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## Identifications of Polyphenols and $\alpha$ -Amylase Inhibitory Activity of Multi herbal Formulation: Cocoa Beans (*Theobroma cocoa*), Buni (*Antidesma bunius L. Spreng*) and Cinnamons (*Cinnamomum cassia*)

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**Abstract.** Chemical compounds from cocoa, buni, and cinnamons are expected to inhibit the activity of the enzyme  $\alpha$ -amylase,  $\alpha$ -glucosidase, and proanthocyanidin as mimetic insulin. Cinnamic acid may inhibit the enzyme activity of HMG-CoA reductase, so that provides benefits for people with diabetes mellitus because it can stimulate pancreatic cells to produce insulin. The objective of this study was to evaluate polyphenols and  $\alpha$ -amylase inhibitory activity of a multiherbal formulation. The multiherbal extract prepares with aqueous, acetone, and ethanol. Total phenolic content was found to be 236.28 mg of GAE/ 100 g (cacao fat extract), 217.94 mg of GAE/ 100 g (cacao free fat extract), 159.61 mg of GAE/ 100 g (cinnamons extract), and 181 mg of GAE/ 100 g (buni extract).  $\alpha$ -amylase inhibitory activity found to be 88.74 ppm (cacao extract), 85.32 ppm (cinnamons extract), 83,49 ppm (buni extract), and 13.07 ppm (acarbose). All compounds revealed inhibition potential with IC<sub>50</sub> when compared to the standard acarbose.

### 1. Introduction

Treatment of diabetes mellitus with insulin or oral chemical medications in the long term shows a tendency to increase blood sugar, resistance to drugs, side effects, and subsequent complications affecting the body's immunity. In order to prevent side effects of anti-diabetic medications and hypolipidemic drugs such as flatulent, diarrhea, and cramps in the abdomen, new herbal anti-diabetes compounds (phytotherapy) intensively performed and produced from raw materials, technical method, and product type [1,2]. Natural ingredient products, as well as the active compounds contained therein, alternative ingredients for type 2 diabetes therapy and other complications (such as heart disease, kidney, mental disorders, inflammation, and cardiovascular attack) without adverse side effects. Several herbal plants and natural bioactive molecules have been reported as a natural remedy for type-2 diabetes disease therapy. A Polih herbal Polar formula extract consisting of nine herbal plants also demonstrates antioxidant activity and inhibitory activity against  $\alpha$ -amylase enzymes. Polyherbal Formula contains polyphenols, flavonoids, and antioxidant compounds [3,4]. Another study reported that a combination of several herbal extracts resulted in a synergistic effect of its bioactive compounds against the research of diabetes on cells and animals as models. Even phytochemical compounds exhibit favorable effects in various studies in vitro [5,6].



This research utilizes cocoa beans (*Theobroma cocoa*), buni (*Antidesma bunius L. Spreng*), and cinnamon (*Cinnamomum cassia*), which is a potential nutraceutical herbal plant. Nutraceutical is a term derived from the words “nutrition” and “pharmacy.” Nutraceutical products are defined as substances with physiological benefits or providing protection against chronic diseases, delay the aging process, and increase life expectancy. Nowadays, Nutraceutical gets much attention because it has the potency of nutrients, safety, and therapeutic effects. Some research suggests that Nutraceutical may prevent and cope with various diseases such as diabetes, atherosclerosis, osteoporosis, cardiovascular disease, cancer, and neurological diseases. Most nutraceuticals compounds have activity as antioxidants. In cocoa beans without fermentation contain various compounds of polyphenols, approximately 60% of the total polyphenols in cocoa beans are monomer-monomer flavanol (Epikatekin and Catechin) and oligomers Procyanidin (dimer and decamer) with varying concentrations. The component of this compound has vigorous antioxidative activity with physiological properties that inhibits the activity of  $\alpha$ -amylase,  $\alpha$ -glucosidase. It also exhibits anti-diabetic properties in test animals and is an insulin-mimetic agent [7]. Other benefits of cocoa polyphenols are protecting the body from free radicals, reducing stress and depression, heart disease, high blood pressure, anti-cancer, lowering cholesterol, and the risk of a heart attack. Previous research found an anti glyceemic effect from cinnamon plants (*Cinnamomum cassia*). Cinnamon also has the potential of anti-dyslipidemia; prior studies reported that the content of Proanthocyanidin type polyphenols as mimetic insulin and cinnamic acid that can inhibit the activity of HMG-CoA reductase enzyme. The liver thus produces a hypolipidemic effect. Also, cinnamon can provide benefits for people with type-2 diabetes through antioxidant activity and the propagation of pancreatic cells to produce insulin [8].

Buni (*Antidesma bunius L*) is one of the herbal medicines that have been used a long time ago. A study has shown that ethanol extracts 80% of buni has inhibitory activity against  $\alpha$ -glucosidase with an  $IC_{50}$  value of 3.90 ppm [9]. It shows that the bark of the buni can be efficient as an anti-diabetic. The bark of this plant contains alkaloids, saponins, tannins, and flavonoids [10,11]. The exploration and utilization of these crops to be a herbal food product rich in polyphenols or functional beverages is the goal to be achieved in this research. For that, the stage of research been conducted is extraction, determination of total levels of polyphenols, and evaluation of the potency of multi herb (cocoa, cinnamon, and buni) through the inhibitory test of  $\alpha$ -amylase as anti-diabetes.

## 2. Methodology

### 2.1. Materials

The cocoa samples evaluated in this study were made and kindly supplied by Cocoa varieties Forastero from Bulukumba Regency, South Sulawesi, Indonesia, as well as the bark of the buni from Makassar City, South Sulawesi, and cinnamon from Ternate Regency, Maluku Indonesia. Pro quality chemicals analysis grade: hydrochloric acid (CAS: 109063), hexane (CAS: 110-54-3), sodium carbonate (CAS: 497-19-8), starch soluble (CAS: 9005-84-9), acetone (CAS: 67-64-1), ethanol (CAS: 64-17-5), potassium sodium tartrate tetrahydrate (CAS 6381-59-5), sodium phosphate (CAS: 10049-21-5) supplied by Merck Millipore (Burlington, Massachusetts, United States), DPPH (D4313, CAS: 1898-66-4) was from Tokyo Chemical Industry (Tokyo, Japan). Enzyme  $\alpha$ -amylase (CAS: 9000-90-2), aluminum chloride (254134, CAS: 12125-02-9), sodium nitrate (CAS: 7631-99-4), Folin Ciocalteau (CAS: 109-00-1), Gallic acid (CAS: 149-91-7), sodium hydroxide (CAS: 1310-73-2), reagent 3,5-Dinitrosalicylic acid/DNS (CAS: 609-99-4) and glucobay/acarbose  $\geq 95\%$  (CAS: 56180-94-0) were from Sigma-Aldrich.

### 2.2. Preparation of cocoa beans non-fermentation, cinnamons powder, buni powder, and fat-free cocoa powder

Cocoa beans without fermentation are roasted in (roasting machine KL Protech Type Number 043.13P033 capacity 15 kg) at 100-105°C for 1 hour. In contrast, the cocoa beans without fermentation are injected at 80°C for 40 minutes to form a distinctive aroma and flavor of chocolate. Minimum roasting machine capacity 8 kg. After the roasting stage complete, then proceed with cooling up to reach the temperature 40-50°C. Then samples of cocoa beans were inserted in winnower (nibs separator

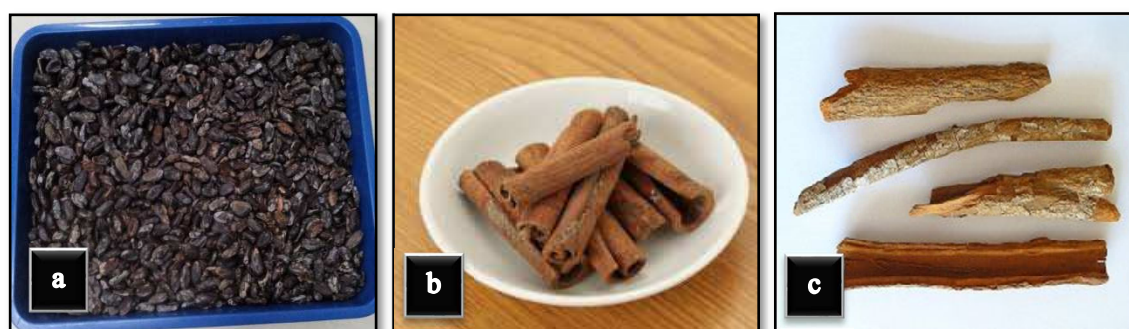
machine KL Protech Type Number 049.13P043) to separate between nibs and shell (seeds and outer skin). Once separated from the seeds and outer surface, nibs and shells are weighted, nibs then ground using a stone mill (KL Protech Type Number 066.13P063) to destroy nibs that initially shaped coarse solid granular into the cocoa paste (cocoa liquor).

Furthermore, cocoa liquor takes in a ball mill (ball mill mini KL Protech Type Number 041.,13P028), which is useful to smooth the still rough cocoa liquor at 50°C for 30 hours. The cocoa paste is pressed at temperature 50°C with a pressure of 58 MPa for 60 minutes until the whole fat is separated by cake. Then the cake in the puree with hammer machine using a sieve measuring 0.8 mm until fine cocoa powder obtained.

The dried cinnamon is crushed with a hammer machine with a filter size of 0.8 mm, then stored for analysis and processed further.

The bark of the buni is cleaned and dried with sunlight and then crushed with a hammer machine using a filter measuring 0.8 mm. The Buni bark powder is stored for analysis and processed further.

Twenty-five grams of cocoa powder samples were weight, then the fat was reduced by the addition of 100 mL of hexane solvent and in incubator Shaker for 24 hours at room temperature. They were followed by a centrifuged process with a speed of 1000 rpm for 10 min, the work repeat three times. Then the hexane content at the room temperature for 48 hours. Samples of cocoa powder and cocoa fats weighed and in the analysis of fat content in cocoa powder.



**Figure 1.** Main ingredient multiherbal: a) cocoa beans, and b) cinnamons; and c) buni

**Table 1.** Formulations of double and multiherbal

Formulations	Samples	Cocoa Powder (%)	Cinnamons Powder (%)	Buni Powder (%)
Multi herbal	F1	70	20	10
	F2	60	30	10
	F3	50	40	10
	F4	40	50	10
Double herbal	F5	70	30	-
	F6	60	40	-
	F7	50	50	-
	F8	40	60	-
	F9	30	70	-

### 2.3. Preparation extraction of polyphenols on samples

The procedure of extraction of polyphenols for cocoa powder refers to the method [12], by dissolving samples into acetone solvents. In the incubator, Shaker for 24 hours at room temperature in a closed state and spared from direct contact with the light, centrifuged at 3500 rpm for 20 min, and the supernatant is separated. Total extracts than in the rotary evaporator at a temperature of 41°C to vaporize acetone, so the polyphenol condensed obtained from the extract. The polyphenols extract dried with a freeze dryer until obtained by polyphenol extract powder. In comparison, the procedure of extraction of polyphenols to the skin samples of buni stem and cinnamon powder has the same stages of process as preparation of cocoa powder [13,14].

#### **2.4. Total polyphenols content**

The total phenolic content of samples was determined using the Folin-Ciocalteu assay [15]. An aliquot 1 g extract or standard solution of Gallic acid (2, 4, 6, 8, 10 and 12 ppm) added to a 25 ml volumetric flask containing 9 ml of distilled deionized water (dd H<sub>2</sub>O). A blank reagent prepared using dd H<sub>2</sub>O. One milliliter of Folin-Ciocalteu phenol reagent has been applied to the mixture and shaken. 10 ml of 7% Na<sub>2</sub>CO<sub>3</sub> solution added to the mixture after 5 min. The solution was diluted in 25 ml (volume) with dd H<sub>2</sub>O and mixed. After incubation at room temperature for 90 min, the absorbance against the prepared reagent blank was determined at 750 nm with the UV-Vis Spectrophotometer lambda 5. The total phenolic content of cocoa powder extract powder, buni steam and cinnamon powder was expressed as mg GAE/100 g fresh weight. All samples have been analyzed in duplicates.

#### **2.5. Analysis activity of $\alpha$ -amylase enzymes by DPPH assay**

Activity was detected using a 1% solution of starch in a 20 mM phosphate buffer (pH 6.9) containing 6.7 mM sodium chloride as a substrate. The enzyme solution was pre-incubated with different concentrations of samples (25, 50, 75 and 100 ppm) at 37°C for 10 and 30 min to achieve optimum conditions and 30 min. Optimum pre-incubation time for an enzyme inhibitor mixture of 100  $\mu$ L of enzyme solution (pre-incubated) added to 100  $\mu$ L of starch solution. The reaction was stopped by adding a double-dinitrosalicylic acid solution (DNS) and boiled in a boiling water bath for 5 min. After the mixture was cooled, 2 mL of distilled water was added and the enzyme activity was determined to measure the acarbose equivalents released from starch at 540 nm — all antioxidant experiments in two replications and the solvent control sample anti-diabetic activity expressed in IC<sub>50</sub>.

### **3. Results and Discussion**

#### **3.1. Extraction of polyphenols**

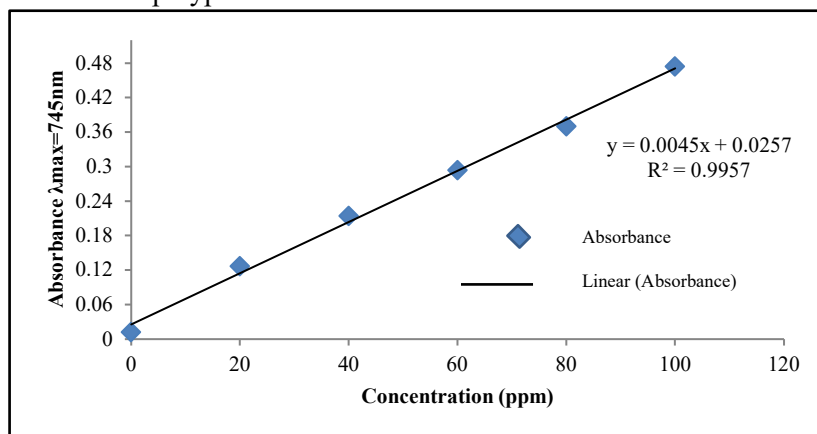
This study uses the maceration method to extract polyphenols from cocoa powder without fermentation, cinnamon, and bark. This method is selected because the process stage uses a temperature of 30°C. At high temperatures and prolonged heating will cause the content of polyphenols from cocoa beans to be lost or decreased. Extraction of polyphenols using three types of solvent, namely acetone solvent: water (7:3), ethanol 80%, and water in each cocoa sample without fermentation, cinnamon, and the bark of the Buni. The use of acetone solvent: water (7:3) is an excellent solvent for the application of polyphenols, especially proanthocyanidin. Ethanol and water are polar, universal, and easily obtainable solvents and are capable of extracting almost all-natural chemical content. The polar compound is water-soluble. The polyphenol extract of each ingredient is centered and overgrown by using a freeze dryer resulting in a polyphenol extract powder.

#### **3.2. Total phenolic content**

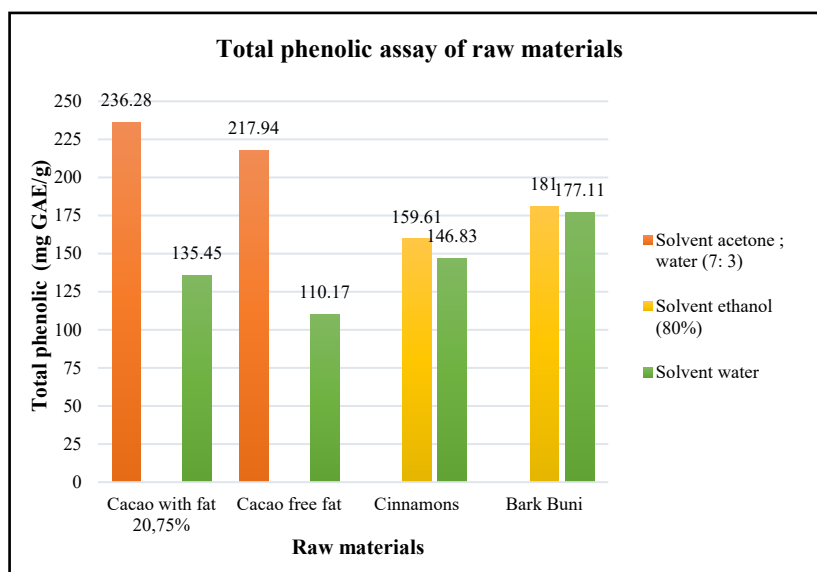
Determination of polyphenol levels using the Folin-Ciocalteu method, where total polyphenol levels are expressed as gallic acid, which is equivalent to gallic acid (GAE). GAE is a general reference for measuring some phenolic compounds contained in an ingredient [16]. Standardized acid error curves calculate the total levels of polyphenols. Based on the standard curve measurements obtained, the equation of a linear line regression  $y = 0,0045x + 0.0257$ . The results of the analysis of the total content of polyphenols can see in Figure 2, 3, and Table 2.

The content of cocoa polyphenols with the solvent of acetone and aqueous amounted to 236.28 mg GAE/g, higher than by using solvent water of 135.45 mg GAE/g. Acetone and water solvents are more

useful to attract more polar compounds than cocoa. Meanwhile, the content of cinnamon and bark polyphenols using ethanol solvents has a value of 159.61 mg GAE/g and 181 mg GAE/g, comparable with the solvent water of 146.83 mg GAE/g and 177.11 mg GAE/g. Ethanol and water solvents can extract most of the content of polyphenols and have the same level.



**Figure 2.** Standard curve of polyphenols at wavelength  $\lambda_{\max}=745\text{nm}$



**Figure 3.** Polyphenols of raw materials extract with various solvents

**Table 2.** Total polyphenols and antioxidant activity of double and multiherbal

Formulations	Samples	Total phenols mg GAE/g)	Antioxidant activity IC <sub>50</sub> (ppm)
Multi herbal	F1	106	99.68
	F2	82.39	112.98
	F3	122.39	92.20
	F4	112.94	97.04
Double herbal	F5	79.61	118.46
	F6	91.28	108.79

F7	95.72	104.78
F8	105.72	96.64
F9	149.06	87.33

### 3.3. Antioxidant activity assay

$\alpha$ -amylase is an enzyme that acts on the catabolism of starch into simpler sugars. The inhibitory analysis of  $\alpha$ -amylase enzymes aims to determine the decrease in the activity of  $\alpha$ -amylase enzymes in a starch break, thereby lowering the digestibility of starch. Research [17], a polyphenol compound, can act as an enzyme inhibitor to digest  $\alpha$ -amylase and  $\alpha$ -glucosidase. Polyphenols can be a natural inhibitor of enzymes that play a role in hydrolyzing carbohydrates to help inhibit the increase in blood glucose levels. Radical scavenging activity of cacao powder, cinnamons, bark buni, instant powder multi herb, and double herbs was performed by DPPH assay using acarbose as standard, with a sample concentration (25, 50, 75, and 100 ppm).

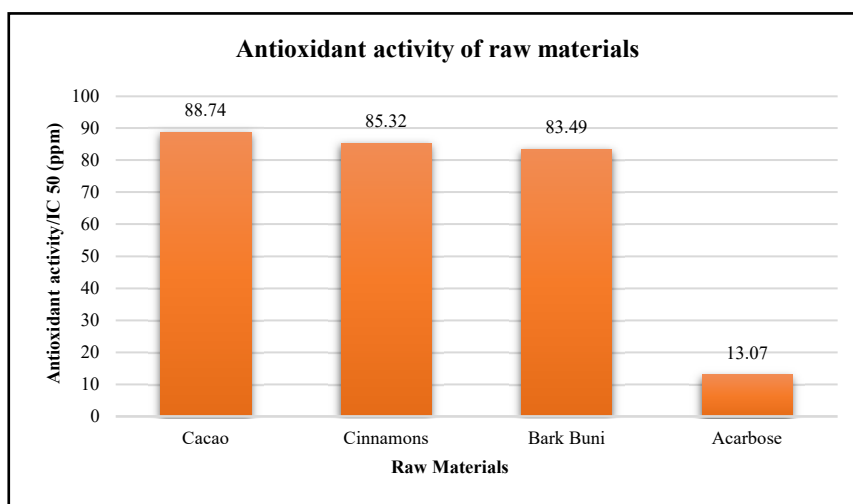


Figure 4. Antioxidant activity of raw materials extract

The results of the analysis of  $\alpha$ -amylase ( $IC_{50}$ ) enzyme inhibitory activity in Figure 4 show that the polyphenol extracts in buni and cocoa with values ranging from 83.49 to 88.74 ppm. In comparison, the control sample (acarbose) has an  $IC_{50}$  value of 13.07 ppm. The  $IC_{50}$  value of the third sample includes an active anti-diabetes category because it has an  $IC_{50}$  value ranging from 50 to 100 ppm. According to [18], that test material said to inhibit the activity of a potent enzyme if it has a low  $IC_{50}$  value of 50 ppm, an active category ranges from 50-100 ppm, lowly category 100-150 ppm and weak types ranged from 151-200 ppm. Herbal powder formula has inhibition to the activity of the  $\alpha$ -amylase enzyme with a value of  $IC_{50}$  ranging from 87,33 to 118,46 ppm. The best formula for multiherbal powders is the F3 sample with an  $IC_{50}$  value of 92.20 ppm, while the best procedure for double herbal powders is the F9 sample with an  $IC_{50}$  value of 87.33 ppm. The synergy effect occurs if the interaction between the mixture of several components has increased inhibitory activity against the enzyme  $\alpha$ -amylase compared to its single part.

## 4. Conclusion

Double and multi-herbal formulations with variations in the addition of extracts of cocoa beans, buni, and cinnamon, can increase the content of polyphenols and antioxidants and play a role in the release of  $\alpha$ -amylase enzymes, which are very potential as functional foods for people with diabetes mellitus. This research can be useful in developing cocoa instant drink that has an equivalent nutritional value, so that can be a reference for chocolate products that are worth the functional food.

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