

Hair Growth Cream Formulation From Shoe Flower Leaf Ethanol Extract (*Hibiscus rosa-sinensis* L.) As a Hair Grower in Rabbit (*Oryctolagus cuniculus*)

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ABSTRACT

Hair loss is one of the hair problems that often happens to anyone. hibiscus leaves (*Hibiscus rosa-sinensis*L.) have traditionally been recognized as having an activity that stimulates hair growth. To make it easier to use, it needs to be made in preparations that are practical and comfortable to use, such as in the form of cream. This study aims to determine the hair growth activity of cream preparations of ethanol extract of hibiscus leaves with concentrations of 10%, 15%, and 20%. In addition, physical quality tests and stability tests of cream preparations were also carried out. Observation of hair activity was carried out by calculating the rate of hair growth in 3 white rabbits. The Stability test was carried out at room temperature storage ($25^{\circ}\text{C} \pm 2^{\circ}\text{C}$). The results of statistical analysis using way ANOVA Repeated measures obtained a significant value of 0.000 ($p < 0.05$). From this significance value, it can be interpreted that there is a significant difference in hair growth activity between all treatment groups. The results showed that hibiscus leaf extract hair growth cream showed hair growth activity the best at a concentration of 20%. The results of the physical quality test of cream at FI had the best characteristics because it produced a physical quality value that was better than FII and FIII. The results of the physical stability test showed that the ethanol extract of hibiscus leaf hair growth cream had good physical stability.

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1. INTRODUCTION

Hair has various important functions in life, for example, hair that grows on the head can serve as protection from hot or cold weather and has an aesthetic value that supports one's appearance [1]. With this important function, hair care needs to be done to keep hair healthy. One way to care for hair is to provide nutrients and vitamins for hair growth. However, an excess of several types of vitamins or nutrients harms hair, such as an excess of vitamin A, vitamin E, and omega 3 which has a side effect, namely hair loss [1].

The prevalent issue pertaining to hair is the gradual loss or thinning of hair. Hair strand abnormalities can be observed in certain individuals, often resulting from excessive outdoor activities, and in rare cases, congenital abnormalities may also be a contributing factor [2]. Hair loss is an inherent process that is universally experienced, as hair undergoes a cyclical pattern. The typical cycle of hair growth encompasses three distinct phases, which are identified as the anagen phase (growth phase), the catagen phase (resting phase), and the telogen phase [3].

The increasing prevalence of adverse effects associated with the utilization of synthetic materials has led to a growing interest among the general populace in the notion of reverting back to natural alternatives. Utilizing natural ingredients presents a viable alternative for addressing Alopecia. Natural plant extracts are a cost-effective and easily accessible alternative to synthetic drugs, with the added benefit of minimal adverse effects, rendering them a safer option. Hibiscus leaves are a viable natural ingredient for promoting hair growth.

Prior research has established the hair growth and anti-graying properties of hibiscus leaves and flowers (*Hibiscus rosa-sinensis* L.), as acknowledged by [4]. The subject of discussion is the *Hibiscus rosa-sinensis* L. plant. The specimen exhibits conspicuous red flowers of considerable size. Flavonoids are present in the leaves, flowers, and roots. According to [5], hibiscus leaves are composed of various

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compounds, including but not limited to alkaloids, glycosides, flavonoids, tannins, phenols, and saponins. According to [6], there exist various compounds that possess properties conducive to hair growth, including flavonoids, saponins, and phenolic compounds. According to [6], prior research has demonstrated that extracts derived from both hibiscus flowers and leaves exhibit comparable properties in promoting hair growth, while also presenting a lower likelihood of causing significant adverse effects. Some research results prove that hibiscus leaf extract is more potent in stimulating hair growth compared to hibiscus flower extract [7].

The mechanism of flavonoids for hair growth is that they can increase hair growth by strengthening the capillary walls of the small blood vessels that supply hair follicles [8]. The mechanism of saponins for hair growth is that saponins can form a foam which means they can clean the skin of dirt, besides that it functions to increase blood flow to the hair follicles so that it can increase, if blood flow to follicles decreases it will affect the hair follicles and cause hair loss [3]. The mechanism of phenol for hair growth is that it has keratolytic activity. Keratolytic itself is a substance that can remove excess dead skin cells produced by the epidermal layer, in the form of rough skin scales on the scalp.

In this study, using rabbits as test animals because they have several advantages, namely body size (including the back) which is wide enough as a test area making it easier to shave hair, ease in handling it when given treatment (not easily stressed)[9]. This research is expected to make the application of the use of hibiscus leaf extract more practical, as well as be able to make hair growth activities more optimal.

2. METHOD

Tools and materials

The tools used in this research are analytical scales, erlenmeyer, measuring cups, beaker glass, mortar, and stamper, porcelain cup, dropping pipette, stir bar, shakers, water bath, pH meter, watch glass, spatula, calipers, aluminum foil, parchment paper, oven, ointment pot, desiccator, test tubes, test tube rack, object glass, cloth flannel, conductor lamp, glass funnel, and hair clipper.

The material used in this study was the simplicity of hibiscus leaves (*Hibiscus rosa-sinensis*), hibiscus leaf extract (*Hibiscus rosa-sinensis*), stearic acid, cetyl alcohol, glycerin, TEA, propylparaben, methylparaben, distilled water, 70% ethanol.

Preparation of hibiscus leaf extract

Hibiscus flower leaves are extracted by maceration. 300 grams of hibiscus leaf simplistic powder is put in an Erlenmeyer, then soaked in 3000 ml of 70% ethanol, and then closed. Maceration is carried out for about 5 days by shaking or frequently stirring. After 5 days, filtered with flannel and squeezed. The macerate obtained was concentrated in the water bath (the temperature is maintained at 40°C – 50°C) until a thick extract is obtained [10].

Identification of Flavonoids

The concentrated extract was dissolved in ethanol. Then put a little into the test tube, then add Mg powder. After that, 1 ml of concentrated HCl was added to the test tube. Changes in the color of the sample to color yellow, orange, red, and green indicate the presence of flavonoids[11].

Phenol Identification

The thick extract was diluted without a little into a test tube, then 3 drops of FeCl reagent were added 31%. A change in the color of the sample to green, blue, or purple indicates the presence of phenolic compounds[11].

Identification of Saponins

The thick extract is dissolved first with warm water. After that, put a little into a test tube, then add 10 ml of water, after that, it is cooled and shaken vigorously until foam forms. The 1 cm high froth that formed indicated the presence of saponins[11].

Cream formulation

Weighing all the ingredients, then preparing a hot mortar. Oil phase ingredients (stearic acid, cetyl alcohol) are put in and melted in a water bath with a temperature of 70°C – 80°C (Mixture 1). Dissolve methyl paraben and propyl paraben with glycerin (Mixture 2) in a beaker glass, stir, and dissolve. Added TEA in mixture 2 stir ad dissolved, put mixture 2 and TEA into the mortar. The oil phase is introduced into the water phase gradually by stirring and formed creamy mass. Enter the hibiscus leaf extract which has been dissolved with distilled water into the cream base that has been formed little by little, and stir until homogeneous. The cream is then put in a tightly closed container[12].

Table 1. Formulation of cream preparations

Material name	Utility	Concentration			
		K (-)	F1(%)	F2(%)	F3 (%)
Leaf extract Hibiscus	Active substance	8	8	8	8
Stearic acid	Emulsifying agent	8	8	8	8
Cetyl alcohol	Emulsifying agent	5	5	5	5
Glycerin	humectants	15	15	15	15
TEA	Alkalizing agent	1	1	1	1
Methyl paraben	Water phase preservative	0,2	0,2	0,2	0,2
Propyl paraben	Oil phase preservative	0,2	0,2	0,2	0,2
Aquadest	Solvent	100	100	100	100

EVALUATION OF CREAM PREPARATION

Organoleptic Test

Organoleptic tests include observing changes in shape, smell, and color in cream preparations[13].

Homogeneity Test

The cream is taken from each formula to taste and smeared on the glass plate, touched, and when rubbed the cream mass must show a homogeneous composition, that is, no solid material is felt on the glass[13].

pH test

Measurement using pH preparations done. pH meters first calibrated with a buffer solution of pH 4 and pH 7. Take 1 gram of cream preparation and dilute it with 10 ml of distilled water. Then measure the pH using a pH meter, that has been calibrated with a buffer solution using pH[13].

Cream Type Test

The cream that has been made is put into a beaker glass, then connected to an electric current. An incandescent lamp indicates the type of cream is oil in water[14].

Spreadability Test

A quantity of 0.5 grams of the cream formulation was measured and applied onto a graph paper-coated glass surface. Subsequently, a petri dish was positioned over the cream and allowed to remain for a duration of 1 minute. The surface area occupied by the cream preparation was then determined through calculation. Subsequently, a load of 50, 100, 150, 200, and 250 grams was applied successively to the preparation, followed by a 60-second rest period, after which the area of the resulting preparation was determined[15].

Stickiness Test

As much as 0.5 grams of cream smeared on top Object glass. laid Object glass the other on the cream is then pressed with a load of 1 kg for 5 minutes. Object glass was installed on the test equipment and then given a load weighing 80 grams, and recorded the time until the two glass objects were separated[15].

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Acceptability Test

The acceptability test was carried out for 1 day on 10 panelists to find out which formula the volunteers liked the most as a hibiscus leaf extract hair growth cream[16].

Stability Test

The stability test was carried out for 1 month and stored at room temperature. in the 7th week

3. RESULTS AND DISCUSSION

The results of the phytochemical screening showed that hibiscus leaves positively contained flavonoids, phenols, and saponins. Results of Physical Quality Evaluation of Cream Preparations.

Organoleptic test

The organoleptic test was carried out to see the physical appearance of the cream preparation with 0, the 2nd week, and the 4th week a physical quality test is carried out. The preparation is said to remain stable if it does not experience changes such as organoleptic, homogeneity, pH, spreadability, and adhesion.

Hair Growth Activity Test

Hair growth activity test was carried out by preparing 3 rabbits which were grouped into rabbit 1 for control (+) and control (-), rabbit 2 for P1 (formulation 1), P2 (formulation 2), and P3 (formulation 3). The rabbit's back was shaved, then measured 2x2 cm in each area, one area with another area was given a distance of 1 cm. The basting of the preparations was carried out 2 times a day, which was carried out for 21 days. Measure the hair that grows in the test area on days 8, 15, and 22. The longest hair is taken then placed on tape and black cloth and then measured from the base to the tip of the hair using a caliper[17].

Results of Phytochemical Screening

Flavonoid compounds formed orange color, phenol formed brown color, and saponins formed a stable froth. Make observations of shape, color, and smell visually. The results of the observations showed that the negative control was in the form of a semisolid, white color, odorless, whereas formulations I, II, and III are semisolid, green in color, and have a characteristic odor of extracts.

Homogeneity test

The homogeneity test was carried out to see whether there were coarse grains in the cream preparation[18]. The results of the observations showed that the negative control cream preparations and formulations I, II, and III were homogeneous and free of coarse particles. In the cream preparation, there are no coarse particles indicating that the ingredients are mixed evenly.

pH test

The pH assessment is conducted to ascertain the pH level of the cream in relation to the pH level of the skin, with the aim of avoiding any potential harm to the skin caused by excessive acidity or alkalinity. Low pH levels or acidity can lead to skin irritation, while high pH levels or alkalinity can result in skin dryness. et al., 2020). The pH test results obtained had an average pH of 7.6 ± 0.152 in the negative control, formulation I 7.4 ± 0.152 , formulation II 7.0 ± 0.208 , formulation III 7.1 ± 0.100 . From the results of the pH value, the cream preparation met the requirements for the scalp pH range, which ranged from 5.0 to 9.0[19].

Table 2. pH Test Results

Formulation	Replication	pH test	Average
K(-)	1	7,5	7,6 ± 0,152
	2	7,7	
	3	7,8	

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FI	1	7,4	7,4 ± 0,152
	2	7,3	
	3	7,6	
FII	1	7,3	7,0 ± 0,100
	2	7,0	
	3	6,9	
FIII	1	7,0	7,6 ± 0,100
	2	7,1	
	3	7,1	

Spreadability test

The spreadability test was carried out to determine the extent of the spreading of the cream preparation. The spreadability requirements for topical preparations are 5-7 cm [20]. From the results of the power distribution test, hibiscus leaf extract cream had an average negative control of 5.76 ± 0.301 , formulation I 5.83 ± 0.230 , formulation II 5.8 ± 0.229 , formulation III 5.53 ± 0.208 . The test results for the spreadability of the cream preparations met the requirements for good spreadability of topical preparations, namely 5- 7cm. Good spreadability causes contact between the drug and the skin to be extensive, so that the absorption of the drug into the skin takes place quickly.

Table 3. Spreadability Test Results

Formulation	Replication	Spreadability Test Diameter(cm)					Average
		50g	100 g	150g	200 g	250g	
K(-)	1	4,8	4,95	5,3	5,7	6,05	5,76 ± 0,301
	2	4,65	5,05	5,25	5,45	5,8	
	3	4,65	4,85	5,1	5,2	5,45	
I	1	4,35	4,7	5,1	5,45	5,7	5,8 ± 0,230
	2	4,3	4,65	5,2	5,6	6,1	
	3	4,6	4,9	5,1	5,45	5,7	
II	1	4,8	5	5,2	5,5	5,75	5,8 ± 0,229
	2	4,8	5,25	5,5	5,8	6,05	
	3	4,3	4,75	5,05	5,35	5,6	
III	1	4,85	5,05	5,25	5,45	5,7	5,53 ± 0,208
	2	4,4	4,65	5,05	5,35	5,6	
	3	4,25	4,6	5	5,05	5,3	

Stickiness test

Stickiness test done for Find out how long it takes the cream to stick to the skin. The requirement for good adhesion for topical preparations is more than 4 seconds [20]. The results of the observations obtained an average negative control of 5.13 ± 0.032 , formulation I 4.39 ± 0.085 , formulation II 4.18 ± 0.055 , formulation III 4.16 ± 0.080 . The results of the adhesiveness test for cream preparations met the requirements for good adhesion of topical preparations, namely more than 4 seconds. Good adhesion means that the drug is not easily separated and sticks to the skin longer, so it can produce the desired effect.

Table 4. Stickiness Test Results

Formulation	Replication	Power Test sticky	Average
K(-)	1	5,17	5,13 ± 0,032
	2	5,12	
	3	5,11	
FI	1	4,48	4,39 ± 0,085
	2	4,39	
	3	4,31	

FII	1	4,24	4,18 ± 0,055
	2	4,19	
	3	4,13	
FIII	1	4,08	4,16 ± 0,080
	2	4,18	
	3	4,24	

Cream type test

Cream type test done for know the type of cream M/A (oil in water) or A/M (water in oil). The conductivity test or electrical conductivity test carried out revealed that the light bulb used was glowing because water as the outer phase is capable of conducting electricity (Armini, 2014). The test results, Proves that all cream preparations are included in the M/A type (oil in water) and also do not show phase inversion after accelerated storage conditions.

Acceptability test

The acceptability test was carried out to find out which formulation the panelists liked the most in hair growth cream preparations. This acceptability test was carried out for 1 day on 10 panelists. Based on the panelist test results, the researcher wanted to see which of the 3 formulations the panelist preferred. Assessment parameters include color, smell, shape, and ease of applying the cream to the skin.

Table 5. Acceptability Test Results

Formulation	Indicator	% Panelist Like Rate			
		SS	S	KS	TS
FI	Color	80	10	10	-
	Smell	70	20	10	-
	Form	70	10	20	-
	Easy to grease	80	20	-	-
FII	Color	70	10	10	10
	Smell	60	10	20	10
	Form	50	30	20	-
	Easy to grease	70	30	-	-
FIII	Color	60	10	10	20
	Smell	50	10	20	20
	Form	40	10	20	30
	Easy to grease	60	10	30	-

Stability Test

Stability test done for determine whether the cream formulation is stable in storage. Evaluation of the stability of the cream preparations in terms of physical quality includes organoleptic tests, homogeneity tests, pH tests, spreadability tests, and adhesion tests. This is a factor that must be considered because it can be used to determine the effect of the environment during storage. Observation of the stability of this cream preparation was carried out for 1 month every 2 weeks, observations were carried out, namely at week 0, week 2, and week 4.

In the organoleptic test week 0 and week 2 the negative control cream and formulations I, II, and III did not show changes in color, shape, and smell both before and after storage. During the 4th week of observation, the negative control did not change color, smell, and shape before and after storage. Meanwhile, in formulations I, II, and III, the color and smell did not change before and after storage, but the form changed to become solid after storage. In the homogeneity test of week 0, week 2, and week 4, negative control cream preparations and formulations I, II, and III showed good results, namely homogeneous. This indicates that the preparation is stable inhomogeneity during storage either before or after.

Observations on the week 0 pH test of negative control cream preparations were 7.6 ± 0.152 , FI 7.4 ± 0.152 , FII 7.0 ± 0.208 , and FIII 7.1 ± 0.100 . Week 2 negative control cream preparations $7.5 \pm$ *Hair Growth Cream Formulation From Shoes Flower Leaf Ethanol Extract (Hibiscus Rosa-SinensisL.) As A Hair Grower In Rabbit (Oryctolagus Cuniculus)*. **Munifatul Lailiyah**

0.000, FI 7.0 ± 0.115 , FII 6.8 ± 0.057 , and FIII 6.8 ± 0.152 . Week 4 negative control preparations 7.5 ± 0.208 , FI 7.3 ± 0.100 , FII 7 ± 0.000 , and FIII 7.0 ± 0.152 . It can be seen from the weekly pH value that has decreased and increased during storage, but the results still meet the pH standards for topical preparations.

Observation of the spreadability test on week 0 of the negative control cream was 5.76 ± 0.301 , FI 5.83 ± 0.230 , FII 5.8 ± 0.229 , and FIII 5.53 ± 0.208 . Week 2 negative control cream preparations 5.2 ± 0.05 , FI 5.35 ± 0.312 , FII 5.46 ± 0.361 , and FIII 5.35 ± 0.086 . Week 4 negative control preparations 5.36 ± 0.160 , FI 5.25 ± 0.1 , FII 5.15 ± 0.1 , and FIII 5.33 ± 0.160 . Judging from the spreadability value obtained every week it has decreased and increased but still meets the standard topical spreadability value.

Observation of adhesion test on the 0th week of negative control cream preparations 5.13 ± 0.032 , FI 4.39 ± 0.085 , FII 4.18 ± 0.055 , and FIII 4.16 ± 0.080 . Week 2 negative control preparations 4.23 ± 0.091 , FI 4.18 ± 0.070 , FII 3.87 ± 0.200 , and FIII 3.51 ± 0.160 . Week 4 negative control preparations 4.57 ± 0.111 , FI 4.23 ± 0.080 , FII 4.08 ± 0.036 , and FIII 3.77 ± 0.245 . It can be seen from the adhesive power values obtained every week that have decreased and increased.

Hair Growth Activity Test

The hair growth activity test in rabbits was carried out in 5 groups, namely positive control, negative control, formulation I, formulation II, and formulation III. Assessment parameters include hair length. Based on the data in Table 15, it can be explained that the smallest average hair length was shown by the negative control treatment group, namely 2.44 mm on the 8th day, 4.23 mm on the 15th day and 5.54 mm on the 21st day. Meanwhile, the treatment group of hibiscus leaf ethanol extract cream formulation III with a concentration of 20% showed the largest average hair size of 6.01 mm on the 8th day, 8.70 mm on the 15th day, and 12.43 mm on the 21st. 10%, 15%, and 20% on rabbit hair growth.

The results of statistical analysis using One way ANOVA Repeated Measure Based on the multivariate test, there was a significant difference between the average hair length of the rabbits in the first week to the third week. In testing pairwise comparisons, the hair length of the first, second, and third weeks there is different. Based on the effect, the variance of the first, second, and third weeks there is a significant difference.

From the resulting data it can also be concluded, the hair growth activity test for 22 days showed that 10%, 15%, and 20% hibiscus leaf extract cream had an activity that stimulated hair length growth which was equivalent to minoxidil. Cream of hibiscus leaf ethanol extract 10%, 15%, and 20% and the positive control (minoxidil) showed stimulation of rabbit hair growth compared to the negative control. In 20% hibiscus leaf extract cream, hair length growth activity was greater than that of 10% and 15% hibiscus leaf ethanol extract cream. Several studies have shown that the compound Polar compounds such as flavonoids have the activity to promote hair growth by strengthening the walls of the capillaries in the hair hair follicle blood vessels[7].

Table 6. Hair Growth Activity Test Results

Test group	Average hair length (mm)		
	1st week	2nd week	3rd week
Positive control	6.57 ± 0.025	9.85 ± 0.032	14.32 ± 0.147
Negative control	2.44 ± 0.02	4.23 ± 0.03	5.54 ± 0.03
FI	4.04 ± 0.035	6.36 ± 0.03	10.23 ± 0.025
FII	5.03 ± 0.030	7.63 ± 0.03	11.46 ± 0.025
FIII	6.01 ± 0.015	8.70 ± 0.015	12.43 ± 0.035

The mechanism of action of the chemical compounds responsible for the hair growth activity of the ethanol extract of hibiscus leaves could not be determined in this study. From the results of the identification of phytochemicals, the ethanol extract of hibiscus leaves contains flavonoids, phenols, and saponins. Several other studies show that flavonoids have activities that can increase hair growth by strengthening the capillary walls of small blood vessels that supply hair follicles, increasing blood circulation to nourish hair follicles to increase hair growth[7].

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4. CONCLUSION

Differences in concentrations of ethanol extract of hibiscus leaves (*Hibiscus rosa Sinensis L.*) affect physical quality characteristics of hair growth cream preparations. Differences in concentrations of hibiscus leaf extract (*Hibiscus rosasinensisL.*) affect hair growth activity in rabbits. Cream preparation of ethanol extract of hibiscus leaves with a concentration of 20% showed the best hair growth activity in rabbits compared to cream extract. hibiscus leaf ethanol concentration of 10%, 15%, and negative control.

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