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ANALYSIS PHENOLIC COMPOUND AND ANTIOXIDANT ACTIVITY OF ETHYL ACETAT PURIFICATION EXTRACT OF SAWO MANILA (*Manilkara zapota* L.) LEAFS

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Abstract

Antioxidant utilization can reduce oxidation stress or as antioxidant substance in food product. Natural antioxidant is more safe than synthesis antioxidant because it is toxic and carsinogenic. The plant is source of natural antioxidant where it is related by content it's polyphenol compound. Antioxidant activity of extract ethanol of *Manilkara zapota* L. (chiku) (sawo manila) had been researched. The different of solvent and purification process displays the different of antioxidant activity. So the aim of this research are determination phenolic compound and antioxidant activity of ethyl acetat purification extract of *Manilkara zapota* L. (chiku) (sawo manila) leafs. This research had been done and acquired the yield value of ethyl acetat purification extract from extract ethanol *Manilkara zapota* L. (chiku) leafs is 2,524 %, its phenolic compound is 103,827 mg equivalen gallic acid/g extract , and IC_{50} antioxidant activity is 124,516 ppm.

Key word : : Antioxidant, Phenolic, Sawo Manila leafs, and purification extract

Introduction

Antioxidant utilization can reduce oxidation stress that relate to disease. Beside that antioxidant can be used as radical preventive if it is added to the food, so can postpone or obstruct of oxydation prosess and increase life time with obstruct peroxidation prosess of lipid. Currently, maker and user of antioxidant more interest to natural antioxidant, cause it's safety. While synthesis antioxidant such as butyl hydroxilanisol (BHA) and tetra butyl hydroquinone (TBHQ) have toxic and carsinogenic feature, where using TBHQ in Japan and Europe had been forbided (1,2).

Source of natural antioxidant can be obtained from a plant, may be from all of plant or one part of plant, such as fruit, seed, leaf, stem, bark , or root, but commonly, had be reported that leaf is the best choise to antioxidant test (3). Several researce had reported that content polyphenol compound of plants have the important role in antioxidant activity and many study Pharmacology and Biochemist had reported that polyphenol compound from a variety plants useful for health (1,2).

Manilkara zapota L. (chiku) is a plant from sapotaceae family. This plant had been used in natural therapy system to lots of disease. The different parts of this plant sucs as leaf, stem, and seed, had been much deeper researched about antimicrobe activity, where it's leaf had been determined have antioxidant activity (4). Some research had been done to determine antioxidant activity of extract this plant, such as extract ethanol of leaf this plant given radical DPPH scavenging with IC50 value is 68,27 μg/ml (5).

Beside that, had been researched about using the different solvent, where water extract, toluene extract, and aceton extract, given IC50 value of radical DPPH scavenging activity, respectively are 160 μg/ml, 93 μg/ml, and 20 μg/ml. This fact exhibited that the different solvent showed the different antioxidant activity. It is caused by every solvent will extract the different content of fitochemist compound (3), and purification process of crude extract given the different fitochemist compound, where can be done by use liquid-liquid partition method or solid phase extraction (SPE) (6).

By virtue of, so we was interested to further develop the result of extract ethanol *Manilkara zapota* L. (chiku) research by using extract purificated. This case was done caused consideration that quantity content of lipid or non polar compound in crude extract could be lose, so using this extract become more little with active compound many more. Result of this research was expected would produce extract purificated ethyl acetat of *Manilkara zapota* L. (chiku) as natural antioxidant with better activity, so this research would be done to analysis phenolic content and antioxidant activity of ethyl acetat purification extract of *Manilkara zapota* L. (chiku).

Materials

Sawo Manila Leaf (*Manilkara zapota L.* (chiku)), Folin-Ciocalteu's Reagent and Gallic acid was purchased from Merck, 1,1-Diphenyl-2-Picryl Hydrazyl (DPPH) was purchased from Sigma.

Procedures

1. Sample Preparation
   This research used sawo manila leafs (*Manilkara zapota* L. (chiku)) as sample. It obtained from Sidrap regency, south Sulawesi, with simple random sampling method. This sample was prepared by way it were washed with tap water and dried, then were ground into fine powder.
2. Extraction Procedure
   The sample powder (70 gram) was extracted by maceration method with ethanol at room temperature for 3 days. After that it were filtered and residue was re-extracted with equal volume of solvents. Supernatants were combined and evaporated to dryness, after that stored in refrigerator to further using
3. Purification Extract Procedure
   Crude ethanol extract (2 gram) were soaked with 100 mL boiling water then cooled and stored in refrigerator for 12 hours. Extract were filtered and supernatants were liquid-liquid partitioned with ethyl asetat (3 x 100 mL). The ethyl asetat extracts were evaporated to dryness and then it was called purification extract (6,7).
4. Determination of Phenolic Compound
   The concentration of phenolic was determination by using Folin reagent. Ethanol solution of purification extract in the concentration 400 ppm was used in the analysis. The reaction mixture was prepared by mixing 300 μL of ethanol solution of purification extract, 1.2 mL of Folin-Ciocalteu's reagent in water (1 : 10) and 1.5 mL of 7% $NaHCO_3$. The mixture samples were incubated at room temperature for 30 minutes. The absorbance was determined using spectrophotometer at λmax = 756 nm. The samples were prepared in triplicate and the concentration of phenolic was expressed in terms of gallic acid equivalent (mg of GA/g pf extract) (8).

5. Determination of Antioxidant Activity

The purification extract solution were prepared the final concentration 120, 140, 160, and 180 ppm in methanol. It (1 mL each) were added in the mixture solution of 1 mL of 0.4 μM DPPH and 1 mL of methanol. The mixture was shaken and left to stand at room temperature in the dark for 10 minutes. Absorbance was measured at 520 nm against a blank containing all reagents except the test sample (1). Vitamin C was used as the positive control. The percentage of inhibition of DPPH (I%) was calculated using the following equation:

$$I\% = \frac{A_b - A_s}{A_b} \times 100\%$$

A_b is the absorbance of the blank solution and A_s is the absorbance of sample solution. The IC_{50} was obtained from a graph of I% versus extract concentration.

Results and Discussion

Ethanol extract sawo manila leafs had been researched given antioxidant activity, so this research use ethanol to obtain crude extract. The crude extract was obtained 3.529 gram from 70 gram dried sample leafs. From the crude extract was done purification extract process with use liquid-liquid partitioning method. This method is most used, simple, and cheap, but the result is not significant with solid phase extraction (SPE) method (6). Some solvent is most used to extract phenolic compound were ether, chloroform, aceton and ethyl acetat, where for this research was used ethyl acetat.

This extract was called purification extract cause when was done liquid-liquid partitioning process, occur separation between nonpolar and polar compound. To remove non polar from polar compound was used hot water, and to obtain phenolic compound from water, used ethyl acetat by way liquid-liquid partitioning. This research acquired the yield value of ethyl acetat purification extract was 2.524 %.

Several research had notified that phenolic compound have important role to antioxidant activity, so this research was done to determine concentration of phenolic. It was done by using Folin reagent method, with it's mechanism is reduction of Folin reagent by phenolic compound to blue compound. Absorbance result of reaction was measured by using spectrophotometer UV-Vis with max wavelength 756 nm (9). Calculation of concentration of phenolic compound can be see at the following table 1 :

Table 1. Calculation of phenolic concentration of ethyl acetat purification extract

Rep.	Absorbance	Phenolic concentration (ppm)	Mean of Phenolic concentration (ppm)	Phenolic concentration of ethyl acetat purification extract
1	0,558	45,111	41,531	103,827 mg GAE/gram extract
2	0,498	39,556		
3	0,502	39,925		

Whereas for antioxidant activity of ethyl acetat purification extract was done by using scavenging 1,1-Diphenyl-2-Picryl Hydrazyl (DPPH) method. It's mechanism is donor proton a substance to DPPH radical compound become diphenylpicrylhydrasine non radical compound.

This reaction is marked by alteration of purple colour of solution to yellow colour and it's absorbance would be measured at max wavelength 520 nm. The ability of antioxidant activity of ethyl acetat purification extract was stated in IC_{50} (10). Determination of antioxidant activity of Vitamin C and ethyl acetat purification extract can be see in the following table 2 and 3:

Table 2. Determination of antioxidant activity of Vitamin C

No.	Vitamin C concentrastion (ppm)	Absorbance	Absorbance blank (DPPH)	Inhibisi Persentage (%)	IC_{50} (ppm)
1	4	0,768	0,905	20,110	9,618
2	6	0,633		30,055	
3	8	0,525		40,989	
4	10	0,448		50,497	

Table 3. Determination of antioxidant activity of ethyl acetat purification extract

No.	Vitamin C concentrastion (ppm)	Absorbance	Absorbance blank (DPPH)	Inhibisi Persentage (%)	IC_{50} (ppm)
1	120	0,472	0,905	47,845	124,516
2	140	0,396		56,243	
3	160	0,321		64,530	
4	180	0,293		70,939	

IC_{50} of Vitamin C was 9.618 ppm, shown that have the strong antioxidant activity, whereas IC_{50} of ethyl acetat purification extract was 124.516 ppm, shown that have the medium antioxidant activity. It is accord with parameter IC_{50} antioxidant activity by using DPPH, such as : very strong if < 50 ppm, strong if 51 – 100 ppm, medium if 101 – 150 ppm, low if 151 – 200 ppm, and very low if > 200 ppm. IC_{50} of ethyl acetat purification extract bigger than IC_{50} ethanol extract on the previous research by Islam M.R., et. al., it is caused by there were non phenolic compound have antioxidant activity in ethanol extract and did not soluble in ethyl acetat, beside that phenolic concentration in ethanol extract smaller than in ethyl acetat purification extract (5).

Conclusion

From this research, could be concluded that phenolic concentration of ethyl acetat purification extract was 103.827 mg gallic acid equivalent each gram extract and it's antioxidant activity was 124.516 ppm.

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