

# Anti-alopecia activity of waste cacao (*Theobroma cacao* L.) peels

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## ABSTRACT

**Objective:** Hair loss (alopecia) is the most common hair problem. The objective of this study was to determine the activity of growth stimulant activity of cacao (*Theobroma cacao* L.) fruit peels. **Materials and Methods:** The cacao peel extract was extracted with ethanol 96% solvent and fractionated with water, n-hexane and ethyl acetate solvents and then tested for hair growth stimulant activity of extracts and waste cacao peels fraction with modified Tanaka *et al.* method. In this test, the concentration of ethanol extract of cacao fruit peel was diluted to 5%, 10%, 15%, 20%, and 25% which were tested in test animals, namely, rabbits. **Results and Discussion:** The results showed that the test results showed that the ethanol extract of cacao fruit peel effluent had a hair growth stimulating activity starting at a concentration of 15%. In testing the fraction of cacao fruit peel fraction, it was found that the n-hexane fraction had the best activity compared to the other fractions and positive controls (minoxidil 2%). All data obtained in the study were analyzed statistically. **Conclusion:** Waste cacao peels ethanol extract had a growth stimulant activity at a concentration above 15% with the n-hexane fraction had the best activity.

**KEY WORDS:** Anti-alopecia, Cacao fruit peel waste, Extraction, Hair loss, *Theobroma cacao*

## INTRODUCTION

Hair is a part of the human body that functions as a head protector from the surrounding environment and has an esthetic function that supports a person's appearance. Hair is also a characteristic of ethnicity and serves as a symbol of social and cultural. It is only natural that severe hair loss can be annoying for those who experience it. The biggest impact that is felt due to hair loss is to reduce self-confidence that can interfere with the psychological sufferers.<sup>[1]</sup> The occurrence of hair loss is influenced by several factors both from within and outside the body. Factors in the body that cause hair loss include systemic diseases, hormonal conditions, nutritional status, and genetic disorders while external factors are caused by stimulation from the environment in the form of sunlight, pressure, and the use of hair cosmetics.<sup>[2]</sup> According to Noruka, about 95% of users of hair straightener in America and 53% of users in Africa reported experiencing damage or loss and hair growth problems.<sup>[3]</sup> Some solutions to

overcome hair loss have been found one of which is using the chemical drug minoxidil that has been proven effective in dealing with hair loss. However, minoxidil has side effects of exfoliation during the first 4 months that cause skin discomfort.<sup>[4]</sup> To avoid these side effects, herbal ingredients can be an alternative solution. Since ancient times, ancestors often used certain medicinal plants to treat diseases including treating hair health and growing hair that is still used today. Cocoa fruit peels are one of the plants empirically used by Dingгаа Linggarjati people who live at the foot of Mount Galunggung, West Java. The skin of cocoa fruit is usually boiled and then the water is clamped and taped to treat the head of children who are corroded and bald. The absence of scientific research on the truth of the properties of cocoa skin is the rationale for this research.

Jusmiati *et al.* examined the antioxidant activity of ripe cocoa fruit skin and young cocoa fruit skin.<sup>[5]</sup> These activities are closely related to the mechanism of plants as hair growers.<sup>[6]</sup> This study reports the activity of waste cacao peels where peels were extracted and fractionated then extracts and fractions obtained to be tested for activities using the modified Tanaka method for experimental animals, rabbits.<sup>[7]</sup>

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## MATERIALS AND METHODS

### Materials

The materials used in this study included waste cacao peels obtained from Dingгаа Linggarjati village at the foot of Mount Galunggung, Tasikmalaya, West Java, and determined at the Taxonomy Laboratory, Biology Department, Universitas Padjadjaran.

### Animals

The experimental animals used were white male rabbits 3–5 months old, healthy, normal activities, having a bodyweight of 1.5–2 kg. All rabbits were adapted first before being treated for about 2 weeks by regulating their environmental conditions so as not to be stressed still being fed and given drinks. For 2 weeks, his health was monitored. Seven white male rabbits used in this study were determined using the Federer formula.<sup>[8,9]</sup> Ethical approval no. 06/UN6. KEP/EC/2018 obtained from the Research Ethics Committee, Universitas Padjadjaran.

### Methods

#### Extraction

The maceration method was used for extraction in this study. This method was selected to prevent the occurrence of damage to the thermolabile chemical compounds contained in the *Theobroma cacao* sample. It was carried out by soaking the sample in the macerator then leaving it for 24 h at room temperature with stirring occasionally. The solvent replacement was carried out during  $3 \times 24$  h.<sup>[10,11]</sup>

#### Phytochemical investigation

Phytochemical screening was applied to the sample based on Farnsworth's method for secondary metabolites.<sup>[7]</sup>

#### General standard parameters of extracts

Examination of standard extract parameters based on the general standard of extract parameters.<sup>[12]</sup> The general standard parameters of extracts carried out included organoleptic extract criteria, extract yield, ash content, and moisture content.

#### Fractionation of crude ethanolic extract

The fractionation method of leaves ethanol extract used was liquid-liquid extraction using two or more non-mixed solvents. This fraction used ethyl acetate and n-hexane solvents concerning the Mustarichie *et al.*<sup>[13]</sup>

#### The activity of stimulating hair growth from the ethanol extract

This test was based on a modification of the Tanaka *et al.* method.<sup>[14]</sup> The test was to find effective concentrations that were efficacious as hair growth.

#### Test of hair growth stimulating activity from the ethanol extract fraction

This test was similar to a test of ethanol extract. It was based on a modified method of Tanaka *et al.*<sup>[14]</sup> This test was applied to find the most effective fraction of hair growth.

#### Data Analysis

Rabbit hair length data obtained from the results of the study per 3 up to 18 days were averaged then processed statistically using the one-way variant analysis (ANOVA) and Kruskal–Wallis methods.<sup>[15]</sup>

## RESULTS AND DISCUSSION

### Phytochemical Screening

Phytochemical screening cacao was carried out on ethanol extract to identify groups of compounds in waste cacao peels. Phytochemical screening results are shown in Table 1.

Sihombing *et al.* mentioned that cacao peel extract contained steroids, triterpenoids, and phenolics.<sup>[16]</sup> Djali *et al.* reported that the fresh and after drying cacao shell contained phytochemical compounds such as polyphenols, tannins, and flavonoids.<sup>[17]</sup> Methanolic cacao leaf extract was reported to contain flavonoids, tannins, triterpenes, saponin, and steroids.<sup>[18]</sup> Rachmawaty *et al.* in their study on Cacao Pod Husk reported that it contained three major components of the compound in ethanol, i.e., methyl-9,12-dienoate octadeca; 9-octadecenoic acid (Z)-, methyl ester; and hexadecanoic acid, 15-methyl, methyl ester, whereas in acetone solvent compounds showed four major components, namely, isopropyl myristate; 1,2-benzenedicarboxylic acid, dioctyl ester; 9-octadecenoic acid (Z)-, methyl ester; and octadecanoic acid, methyl ester.<sup>[19]</sup> Similar to our report that no alkaloids were detected in the cacao peels. Lee *et al.* stated that cocoa had more phenolic phytochemicals and a higher antioxidant capacity than teas and red wine.<sup>[20]</sup>

### Standard Parameters of Extracts

Examination of general standard parameters carried out on thick waste cacao peels extract to ensure extract quality as well as a guarantee of quality stability so that it can be developed as pharmaceutical products in general.<sup>[21]</sup> Table 2 showed the results of the examination of the general parameter standard of waste cacao peels ethanol extract.

According to the WHO, the required limit for ash content was not more than 1% while the total ash content obtained exceeds the requirements limit and acid insoluble ash content met the requirements.<sup>[11,22]</sup> The water content in the thick extract obtained, However, met the requirements of no more than 30%.<sup>[23]</sup> The water content in the extract was related to the purity

of the extract, the less water content in the extract and the less likely the extract were contaminated with microbes or fungi.

### Hair Growth Activities from Waste Cacao Ethanol Peels Extract

Tests were carried out on male rabbits using the Tanaka method which was modified by shaving the rabbit's back hair. The ethanol extract used was extracted with a concentration of 5%, 10%, 15%, 20%, and 25%. The reason why male rabbits were chosen as test animals were because male rabbits had a more stable hormonal system compared to female rabbits that were easily influenced by psychological factors starting from the menstrual cycle, pregnancy, and breastfeeding. Furthermore, the selected rabbit must be an adult rabbit aged 3–5 months healthy without a disability and weighing about 1.5–2 kg.<sup>[24]</sup> Adult rabbits had good and perfect physiological functions that were expected to not interfere with the research process. Rabbits used ought to be healthy without defects because it was feared there were physiological defects that affected the results of this hair growth study.

**Table 1: Phytochemical screening of the ethanol extract of waste cacao peels**

Secondary metabolite	Results
Alkaloids	–
Flavonoids	+
Saponin	+
Polyphenols	+
Tannins	+
Quinone	–
Monoterpene and sesquiterpene	+
Steroids and triterpenoids	+

(+): Detected, (–): Not detected

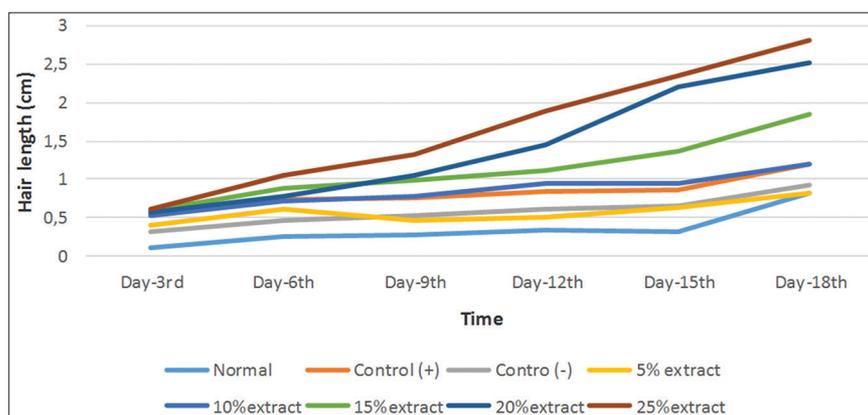
**Table 2: The general standard parameters extract of waste cacao peels**

Parameters	Results (%)
Water content	8.60
Total ash content	5.80
Dissolved ash content in acid	0.64

Tests were carried out on three male rabbits whose backs were shaved and divided into eight plots consisting of normal controls, negative controls (NaCMC 0.5%), positive controls (Minoxidil 2%), test extracts with various concentrations of 5, 10, 15, 20, and 25%. Normal control function as a comparison to determine differences in hair growth in plots that were given normal control and care (not treated) and to determine the effect (activity) of preparation for hair growth. In the negative control plot, 0.5% NaCMC was applied as a basis for extract preparation. This negative control was used as a comparison to prove that NaCMC had no activity to stimulate hair growth during testing. Positive controls used were synthetic drugs that had been on the market, namely, 2% minoxidil. This drug was chosen because it was an food and drug administration-approved hair growth drug and had been shown to have topical hair growth and use.

Test parameters were measured by measuring rabbit hair length which grows every 3 days for 18 days measured using a caliper with an accuracy of 0.05 mm. Measurement of rabbit hair length was carried out on six strands of hair per plot, the more hairs had taken more representative results from hair growth measurements per plot in three rabbits.<sup>[25]</sup> The measurement results are shown in Table 3 and rationalized in Figure 1.

Based on Figure 1, it showed that every day rabbit hair length increases, rabbit hair grew in all groups. On hair measurement data, the longest rabbit hair growth reached 2.765 cm which was found in the treatment group of waste cacao peels extract 25% on the 18<sup>th</sup> day of testing. The graph showed that on the 3<sup>rd</sup>–9<sup>th</sup> days, differences in hair length between groups had not been seen. The difference in hair length between groups began to be seen clearly on the 12<sup>th</sup> day and the 18<sup>th</sup> day. The group that has the highest hair growth was the group given 25% extract. The 10% extract test material showed effects that were almost equivalent to positive controls, while extracts with a concentration of 15%, 20%, and 25% had exceeded the



**Figure 1:** Graph of the results of testing the activity of hair growth stimulator ethanol extract of waste cacao peels

**Table 3: Test results for hair growth stimulant activity extract of waste cacao peels**

Groups	Day to hair length (cm)					
	3	6	9	12	15	18
Positive control						
Rabbit 1	0.320	0.272	0.269	0.586	0.610	1.173
Rabbit 2	0.543	0.783	0.760	0.858	0.886	0.787
Rabbit 3	0.105	0.279	0.528	0.562	0.595	1.042
Mean±SD	0.323±0.219	0.444±0.293	0.519±0.246	0.669±0.165	0.697±0.164	1.001±0.197
Extract 25%						
Rabbit 1	0.476	0.388	1.153	1.092	2.073	2.765
Rabbit 2	0.580	0.990	1.268	1.492	2.162	1.407
Rabbit 3	0.193	0.842	0.741	1.793	1.200	2.223
Mean±SD	0.416±0.200	0.740±0.314	1.054±0.277	1.459±0.352	1.812±0.532	2.132±0.684
Extract 20%						
Rabbit 1	0.417	0.358	1.058	0.765	1.900	2.480
Rabbit 2	0.551	0.786	0.848	1.077	1.951	1.043
Kelinci 3	0.181	0.545	0.643	1.463	0.793	1.862
Mean±SD	0.383±0.188	0.563±0.214	0.850±0.207	1.101±0.349	1.548±0.655	1.795±0.721
Extract 15%						
Rabbit 1	0.371	0.322	0.934	0.660	1.140	1.783
Rabbit 2	0.588	0.862	0.883	0.982	1.313	0.850
Rabbit 3	0.168	0.667	0.591	1.063	0.689	1.468
Mean±SD	0.376±0.210	0.617±0.273	0.803±0.185	0.902±0.213	1.047±0.322	1.367±0.475
Extract 10%						
Rabbit 1	0.309	0.304	0.732	0.476	0.828	0.930
Rabbit 2	0.540	0.614	0.662	0.888	0.898	0.666
Rabbit 3	0.163	0.658	0.403	0.818	0.516	1.190
Mean±SD	0.337±0.190	0.525±0.193	0.599±0.173	0.727±0.221	0.748±0.204	0.929±0.262
Extract 5%						
Rabbit 1	0.255	0.299	0.275	0.310	0.495	0.714
Rabbit 2	0.406	0.641	0.473	0.535	0.634	0.487
Rabbit 3	0.151	0.261	0.310	0.322	0.346	0.785
Mean±SD	0.271±0.128	0.400±0.209	0.353±0.105	0.389±0.127	0.492±0.144	0.662±0.156
Negative control						
Rabbit 1	0.208	0.252	0.263	0.345	0.322	0.908
Rabbit 2	0.384	0.486	0.563	0.642	0.682	0.495
Rabbit 3	0.091	0.233	0.291	0.269	0.358	0.727
Mean±SD	0.228±0.148	0.323±0.141	0.372±0.166	0.418±0.197	0.454±0.198	0.710±0.207
Normal control						
Rabbit 1	0.095	0.250	0.230	0.301	0.302	0.792
Rabbit 2	0.105	0.200	0.285	0.333	0.329	0.496
Rabbit 3	0.075	0.215	0.260	0.272	0.299	0.728
Mean±SD	0.092±0.015	0.222±0.026	0.258±0.028	0.302±0.030	0.310±0.017	0.672±0.156

SD: Standard deviation

**Table 4: Least significant difference test results**

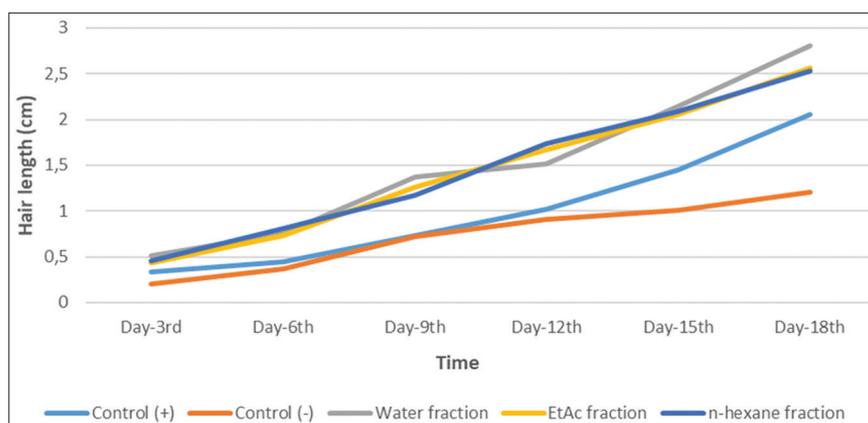
Group (I)	Group (J)	Mean difference (I-J)	Std. error	Sig.	95% Confidence interval	
					Lower bound	Upper bound
Extract 15%	Extract 5%	0.512667*	0.187706	0.015	0.11475	0.91059
	Extract 10%	0.174333	0.187706	0.367	-0.22359	0.57225
	Extract 20%	-0.200000	0.187706	0.302	-0.59792	0.19792
	Extract 25%	-0.557333*	0.187706	0.009	-0.95525	-0.15941
	Positive control	0.233000	0.187706	0.232	-0.16492	0.63092
	Negative control	0.483000*	0.187706	0.020	0.08508	0.88092
	Normal control	0.599667*	0.187706	0.006	0.20175	0.99759

effects of positive control. This verified that the ethanol extract of waste cacao peels had stimulant activity in hair growth in male rabbits. At a concentration of 15%, the ethanol extract of waste cacao peels had a higher effect than the drug minoxidil 2% as a positive control.

#### Analysis with Statistical Methods for Ethanol Extract with the Following Stages

Statistical analysis was performed to see the differences in hair growth stimulant activities between groups so

that the dose of waste cacao peels extract could be determined which provided hair growth stimulant activity. Statistical analysis was performed using IBM SPSS version 22 software. Before further testing, the data were tested for normality and homogeneity to determine the follow-up test to be used. The normality test was carried out by the Shapiro–Wilk method and the homogeneity test using the Levene Statistics method. In the hypothesis normality test  $H_0$ , the population of rabbit hair length data was normally



**Figure 2:** Graph of the results of testing the activity of hair growth stimulator fractions of waste cacao peels

distributed, while H1 is the population data of rabbit hair length not normally distributed. The results of the Shapiro–Wilk normality test show a significance of  $>0.05$  indicating that H0 is accepted so that the data were normally distributed. Data homogeneity testing was carried out by the Levene statistical test where H0 stated that the population of rabbit hair length data was homogeneously distributed and H1 stated that the population of rabbit hair length data was not homogeneously distributed. The results of the analysis show significance  $>0.05$  which indicates that H0 was accepted so that it was stated that the data were distributed homogeneously.

Based on the results of ANOVA analysis, the significance value obtained is  $<0.05$  which indicated that there were differences due to the treatment given. However, ANOVA had not shown the extent to which differences were given between groups. Therefore, further testing was carried out using the least significant difference (LSD) method. The results of LSD analysis showed that there were significant differences with significant ( $<0.05$ ) between extract groups of 15%, 20%, and 25% with negative controls and normal controls. This showed that the waste cacao peels ethanol extract started at a concentration of 15% had a stimulating activity for hair growth. Table 4 showed an example of the statistical results that support decision making in this study.

#### Test of Hair Growth Activity of Water, N-hexane, and Ethyl Acetate Fractions

After the waste cacao peels extract activity was known, then proceed with testing the activity of its fractions. A similar method has applied the result of which is shown in Figure 2.

Using statistical methods similar to the determination of ethanol extract activity, namely, by the normality test using the Shapiro–Wilk method, homogeneity was tested by the Levene Statistic method, followed by ANOVA and LSD tests; it was found that the n-hexane fraction of waste cacao peels had the best

activity compared to the other fractions and positive controls (minoxidil 2%).

## CONCLUSION

The results showed that the ethanol extract of cocoa waste skin and its fractions had stimulant activity in hair growth on rabbit hair. The effect of hair growth starting from 15% concentration of ethanol extract and n-hexane fraction showed the best effectiveness compared to other fractions and positive controls (minoxidil 2%). Testing of volunteers is suggested that this waste cocoa peels extract can be marketed to humans.

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