) respectively had the following values: 94.92 GAE/g, 49.92 GAE/g. while the antioxidant activity of 70% ethanol extract and acetone extract: water respectively had the following values: 88.16%, 44.11%.

1. Introduction

Antioxidants are electron donors or reductants. This compound has a small molecular weight, but is able to inactivate the development of oxidation reactions, by preventing radical formation. Antioxidants are also compounds that can inhibit oxidation reactions, by binding to free radicals and highly reactive molecules (Winarsi, 2007).

In the nature, there are abundant of antioxidants, according to (D Sarastani, ST Soekarto, 2002), many foods that can be a source of natural antioxidants, such as spices, tea, chocolate, leaves, seeds, vegetables, enzymes and proteins. The source of natural antioxidants are dominated by plants and generally contains phenolic compounds that are spread throughout of the plant parts.

Cocoa pod skin is the part of mesocarp or part of the cocoa fruit wall, which includes the outer shell until the pulp before the collection of seeds. Cocoa pods is the largest part of cocoa fruit (75.52% of fresh cocoa fruit). Every years cocoa bean production have been increase, this results in increased waste of cocoa pods (Research Center for Coffee and Cocoa). Cocoa pods have not been used optimally, and most of them are still cocoa plantation waste because they are only collected in holes and then dumped or disposed of around the cocoa plants. For this reason, it is necessary to looking for the ways to use cocoa pods becomes more efficient and has a higher economic value.

The part of Cocoa pods skin consists mostly of polysaccharides (cellulose and hemicellulose) and lignin, and a small portion consists of phenolic compounds, tannins, purine alkaloids, and cocoa butter (Jusmiati, Rusli, & Rijai, 2015). Based on the chemical composition of the cocoa pods, it was suspected that the cocoa pods had antioxidant activity. The cacao fruit skin used in this study is the

skin of cooked cacao fruit. The aim of the study was to determine total of phenolic and test the free radical scavenging activity of ethanol extract and acetone extract: water (7: 3) on the skin of cocoa pods (*Theobroma cacao* L).

2. Method

2.1. Materials

The ingredients used are cocoa pod husk taken from Majene Regency (West Sulawesi), petroleum ether, ethanol, aquadest, sodium carbonate (Na2CO3), FolinCiocalteu reagent, DPPH (1,1 diphenyl-2-picrylhydrazyl) and acetone.

2.2. Tools

The tools used are laboratory glassware, magnetic stirrers, ovens, vortices, water baths, analytical scales, modified evaporators, spectrophotometers UV-Vis Milton Roy 501.

2.3. Preparation of Sample

Sorting the cocoa pods and then dry it in an oven at 55ºC for 72 hours until the sample water content is + 10%. Smoothed the sample size to 60 mesh. Sample extraction (maceration) was prepared as much as 500 grams of sample then extracted using acetone solvent: water (7: 3) and 70% ethanol with a sample-solvent ratio (1: 3) for dry samples for 48 hours. Then the filtrate is separated and the residue is extracted with filter paper. The filtrate was taken and then concentrated with a rotary evaporator at a temperature of 45ºC until a thick extract of cocoa pod husk was obtained.

2.4. Determination Total of Phenolic Content

According to (Singleton and Rossi, 1965), Determination total of phenolic content has been modified were each of 0.1 mL of 200 µg/mL of cocoa fruit peel extract was put into a test tube, and added 0.1 Folin Ciocalteu reagent 50% solution and then vented for 1 minute. The solution was added 2 mL of sodium carbonate solution (Na_2CO_3) 2%. This mixture is stored in a dark room for 30 minutes. The absorbance of the extract solution was read at a wavelength of 750 nm with a UV-Vis spectrophotometer. The results are expressed as mg gallic acid/g extract.

2.5. Determination of Antioxidant Activity of Cocoa Pod Husks Extract

Determination of antioxidant activity of extracts cocoa pods using DPPH. 0.5 ml of 200 µg/ml of cocoa pods extract were poured into a test tube and 2 ml of 0.2 mM of DPPH solution were added. The blank solution is made by 0.2 mM of DPPH solution as much as 2 ml pipette and then put into a test tube and added with 0.5 ml ethanol. DPPH absorbance was measured by UV-Vis spectrophotometer at a wavelength of 517 nm, after incubation in a room without light for 30 minutes. Antioxidant ability was measured as a decrease in DPPH solution absorption due to the addition of samples. The uptake value of DPPH solution before and after the addition of the extract was calculated as a percent of antioxidant activity with the following formula:

$$\% \text{ Antioxidant activity} = \left[1 - \frac{Asample}{AControl} x\ 100\right] \quad (1)$$

Were, A control = Absorbance does not contain a sample
A sample = Absorbance of the sample

3. Results and Discussion

3.1. Total Phenolic Content of Cocoa Pod Husk Extract

Determination of total phenolic extracts of cocoa pod husks was conducted to determine the potential as an antimicrobial. Analysis of total phenolic acid using standard Gallic Acid Solution The use of gallic acid as a standard solution because gallic acid is a derivative of hydroxybenzoic acid which is

classified as a simple phenolic acid (Lawalat *et al*., 2012). Total phenolic of each extract expressed as Gallic Acid Equivalent (GAE). GAE is a common standard to measure the phenolic compounds contained in a substance (Brinkmann & MüllerGoymann, 2003). From the calculation of the data, acetone extract had the highest total phenolic which was 94.92 GAE / g extract compared with ethanol extract (49.92 GAE / g). Solubility of phenolic compounds depends on the solvent used. Cocoa pod husks extract with the highest phenolic levels found in an extract of acetone: water (7: 3), while the lowest levels were extracted in ethanol 70%. Phenolics are bound to a sugar molecule at position 3-O-glycoside will tend to dissolve in polar solvents. hydrogen bonds between acetone O atoms and H atoms from glycosylated phenolic compounds also affect solubility. The presence of a methyl group which is an electron activator causes O atoms to become rich in electrons. Thus the hydrogen interaction with phenolic compounds become easier. Although the same thing occurs in acetone 70%, but with the amount of water available, possible interactions of hydrogen tends to occur between acetone and water. In ethanol, H atoms in the solvent would reduce the possibility of hydrogen interaction with the sample for a stronger interaction with O atoms in the molecule itself. This will reduce the possibility of hydrogen bonding with H atoms from the sample phenolic OH group (Agustin *et al*., 2016). Hydrogen bonding will occur between oxygen atoms have a free electron pair of phenolic compounds and hydrogen atoms solvent or vice versa. Phenolic still bound to a sugar molecule in a polar cocoa fruit skin will tend to dissolve diluted with distilled water. Because glycosides that are bound have a good solubility in water, when compared to the pure glycosides, the type of solvent that would be able to extract more phenolic compounds (Khotimah, Darius, and Sasmito, 2013). Data total phenolic content can be seen in Figure 1.

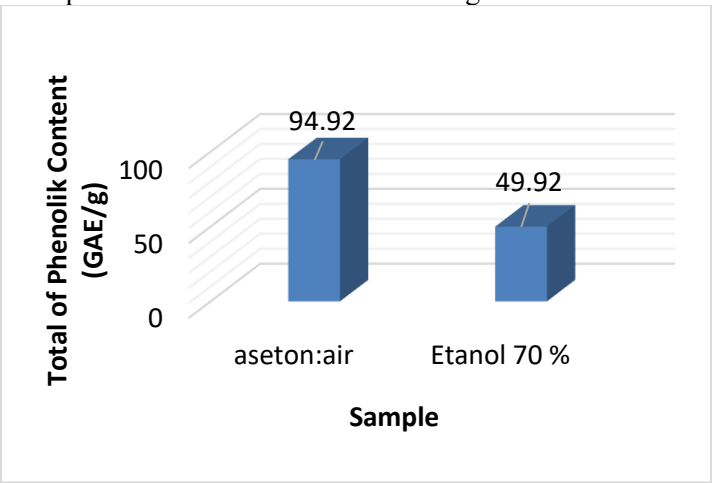

Figure 1. Diagram of total phenol content in cocoa pods husks using two solvents

3.2. Antioxidant Activity of Cocoa Pods Extract

Antioxidant testing is a parameter that can describe the percentage ability of a substance to inhibit free radicals. DPPH test is one method of measuring antioxidant activity in foodstuffs. Measurement of total antioxidants helps to understand the functional properties of foodstuffs.

Observation results of antioxidant activity of cocoa pods extract using acetone: water and extract ethanol 70% showed that the antioxidant activity of acetone : water extract of cocoa pods husks was higher than ethanol 70% extract of cocoa pods husks, show in Table 1.

Table 1. Antioxidant activity of Cocoa podsextract

Sample	Persentage of inhibiting of DPPH (%)	Capacity of antioxidant (mg/L)
Ethanol	61,583 ± 0,0045	58,014 ± 0,0039
Aceton : water	69,190 ± 0,0031	63,230 ± 0,0014

Antioxidants are additional ingredients used to protect unsaturated food components (having double bonds), especially fats and oils. The potential of cocoa as a source of antioxidants is quite large, given the high content of polyphenols. There are three components of polyphenols in cocoa, they arecatechins (37%), anthocyanins (4%) and proanthocyanidines (58%). The mechanism of catching free radicals by polyphenols is by releasing hydrogen atoms from their hydroxyl groups (Stephen *et al.,* 2011).

Cocoa has the potential as a natural antioxidant. Cocoa contains lignin, phenolic, tannin and alkaloid compounds which are components of antioxidant compounds(Jusmiati *et al*., 2015). Antioxidant activity in extracts of cocoa pods was measured by spectrophotometer using DPPH method. It used of DPPH method, because this method is simple, easy and uses a small number of samples and a short measurement time(Jusmiati *et al*., 2015). DPPH radical capture capability by an antioxidant is expressed by the percentage of radical capture. Higher values indicate that the sample of compounds used is indeed potential as an antioxidant(Jusmiati *et al*., 2015). Based on the research that has been done, the addition of DPPH solution in the sample is marked by the change of purple to yellow which means the process of catching free radicals.

In Table 1 shows the inhibition percentage of acetone extract is greater than methanol extract as well as greater antioxidant capacity of acetone solvents used to extract cocoa pods. DPPH free radical scavenging activity of acetone extract of cocoa pods is determined by various antioxidant compounds found in cocoa pods. Acetone extract tends to have DPPH damping activity greater than ethanol extract, this is due to the content of phenol compounds which are easily soluble in acetone compared to ethanol. Whereas the ethanol extract is probably caused by the content of phenol and betasianincompounds which are more easily partitioned into ethanol than in acetone. To find out the active compounds that provide antioxidant activity, further research is needed in the form of screening and isolation of active compounds guided by activity test guided isolation.

Antioxidant activity is caused due to the presence of phenolic compounds. According to (Nakibogl*uet al.*, 2007) the ability of DPPH free capture is strongly influenced by OH groups contained in phenolic compounds. The difference in the activity of phenolic antioxidants is determined by the chemical structure, the number and position of the hydroxy and methyl groups in the ring. The more hydroxyl groups substituted in the molecule, the stronger the ability to capture free radicals because more and more hydrogen atoms can be donated(Lin*et al.*, 2009).

4. Conclusion

Acetone extract: water of cacao pods has a higher of total phenolic content and antioxidant activity of 94.92 mg / kg and 69.190% compared to ethanol extract of cocoa pods which has a total phenol content of 49.92 mg / kg and antioxidant activity of 61.583%.

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