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Effects of *Theobroma cacao* Bean Onantioxidant Activities and Re-Epithelialization in Burn Wound in Pre Clinical

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Abstract : Cocoa beans contain flavanoid compounds that can improve blood circulation, anti-inflammatory, and antioxidant. The aims of this study were to determine the% inhibition of purified extract and the ability to re-epithelialization against burns.This study uses unfermented cocoa beans. The unfermented cocoa beans are extracted using a solvent n.Hexan. The residue of the n-hexan is subsequently extracted using acetone:water (7:3). Cocoa beans in the form of both powder and purified extract were tested for antioxidant content using free radical DPPH. Testing epithelialization of the burn was done by dividing the experimental animals into 5 groups. The first and the second groups are Control group andPositive group.The Group III, IV and V are the group that given the extract with a concentration of 2%, 4% and 8% respectively.The results showed,the evaluation of 80% inhibition at concentrations of 50 ppm are 84.83% for the purified extract and 83.54% for BHT, while the powder form has not reached 80% inhibition. The results of epithelialization ability of purified extract to burn show the percentage reduction of wound are better than the normal group. The groups of purified extract at the concentration of 2%, 4%, and 8% have the epithelialization ability of89.67%, 81.75%, and 63,5% respectively while the normal group only 20.5 %. The purified extract form is better than the powder form.
Keywords : Cocoa bean, purified extract, powder, antioxidant, epithelialisation.

Introduction

Indonesia is one of the places with the most plant species in the world. There are about 35,000 species of higher plants and about 3,500 of them were reported as a medicinal plant[1]. One of the well known plant that has many health benefits is chocolate plant (*Theobroma cacao* L). One compound of cocoa is polyphenol that is obtained by extracting the cocoa beans. The main components of the cocoa beans are flavonoids, especially epicatechin, catechin and polymer form[2]. Flavonoid has a working mechanism that can improve the blood circulation throughout the body and prevent blockages in blood vessels, anti-inflammatory, antioxidant andreduce pain if bleeding or swelling[3,4,5].The levels of polyphenols and flavonoids can be measured by Folinciucalteu and Aluminum chloride colorimetric methods[6]. The flavonoids total in purified extracts of cocoa beans is 272.2 mg/g[7].

Antioxidant substances are thought to be used as a woundhealing and in line of high cholesterol diet[8,9]. Burns wound is a form of tissue damage or loss caused by contact with a heat source such as a fire, hot water, chemicals, electrical, and radiation. Burn is a type of trauma with high morbidity and mortality.Inflammatory phase lasted from injury to approximately the fifth day. The blood vessels severed in the injury will cause bleeding and the body will try to stop it by vasoconstriction, shrinkage the end of broke up vessel (retracted),

and the reaction hemostasis. Clinical signs and symptoms of inflammation became apparent in the form of a reddish color because of dilated capillaries (rubor), warm temperature (heat), pain (dolor) and swelling (tumor)[10].Therefore, this study was conducted to determine the antioxidant activity of cocoa beans in the form of powder and purified extract and to show the epithelialization capabilities of purified extract against burns.

Experimental

Material used in this study were cocoa beans from Enrekang regency of South Sulawesi-Indonesia, distilled water, n-hexan solevent, acetone, cetamin, DPPH (sigma).

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 with solvent acetone: water (70:30; v/v)[7].

Test antioxidant activity

Test antioxidant activity by DPPH method of powder from cocoa beans were made in the concentration of 25 ppm, 50 ppm, 100 ppm, and 200 ppm using methanol. 4 ml were taken fromeach concentrations 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 treatment was also carried out on purified extract of unfermented cocoa beans and BHT (control)[11].

Animal test

Twenty five rats were adapted into laboratory condition and free-pathogen condition for two weeks.On the day 15th, all the mice's backs sheared with an area of 2 x 2 cm and anaesthetized using ketamine 1.233 mg/ml intraperitoneally (IP).Anhot plate with a temperature of 100°C placed on the back skin of mice for two seconds until a second-degree burns.Furthermore, the test animals were divided into 5 groups. Group 1st as the normal control group (Na CMC 1%), group 2nd is a positive control group that were given Madecassol burn ointment containing extracts of *Centella asiatica*.Group 3rd, 4th, and 5thare the treatment groups which applied by purified extract of cocoa bean (*Theobroma cacao* L) with 3 variations of concentration, they are 2% w/v, 4% w/v, and 8% w/v.Each treatment was applied topically 2 times a day for 3 weeks. The measurements of surface area of wounds were observed on the days 3rd, 7th, 11st, 14th, and 21^{st12}.

Result and Discussion

Antioxidant activity

This study used cocoa beans (*Theobroma cacao*) that are made in unfermented condition. The antioxidant activity of the cocoa beans observed in the form of powder and purified extract. Table 1 shows the concentration of 25 ppm antioxidant activity of cocoa in powder form was lower than purified extract and control (BHT). The same conditionswhen the concentration of 50 ppm and 100 ppm. The evaluation of 80% Inhibition of purified extract began at concentration of 50 ppm for purified extracts and positive control (BHT).

Table1. Free radical scavenger activity of extracts of cocoa beans (*Theobroma cacao*)

Sample	% inhibition with DPPH			
	25 ppm	50 ppm	100 ppm	200 ppm
Powder	23,33	40,50	41,47	85,93
Purified extract	74,85	84,83	85,49	87,02
BHT	64,70	83,54	88,66	93,17

Epithelialization test

The measurement results of the average surface area of the wound after treatment with the test preparation can be seen in Table 2, Table 3, and Figure 1. In Table 2 shows that the wound surface area for each test group decreased, where all the test animals have extensive initial wound of 4 cm. The wound surface area of control group on day 21st experienced a diminution of wound size of 3,18 cm. This is caused by the body's homeostatic system that has the ability to heal wounds.The group treated with the test extract concentration of 2% w/v, 4% w/v, and 8% w/v obtained wound surface area around 0.41 cm, 0.73 cm and 1.46 cm respectively. The positive control group at the day 21st gained the wound surface area size of 0.13 cm.

Table 2. Decreasing in wound surface area

No	Groups of mice	Surface wound	Average size of wound after therapy on the day (cm) ± SD					
			3rd	7th	11st	14th	17th	21st
1	Control group	4 ± 0	5,92 ± 0,36	5,26 ± 0,26	5,04 ± 0,57	3,92 ± 0,50	3,79 ± 0,76	3,18 ± 0,71
2	Positive group Madecassol®	4 ± 0	4,60 ± 0,36	3,97 ± 0,95	3,01 ± 0,66	2,13 ± 0,82	0,86 ± 0,15	0,13 ± 0,13
3	Purified extract 2%	4 ± 0	5,59 ± 1,05	5,36 ± 0,75	5,29 ± 0,98	2,77 ± 0,65	1,68 ± 0,70	0,41 ± 0,12
4	Purified extract 4%	4 ± 0	5,26± 0,80	5,56 ± 0,84	4,74± 0,83	3,68± 1,40	1,85± 0,73	0.73± 0,16
5	Purified extract8%	4 ± 0	6,73± 1,98	6,48± 0,92	5,52± 0,34	3,68± 0,35	2,22± 0,52	1,46± 0,76

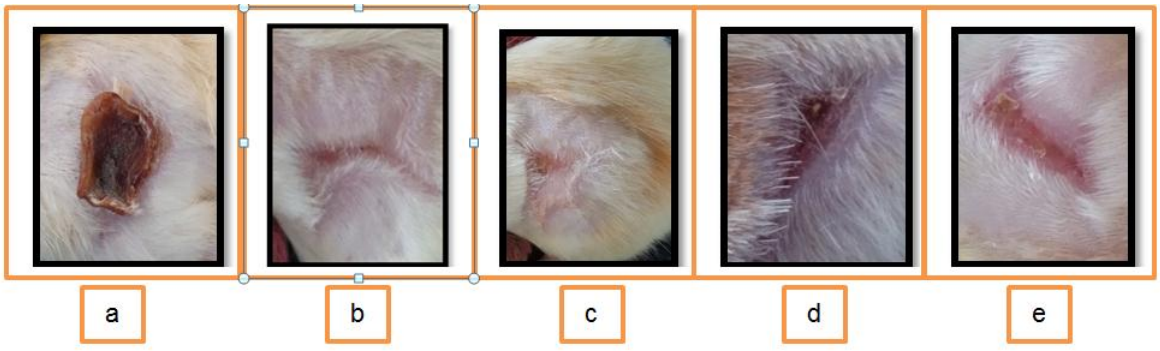

Figure 1. Macroscopic observation on the day 21st: a. Control group, b. Positive group, c. Purified extract 2%, d. Purified extract 4% dan e. Purified extract 8%.

Table3. The percentage reduction in wound surface

Groups	Percentreduction (%)
Control group	20,5%
Positive group (Madecassol®)	94%
Purified extract 2%	89,67%
Purified extract 4%	81,75%
Purified extract 8%	63,5%

The average percentage of the surface area of the wound are: the control group the size of 20.5%, the positive control around 96.75%, the groups of purified extract at a concentration of 2% w/v (size of

89,67%),4% w/v (size of 81,75%), and 8% w/v (size of 63.5%).ANOVA statistical testing of the percentage reduction in wound surface area in the extract group 2%, 4% and 8% showed the results in different significance ($p>0.05$). This shows there is a difference among the treatment groups.

Conclusion

1. The evaluation of 80% inhibition at concentrations of 50 ppm for purified extract is 84.83% and for BHT is 83.54%, while the powder form has not reached 80% inhibition at the concentrations of 50 ppm.
2. Decreasing in wound surface area by macroscopic observation and percentage reduction show the purified extractsare better in diminution of the burnsize for concentration of 2% (size of 89.67%), 4% (size of 81.75%) and 8% (size of 63.5%)compared to normal groupthe size only 20.5%.

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