



Polyphenol total content, IC₅₀ and antioxidant activities of ethanol extract from some cocoa (*Theobroma cacao*) beans in South Sulawesi Indonesia

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ABSTRACT

Antioxidants are compounds that can prevent or inhibit the oxidation process. This study was conducted to determine the total polyphenol, test the antioxidant activity against free radicals (DPPH), determine IC₅₀ of ethanol extract from several cocoa (*Theobroma cacao*) clones that is KDI-3, PA-300, PA-310, and NA-34. The study samples were made with unfermented condition. Each clone was extracted with hexane and ethanol 70%. The results showed polyphenol total of ethanol extract from cocoa seed for KDI-3, PA-300, PA-310, and NA-34 are 84 mg/g, 65.92 mg/g, 86,11mg/g, and 49,25mg/g respectively. The IC₅₀ for KDI-3, PA-300, PA 310, and NA-34 are 9.30 pm, 8.50 ppm, 6.40 ppm, and 10.23 ppm respectively. The results described each clone of cocoa beans has a different total polyphenol content, antioxidant activity against free radicals, and IC₅₀, but they still showed the percentage inhibition of DPPH free radicals above 80 % at a concentration of 25 ppm and showed a very strong antioxidant activity with IC₅₀<50. The total antioxidant activity and the highest polyphenol were indicated by the PA-310 clone.

Keywords: Cocoa beans extract, DPPH, Antioxidant, IC₅₀

INTRODUCTION

One of the compounds have been reported to function as antioxidants is polyphenol. Most of the plants have polyphenols and flavonoids content. A study of *Schizostachyum lumampao* of Philippine bamboo has fenolik and flavanoid compound [1]. One of the plants in Indonesia that is rich in polyphenol compounds are cocoa (*Theobroma cacao*). Cocoa and its products are rich in polyphenols especially in flavonoids, a group that is widely available in various varieties of fruits, vegetables, tea and red wine. In cocoa can be found subgroup of flavonoids with names flavanols (flavan-3-ols) [2,3]. Cocoa beans are the raw materials of food and non-food products (pharmaceuticals and cosmetics). Cocoa beans as food raw materials differ in terms of post-harvest treatment with non-food raw materials. Food raw materials need fermentation process in order to obtain good taste while cocoa beans as raw material for non-food does not require fermentation process in order to obtain a high content of polyphenols. Base on research, the polyphenol/flavonoid compounds can act as antioxidants.

Antioxidants can be divided into primary antioxidant which has the function to prevent the formation of free radicals (eg. superoxide dismutase and glutathione peroxidase). Secondary antioxidant function to capture and neutralize free radicals (eg. Vitamin E, Vitamin C, B-carotene) and the antioxidant tertiary for example, an enzyme that repairs DNA (superoxide dismutase mentionin). Benefits of chocolate has gained the attention of a number of scientists and nutritionists since the last few years [4-5]. Season, altitude a growing influence polyphenol content of the plants [6]. Some plant have antioxidant activities. Research showed some medicinal plants in the treatment of diabetes in Thailand have antioxidant activity [7].

Polyphenols are compounds that can act as an antioxidant. Cocoa beans are one of the export commodity from South Sulawesi Indonesia that consists of several varieties/clones. Therefore, this study aims to determine phenolic

content and antioxidant capacity for some clones of cocoa from South Sulawesi Indonesia, KDI-3, PA-300, PA-310, and NA-34, against free radicals based on the parameters of IC_{50} and percent inhibition of free radicals.

EXPERIMENTAL SECTION

2.1 Tools and materials

A set of tools maceration, rotary and UV-Vis spectrophotometer. The research material consists of several varieties of cocoa beans (*Theobroma cacao*), which is obtained from one of cocoa industry in South Sulawesi, Indonesia. Other materials consist of a solution of 70% ethanol, methanol (p.a), and 1,1 diphenyl - 2 picrylhydrazyl (DPPH), BHT (sigma), Folin-Ciocalteu (E-Merch).

2.2 Preparation Material

Cocoa used consists of four varieties (clones) that are KDI-3, PA-300, PA-310 and NA-34, which was obtained from an industry in Makassar. Cocoa beans are made in conditions not fermented. Each sample has powdered of 250 g. It was extracted with 750 ml of n-hexane for 4 days. Then it was extracted with ethanol 70%. Ethanol extract was collected and then it was evaporated using a rotary evaporator.

2.3 Tannic acid solution preparation

0.5 g of tannic acid were weighed and dissolved in 100 ml flask (methanol: water, 6:4) then made dilution concentration of 25 ppm, 50 ppm, 100 ppm and 250 ppm.

2.4 Measurement of Polyphenols Total

Each sample was taken into a 0.2 ml cuvette. Then it was added 1 ml Folin-Ciocalteu reagent that has been diluted 10 times, and 0.8 ml of 7.5% sodium carbonate. It was homogenized and left to stand for 30 minutes. Each sample was measured absorbance with UV-VIS spectrophotometer at a wavelength of 765 nm [8-9].

2.5 Test Antioxidant activity by DPPH method

Extract from cocoa beans (KDI- clones) were made in the concentration of 5 ppm, 10 ppm, 25 ppm, 50 ppm, 100 ppm, and 200 ppm using methanol. 4 ml were taken from a concentration of 25 ppm, 50 ppm, 100 ppm and 200 ppm then added a solution of DPPH (1 mM in 1 ml of methanol). Furthermore, they were incubated for 30 minutes at 37°C. Absorbance was measured by UV-Vis spectrophotometer at a wavelength of 515 nm. The same way was made for cocoa clones PA-310, PA-300, NA-34 and BHT (control).

Measurement of percentage inhibition of extracts of cocoa beans against free radicals (DPPH) was calculated with the following formula :

$$\% \text{ Inhibition} = \frac{\text{Absorbance blank} - \text{Absorbance of samples}}{\text{Absorbance of samples}} \times 100\%$$

RESULTS AND DISCUSSION

Samples of cocoa bean were studied consisted of four clones that are KDI-3, PA-310, PA-300, and NA-34. The husk of cocoa bean was peeled then powdered. Cocoa beans clones were extracted with n-hexane to remove fat contained in cocoa beans, then extracted with ethanol 70%. Weight extract can be seen in Table 1.

Table 1. Weight of several extracts of Cocoa bean (*Theobroma cacao*) with ethanol 70 %

No	Sample code	Sample weight (g)	Extract weight (g)
1.	KDI-3	250	26.84
2.	PA-310	250	27.94
3.	PA-300	250	25.75
4.	NA-34	250	25.55

Table 2. The results of absorption measurements and polyphenol total of extract ethanol from several cocoa clones

No.	Sample code	Sample weight (mg)	absorption	Content of polyphenol total (mg/g)
1.	KDI-3	10	0.463	84
2.	PA-310	10	0.455	86.11
3.	PA-300	10	0.427	65.92
4.	NA-34	10	0.393	49.25

Determination of the amount of polyphenols total was done by creating a standard solution of tannic acid (methanol:water:6:4) with concentration of 25 ppm, 50 ppm, 100 ppm, 200 ppm, and 250 ppm. Determination of polyphenol total was done by using a standard solution of tannic acid at a wavelength of 765 nm (Table 2).

Testing of antioxidant activity was done by using DPPH free radical scavenging effect. The antioxidant activity was tested by DPPH free radical scavenger compounds. Each sample was prepared in a concentration of 5 ppm, 10 ppm, 25 ppm, 50 ppm, 100 ppm, and 200 ppm. The inhibitory activity of free radicals more than 80 % begins at a concentration of 25 ppm. Based on the literature, an active compound as an antioxidant when providing barriers is above 80 % and $IC_{50} < 50$ (Table 3, Figure 1).

Table 3. Free radical scavenging and IC_{50} extracts from several clones of cocoa bean

Sample	Free radical scavenging (DPPH) % (ppm)						IC_{50} (ppm)
	5	10	25	50	100	200	
KDI-3	32.49	53.11	83	91	94	93	9.30
PA-310	41.33	73.32	84.47	94.70	93.16	92.13	6.40
NA-34	30.69	49.76	84.57	85.80	87.76	87.45	10.23
PA-300	32.18	58.20	85.14	85.14	91.46	93.41	8.50
BHT	51.57	62.98	81.8	81.8	83.85	86.63	4.94

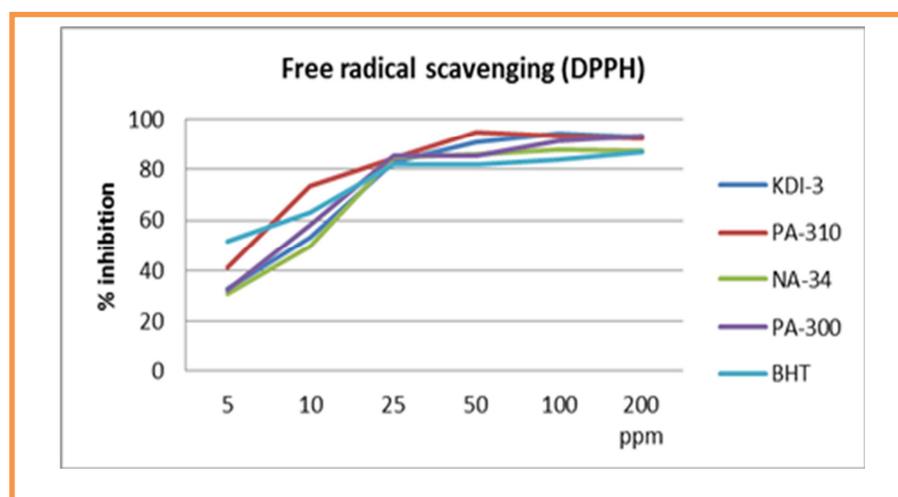


Figure 1. Activity of DPPH free radical extracts from several cocoa beans clones

Table 3 shows the IC_{50} of each clone and control gives a value below 50. They indicate that the extracts of cocoa beans have a very strong antioxidant. A compound acts as a powerful antioxidant if the value of the IC_{50} is less than 50, strong if the IC_{50} value is 50-100, moderate if the IC_{50} value is 100-150, and weak if the IC_{50} appreciating 150-200.

CONCLUSION

The results showed cocoa beans have polyphenol content and antioxidant activity against free radicals. The IC_{50} are different for each clone, but all of them still show percentage inhibition of free radicals (DPPH) above 80 %. Percentage inhibition above 80% starts from a concentration of 25 ppm. They showed a very strong antioxidant activity with $IC_{50} < 50$. Total antioxidant activity and the highest polyphenol were indicated by the PA-310 clone.

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