

## High Levels of Flavonoids and HPLC Profile from Purified Extract of Cocoa Bean from West Sulawesi Indonesia

Andi Emelda<sup>1\*</sup>, Elly Wahyudin<sup>2</sup>

<sup>1</sup>Faculty of Pharmacy Universitas Muslim Indonesia,  
Jl. Urip Sumohardjo Km. 5 Makassar 90231 Indonesia

<sup>2</sup>Faculty of Pharmacy Universitas Hasanuddin,  
Jl. P. Kemerdekaan Km. 10 Makassar 90245 Indonesia

Phone No: +628124110077

\*Corres.author: a\_emelda@yahoo.com

**Abstract:** Cocoa beans have a high content of flavonoids in comparison with some vegetables and fruit. Flavonoid is secondary metabolite in plants. It is a natural phenolic compound which is potential as antioxidants. This study aims to obtain high flavanoid compounds in a purified form of cocoa beans. The extraction and purification were carried out on seeds by using the solvent n-hexan, using the solvent acetone: water (7:3). The measurement of total flavonoids used aluminum chloride colorimetric assay method and the contents of theobromine and kofein of purified extract were conducted using HPLC. The measurement results showed that total flavonoids of purified extract of cocoa beans at 272.2 mg/g with a very low content of caffeine and theobromine.

**Keywords :** Cocoa bean, purified extract, flavanoid compound, HPLC.

### Introduction and Experimental

Cocoa beans are a rich source of flavonoids, especially epicatechin, catechin and polymer form, its monomer called procyanidin. The variety and part of the plant will provide a different polyphenol content<sup>1</sup>. Cocoa flavonoid contents are not easily formed, this depends on several factors such as geography, climate, storage conditions and manufacturing process<sup>2</sup>. Cocoa beans are a rich source of polyphenols<sup>3</sup>. Cocoa beans have a higher polyphenol content than tea, red wine, pomegranates, blueberries and some vegetables that have a high antioxidant capacity as well<sup>4,5</sup>. The results of research by Nicholas N et al showed the differences of geographic location of cocoa beans will give a different effect for different active substance. The active substance compound of a plant is affected by climate and geographic location where the plant growing<sup>6</sup>. Fresh cocoa beans from the tree contain a lot of polyphenols and have antioxidant capacity higher than any other food source. The benefits of chocolate had gained a high attention of many scientists and nutritionists. The consumption of chocolate containing high cocoa is associated with various health benefits, which are closely related to the function as an antioxidant<sup>7,8</sup>. Polyphenolic/flavonoid compounds are damaged by heating, on high temperature and long heating will cause the polyphenol content of cocoa beans to be lost or reduced. Besides that, the content of cocoa polyphenols/flavonoids is not easy to form, this is due to several factors such as geographical location, climate and storage conditions<sup>2</sup>. On previous research, it had been obtained total phenolic content of cocoa beans purified extract from West Sulawesi about 391 mg/g calculated as gallic acid<sup>9</sup>. Based on qualitative analysis using thin-layer chromatography showed no caffeine in the purified extract of the cocoa bean<sup>10</sup>. This result is much higher than that made by tomas-barberin using non-fermented cocoa beans that

obtained a total phenolic amount 80 mg/g. In addition, they found small amounts of caffeine (4.39 mg/g). Therefore, this study is designed to obtain the high levels of flavonoids from the purified extract and observe the content of caffeine and theobromine by using HPLC method.

## Material and Methods

Materials used in this study were cocoa beans taken from Polman regency, West Sulawesi, Indonesia, solvent n-hexane, acetone, distilled water, standard caffeine and theobromine. This study conducted in chemical department of Politeknik Negeri Ujung Pandang, Makassar, Indonesia.

### 1. Extraction and purification

Cocoa beans are extracted by maceration method with n-hexane solvent. Firstly, the powder of cocoa beans is put into a container of maceration and n-hexane is added so that the entire sample is soaked. Then performed purification of polyphenol compounds with solvent acetone: water (70:30; v/v)<sup>3</sup>. The solvent is collected in a container and then evaporated. The next, the water remaining in the purified extracts is eliminated by using a freeze drying to obtain a dry purified extract containing flavonoids compounds.

### 2. Determination of total flavonoid content

Content of flavonoids in purified extract of cocoa beans (*Theobroma cacao*) is determined by aluminum chloride colorimetric assay method. 251,1 mg of purified extract is dissolved in methanol solvent. The volume is added up to 50 ml in a measuring flask. 0.5 ml of sample solution is pipetted and put in a 50 mL measuring flask then added 20 mL of distilled water. 1.5 mL of 5% NaNO<sub>2</sub> was left for 5 minutes. After 5 minutes was added 0.15 mL of 10% AlCl<sub>3</sub>. After 5 minutes it was added 10 mL of 1 M NaOH up to 50 mL in volume with distilled water. Solution absorbance was measured at 510 nm. Standard curve was constructed using various concentrations of catechins. Results are expressed as a percent of catechins.

### 3. The caffeine and theobromine observations of purified extract from cacao beans with HPLC

This research analyse the purified extract by using HPLC (Shimadzu shim-pack ODS C18) with an eluent of methanol: water: 40:60.

## Results and Discussion

### 1. The results of testing the total flavonoids of purified extract from cacao beans

In this study, the extraction and purification of cocoa beans were conducted by using maceration method. Extraction is the process to draw the chemical components contained in either natural materials from plants, animals and marine life. Extraction process is based on the ability of the organic solvent to penetrate into the cell wall and the cavity of the cell that contains the active substance. Active substance will dissolve in organic solvents, due to the concentration difference inside and outside the cell, resulting in the diffusion of the organic solvent containing the active substances go out of the cell. This process happens continuously until there is an equilibrium concentration of active substances inside and outside cells. At high temperatures and long heating will cause the polyphenol content of cocoa beans lost or reduced. Therefore, this research uses maceration method. To obtain the polyphenolic compounds from cocoa beans (*theobroma cacao*), it was conducted purification using solvent acetone: water (7:3). Based on previous research, the phenolic total determination of purified extract of cocoa beans was obtained at 391 mg/g calculated as tanat acid<sup>9</sup>. Flavonoids obtained in this study a total of 272.2 mg/g which are calculated as catechin as showed in Table 1.2 and Figure 1,5.

### 2. The results of HPLC assay

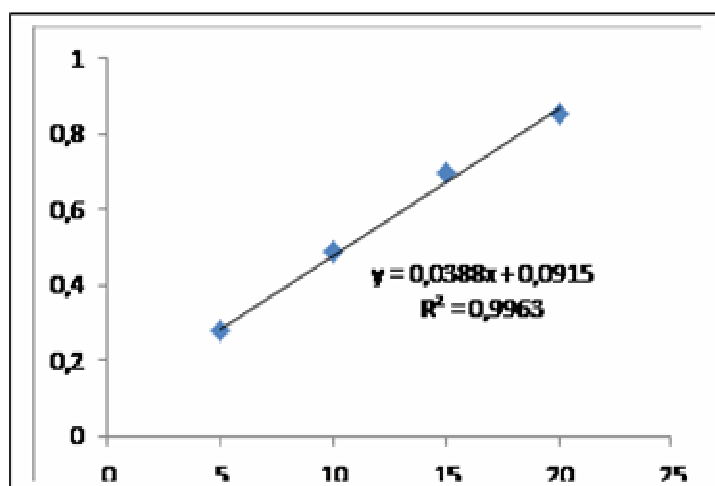
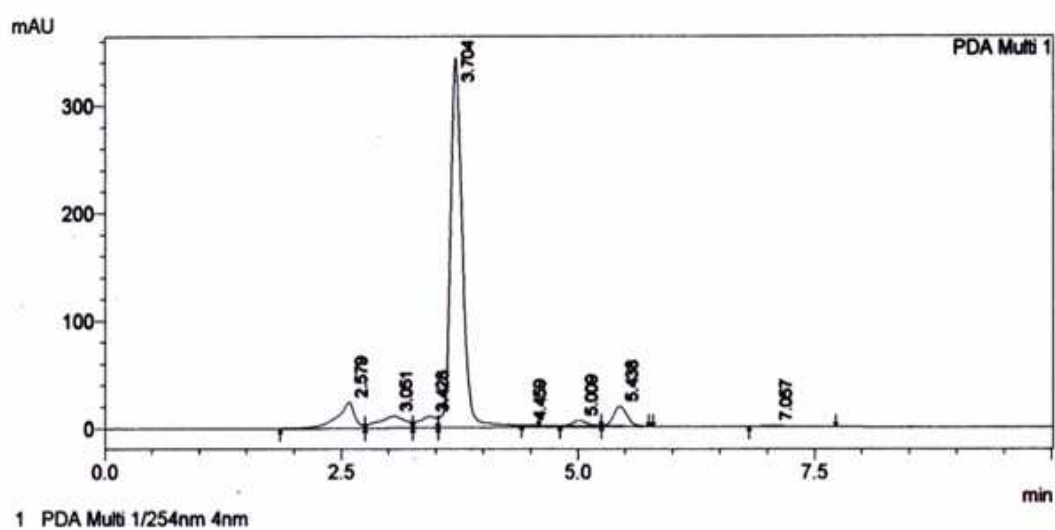
The results of HPLC assay on purified extract of cocoa beans showed that there are caffeine and theobromine with very small levels indicated on Rt. time 3,061 minutes and 3,428 minutes as described in Figure 2, Figure 3, and Figure 4. This very small levels that may lead to the TLC analysis of caffeine is not readily apparent on previous research<sup>10</sup>.

**Table 1.** Concentrations and absorbances of catechin standard

Concentrations (ppm) (x)	Absorbances (y)
5	0,274
10	0,486
15	0,692
20	0,853

**Table 2.** The measurement results in total flavonoids of purified extract of cocoa beans

Absorbance (OD)		Average OD	Average concentration (ppm)	Average level mg/g
I	II			
0,617	0,627	0,622	13,67	272,2 mg/g

**Figure 1.** Calibration curve of catechin standard**Figure 2.** HPLC profile of purified extract of cocoa beans

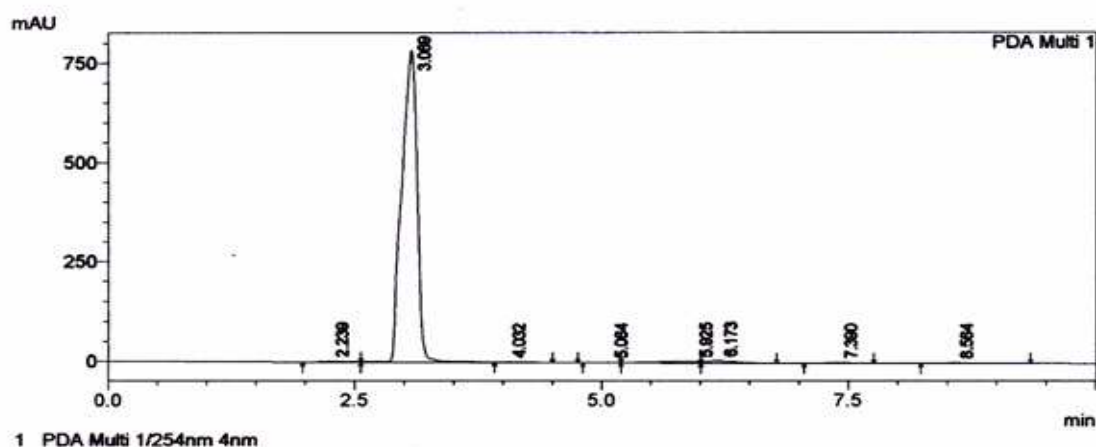


Figure 3. HPLC profile of theobromine standard

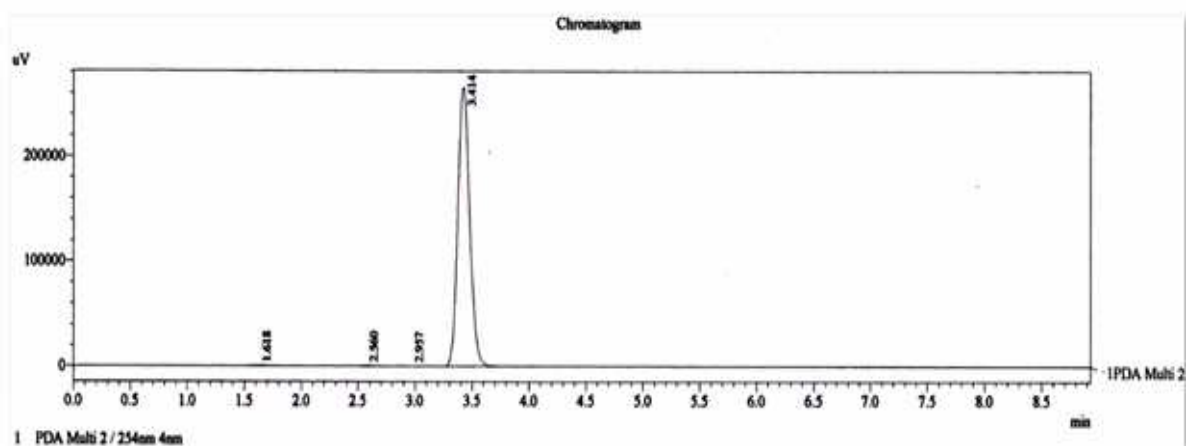


Figure 4. HPLC profile of caffeine standard

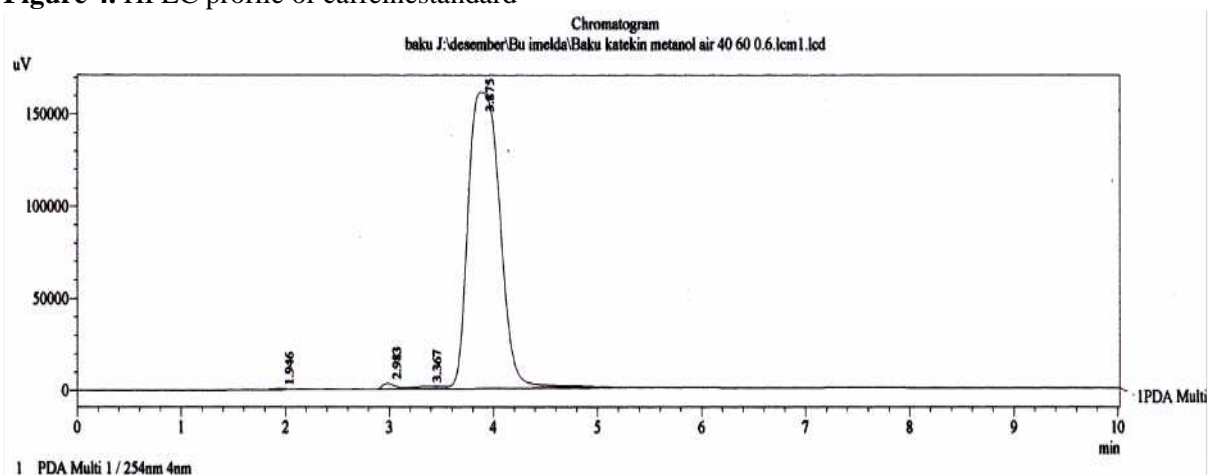


Figure 5. HPLC profile of catechin standard

## Conclusions

The calculation of total flavonoids in purified extract of cocoa beans was 272.2 mg/g calculated as catechin. The purified extract of cocoa beans still have very small levels of caffeine and theobromine shown on Rt. time 3.061 and 3.428 minutes.

## References

1. Jayasekera, S., Molan A.,L., Garg, M, Variation in antioxidant potensial and total polypenol content of fresh and Fully-Fermented Srilankan Tea. Elsevier, Food Chemistry 2011, 125, 536-541.
2. Manach C, Scalbert A, Morand C, Remesy C & Jimenez L, Polyphenols: food sources and bioavailability. Am J Clin Nutr 2004, 79, 727-747.
3. Tomas-Barberan F., A., Cienfuegos-Jovellanos, E., Marín, A., Muguerza, B., Gil-Izquierdo, A., Cerda, B., Zafrilla, P, Morillas, J., Mulero, J., Ibarra, A., Pasamar, M.,A., Ramón, D., Espín, J.,C., A New Process To Develop a Cocoa Powder with Higher Flavanoid Monomer Content and Enhanced Bioavailability in Healthy Humans. Agricultural And Food Chemistry, 2007, 55, 3926-3935.
4. Lee,K.W., Jun Kim Y and Young Lee, Cocoa has more phenolic phytochemicals and a higher antioxidant capacity then tea and red wine. Journal of Agricultural and Food Chemistry, 2003, 51(25), 7292-5.
5. Crozier S.J.,Preston A.G,Hurst J.W.,Payne M.j., Mann J,Hainly L,Miller D.L., Cacao Seeds are a"Super Fruit" A Comparative analysis of various Fruits Powders and Products.,Chemistry Central Journal 2011, 5(5), 1-6.
6. Nicolas,N., Comparatif Study of different cocoa (*Theobroma cacao*) Clones in term of Their Phenolics and anthocyanins contents. Jurnal of Food Composition and Analysis 2006, 6(19), 612-619.
7. McShea A, Ramiro-puig W, Munro S, Casadeus G, Castell M. 2008. Clinical benefit and preservation of flavanols in dark chocolate manufacturing. Nutr Rev, 2008, 66, 630-4.
8. Williams S, Tamburic, Lally C. 2009. Eating chocolate can significantly protect the skin from UV light. J of Cosm Derm,2009, 8, 169-73.
9. Emelda A, Wahid S, Wahyuddin E, Massi N.,(2013).,The total polyphenol content of extracts purified from seeds of cocoa (*Theobroma cacao*) from West Sulawesi, Journal of Nature and Environment., 2013, 4(7), 8-11
10. Emelda A., Qualitative analysis of caffeine on purified extract of cocoa beans(*Theobroma cacao*) in thin layer chromatographyfrom Polman West Sulawesi, Journal of Bertani 2014, 1(9), 38-41.
11. Directorate General of Drug and Food, 1986, Upstream Processing Preparations, Edition II, Ministry of Health of the Republic of Indonesia, Bhakti Husada. Jakarta. 2, 7, 10, 32.
12. Wolgast,J., & Anklam, E, Review on polyphenols in Theobroma cacao, Change in composition during the manufacture of chocolate and methodology for identification and quantification. Food Research International, 200, 37, 423-447.

\*\*\*\*\*