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Research Article

Formulation of Hair Tonic from Ethanol Extract of Sea Hibiscus (*Hibiscus tileaceus* L.) Leaves in Promoting Hair Growth on Guinea Pig (*Cavia porcellus*)

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Abstract

Hair loss is an unavoidable process, as the age increase, the rate of hair growth also slows down. Hair loss is caused by many factors and occur in different areas of body. Since hair become one of the major aspect for women beauty, hair loss will become a serious problem if it is not solved. Many plants or their extracts have been used to reduce hair loss. The leaves of sea hibiscus (*Hibiscus tiliaceus* L.) have been known empirically to promote hair growth and prevent hair loss. The leaves contain saponin, flavonoid, and also polyphenol compounds. The purpose of this study was to formulate sea hibiscus leaf extract into hair tonic preparations and evaluate the activity in triggering hair growth. In this research, tested animals were divided into 5 groups: F0 (blank) as a negative control, F1 (20%), FII (25%), FIII (30%) extract of sea hibiscus leaves and minoxidil as positive control. All preparations made were kept in room temperature for 12 weeks and the pH value test was carried out in first, fourth, eighth and twelfth week. The hair growth activity was done by applying hair tonic to the guinea pig and hair was randomly plucked from test area measuring hair length using calliper on the 7th, 14th, 21st days. On the 21st day, hair tonic of 30% sea hibiscus leaf extract showed a great activity on the growth of hair length with an average of 17.2633 mm and weighed an average of 0.145g, with a significant difference compared to another formula ($p < 0.05$).

Keywords: Hair tonic, *Hibiscus tiliaceus*, Sea Hibiscus Leaves

INTRODUCTION

Hair is a person's crown and is an element that cannot be ignored because hair reflects personality, age and health. Basically caring for hair is very easy, starting with cleaning the hair at least once every 2 days and taking intensive care if you have problems with your hair and scalp [1,2]. Hair has a role in protection against adverse environmental conditions, including cold and hot temperatures, and ultraviolet light. In addition, hair also functions to protect the skin against bad influences, for example, eyebrows protect the eyes so that sweat does not flow into the eyes, while nose hair filters the air [3], in animals (mammals) hair functions as a protector and temperature regulator [4].

Hair growth generally goes through 3 main phases, namely anagen, catagen and telogen. The duration of each phase is different. In humans, anagen lasts 2-6 years (mean three years or 1000 days), catagen is only a few weeks, while telogen averages 100 days. Hair loss is a disorder in which the amount of hair is less or more than normal with or without visible thinning. Normally 80-120 strands of hair are shed per day [5,6]. The normal number of hair follicles on the head is around 100,000, and it is called an abnormality if the number reaches 50% which means about 50,000 strands. Excessive hair loss can lead to baldness [6]. The causes are varied, classified as endogenous, due to systemic disease, hormonal, nutritional status, intoxication, and genetic disorders; and exogenous from stimuli of the environment and the usage of too much hair cosmetics [7,8].

Hair care products are very important to prevent one of the problems of hair damage such as hair loss. Various hair care products, both synthetic and natural, have been developed to overcome the problem of hair loss. One of them on the market is derived from synthetic substances such as minoxidil. The use of minoxidil has the potential to cause side effects in its use such as skin allergies, headaches, vertigo, edema to hypotension. Hair care requires a variety of cosmetics such as hair conditioner, creams, to hair tonics. An easy way to treat hair loss is to do hair care using hair tonic as an ingredient to nourish hair [9].

Sea Hibiscus plants are widely found in Indonesia, on beaches that are not swampy or sandy soils. Sea Hibiscus leaves are also used empirically to treat various diseases such as cough, shortness of breath, boils, hair loss, eye inflammation and fever.The ability to grow hair from Sea Hibiscus leaf extract is caused by the content of saponins, polyphenols and flavonoids. Saponins have the ability to form foam which means they are

able to clean the skin from dirt and are contaminants, resulting in an increase in peripheral blood circulation thereby increasing hair growth. Likewise, polyphenols have keratolytic activity, disinfectants, and flavonoids which have bactericidal and anti-viral activity that can suppress the growth of bacteria and viruses, thereby accelerating hair growth and preventing hair loss [10,11].

Sea Hibiscus leaf extract is made in the form of hair tonic preparations because in daily application, hair tonic preparations are widely used to overcome the problem of hair loss, with several advantages, including easier and less sticky use such as semi-solid preparations so that it does not leave a thin layer that can trigger inflammation. formation of dandruff.

MATERIALS AND METHODS

The ingredients used to make hair tonic are Sea Hibiscus leaf extract, 96% ethanol, propylene glycol, sodium metabisulfite, methyl paraben, menthol, tween 80 and aquadest. Sample of 4 kg fresh and sea hibiscus (*Hibiscus tileaceus* L.) leaves were taken purposively without comparing to another places. The instruments used were guinea pig rearing equipment (cages, feed containers, and drinking containers), caliper, analytical balance, scissors and razors, markers, pH meter, beaker glass, erlenmeyer, measuring cup, aluminum foil, plastic wrap, extraction equipment (blender, oven, filter paper, funnel and evaporator).

Procedure

Preparation of test animals

In this study, the experimental animals used were 15 male guinea pigs weighing 450-600 grams. All of the guinea pigs were acclimatized for 7 days to adapt to the new environment.

Preparation of tonic formulation

The test material used was sea hibiscus leaf extract (*Hibiscus tiliaceaus* L). The sample is cleaned then cut into small pieces and dried in a drying cabinet for 24 hours, then puree with a blender. Fifty grams of the sample was macerated with 500 ml of ethanol for 24 hours. Then filtered using filter paper and the filtrate is stored in a dark and tight closed flask. Then the residue is macerated again with 340 ml of ethanol for 24 hours. After filtering, the residue is macerated again with 240 ml of ethanol. The resulting filtrate is combined and dried using a rotary evaporator until it obtained a crude extract [12,13].

Table 1: Hair tonic formula

Components	Concentration (%)			
	F0	F1	F2	F3
Sea hibiscus leaf extract	-	20	25	30
Ethanol 96%	30	30	30	30
Propylene glycol	15	15	15	15
Sodium metabisulfite	0.01	0.01	0.01	0.01
Methyl paraben	0.25	0.25	0.25	0.25
Menthol	0.10	0.10	0.10	0.10
Tween 80	2	2	2	2
Aquadest ad	100	100	100	100

Description:

F0: hair tonic without sea hibiscus leaf extract
F1: hair tonic containing 20% sea hibiscus leaf extract
F2: hair tonic containing 25% sea hibiscus leaf extract
F3: hair tonic containing 30% sea hibiscus leaf extract

Formulation of hair tonic

The formulation is done by dissolving tween 80 in aquadest, then add the extract of sea hibiscus leaves and stir until completely dissolved. Add sodium metabisulfite in aquadest until dissolved, mix it into the solution before and stir homogeneously by adding propylene glycol. Continue by dissolving each methyl paraben and menthol into ethanol, then mix those solution homogeneously. Mix it with extract solution and add an adequate amount of aquadest until it reach 100 ml for each formula.

Evaluation of hair tonic

a. Determination of physical appearance

Organoleptic test

The color, odor, and homogeneity were being observed since preparations were made and during storage until 12 weeks in room temperature [14].

pH test

The pH of all preparations were found by immersing pH meter into 1% diluted hair tonic. The determinations were carried out in triplicate and the average pH is recorded since the preparations were made and until 12 weeks of storage [15].

b. Activity of hair growth

Preparation of tested animals

Before testing the effectiveness on guinea pigs, the guinea pigs were acclimatized for 2 weeks, then the guinea pigs were divided into 5 groups, where each group consisted of 3 guinea pigs. The hair on the back of each guinea pig was shaved with a hair clipper with an area of 2×2 cm^2. The guinea pigs were

acclimatized for 24 hours and then the test material was applied. Application of hair tonic are based on the treatment group, in which F0 is for application of blank hair tonic (without sea hibiscus leaf extract), F1 for guinea pig with 20% sea hibiscus leaf extract, F2 for 25% concentration, F3 for 30% concentration and F4 for the positive control group. Hair tonic were applied to tested area once daily for 3 weeks observation. The data recorded is the hair length and hair weight which is analyzed before treatment and after 7th, 14th, 21st days of application. For the hair length, hair were randomly plucked and measured by calliper. The test was done triplicate and the average length of hair were analyzed by using statistic to see whether there is a significant difference in each group toward negative control. For the weight determination, hair is weighed on the last experiment period which is on 21st day by cutting the hair from tested area to see whether the volume of hair was increased. Results were recorded and analyzed by statistic [16,17].

RESULT AND DISCUSSION

The plant used in this study was sea hibiscus leaf (*Hibiscus tileaceus* L.). Sea Hibiscus leaves are empirically efficacious to nourish hair. The total weight of fresh leaves used was 4 kg, maceration with 500 grams of simplicia powder resulting 115 grams of crude extract. The extract obtained has a thick consistency, blackish brown in color, has a distinctive smell of sea hibiscus leaves.

The secondary metabolites contained in hibiscus leaves which stimulate the activity of hair growth are flavonoids, saponins and polyphenols. The extraction method used in this research is the maceration method. Ethanol was chosen because it is universal which is able to attract all types of active substances, both polar, semi-polar and non-polar so that active compounds such as flavonoids will be dissolved in the ethanol solvent [18].

Table 2: Organoleptic of hair tonic preparations

Organoleptic			
Formula	**Consistency**	**Color**	**Odor**
F0	Liquid form	Transparent	-
F1	Liquid form	Dark brown	Extract smell
F2	Liquid form	Dark brown	Extract smell
F3	Liquid form	Dark brown	Extract smell

Description:

F0 : hair tonic without sea hibiscus leaf extract F1 : hair tonic containing 20% sea hibiscus leaf extract
F2 : hair tonic containing 25% sea hibiscus leaf extract F3 : hair tonic containing 30% sea hibiscus leaf extract

Based on the results of observations on hair tonic preparations, it was shown that the three formulas had a specific odor of hibiscus leaves, blackish brown color, and it was known that the three formulas did not change during storage for 4 weeks. Therefore, it can be said that the four hair tonic formulas are physically stable at room temperature storage. This stability is influenced by the use of sodium metabisulfite as an antioxidant that is able to prevent oxidation of Sea Hibiscus leaf extract [19].

Table 3: pH value of hair tonic preparations

	Average pH (week)				
Formula	**0**	**1st**	**4th**	**8th**	**12th**
F0	7.0	7.0	7.0	7.1	7.0
F1	6.2	6.2	6.3	6.3	6.3
F2	6.1	6.1	6.2	6.3	6.4
F3	6.0	6.0	6.2	6.2	6.4

Description:

F0 : hair tonic without sea hibiscus leaf extract F1 : hair tonic containing 20% sea hibiscus leaf extract
F2 : hair tonic containing 25% sea hibiscus leaf extract F3 : hair tonic containing 30% sea hibiscus leaf extract

Measurement of the pH of the preparation using a digital pH meter. First, the pH meter was calibrated using a neutral pH and an acidic pH, then washed with distilled water, then dried using a tissue. The measurement of the pH value of the hair tonic preparation is done by dipping the pH meter in the preparation that has been dissolved using distilled water, then waiting for a while until the pH meter shows a constant number. pH examination is one of the test parameters to determine whether the preparation is in the skin pH range or not, which is between 3-7. Where the pH of the preparation used-n can affect absorption on the skin. If the pH of the preparation is too acidic, it can cause skin irritation. And if the pH is too alkaline it can cause scaly skin [20].

Stability test of hair tonic preparation of Sea Hibiscus leaf extract

The physical stability test of the preparation was carried out by storing all the preparations in tightly closed bottle at room temperature (25±2°C) for 12 weeks.

Table 4: Stability of hair tonic preparations during storage in room temperature

Formula	Observation (week)														
	0			1st			4th			8th			12th		
	X	Y	Z	X	Y	Z	X	Y	Z	X	Y	Z	X	Y	Z
F0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
F1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
F2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
F3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Description:

F0 : hair tonic without sea hibiscus leaf extract F1 : hair tonic containing 20% sea hibiscus leaf extract
F2 : hair tonic containing 25% sea hibiscus leaf extract F3 : hair tonic containing 30% sea hibiscus leaf extract
X : color Y : odor Z : consistency - : no change + : change

Based on the observations of hair tonic preparations for 4 weeks of storage at concentrations of 20%, 25%, 30% and the blanks showed a stable condition because there was no change in shape, smell, and color. The preparation remains stable and protected from fungal microbes, namely due to the use of preservative methyl paraben in the preparation.

Test the activity of hair tonic preparations of Sea Hibiscus leaf extract on hair growth

Hair growth activity test in guinea pigs was carried out to determine the effectiveness of hibiscus leaf hair tonic preparations in growing hair. The hair growth activity test is measured based on the results of the average hair length test. This test was conducted to determine the effect of each concentration of extract added to the preparation in accelerating hair growth in guinea pigs. Hair growth in guinea pigs is determined by picking randomly the hair in the test area on the guinea pig's back and measured using a caliper [21].

The results of the measurement of hair length obtained were then calculated the average hair length in each group can be seen in the table 5.

Table 5: The average hair length of guinea pigs on 7th, 14th and 21st day

Group	Average hair length (mm) (SD)		
	Day 7th	Day 14th	Day 21st
F0	5.4733 ± 0.1159	8.2700 ± 0.0200	12.0600 ± 0.2523
F1	6.4367 ± 0.1767*	8.6767 ± 0.1401	14.1167 ± 0.0901*
F2	6.4467 ± 0.1193*	9.1433 ± 0.4186	14.9433 ± 0.9433*
F3	7.4500 ± 0.0755*	11.3600 ± 0.0818*	17.2633 ± 0.2633*
Positive control	7.6433 ± 0.1193*	13.8300 ± 0.8660*	18.2000 ± 0.2751*

Description:

F0 : guinea pig with application of hair tonic without sea hibiscus leaf extract
F1 : guinea pig with application of hair tonic containing 20% sea hibiscus leaf extract
F2 : guinea pig with application of hair tonic containing 25% sea hibiscus leaf extract
F3 : guinea pig with application of hair tonic containing 30% sea hibiscus leaf extract
Positive control: guinea pig with application of minoxidil 2%
*significantly different with negative control (F0) $p < \alpha$ (0.05)

Observations were also made on hair weight on day 21. Hair in each test area of each treatment was shaved and then weighed. This hair weight parameter was used to see the effect of tonic preparations of hibiscus leaf extract on hair growth in guinea pigs. The results of the measurement of hair weight can be seen in table 6.

Table 6: The hair weight of guinea pigs after 21 days

Group	Hair weight (gram) ± SD
F0	0.1061 ± 0.0019
F1	0.1107 ± 0.0023
F2	0.1233 ± 0.0076
F3	0.1450 ± 0.0004
Positive control	0.1514 ± 0.0017

Description:

F0 : guinea pig with application of hair tonic without sea hibiscus leaf extract
F1 : guinea pig with application of hair tonic containing 20% sea hibiscus leaf extract
F2 : guinea pig with application of hair tonic containing 25% sea hibiscus leaf extract
F3 : guinea pig with application of hair tonic containing 30% sea hibiscus leaf extract
Positive control: guinea pig with application of minoxidil 2%

To see the difference in hair weight can be known by means of statistical calculations. Based on statistical calculations, it is known that the data is normally distributed and homogeneous. Followed by statistical test with ANOVA test. The test results showed that there was a significant difference ($p<0.05$) between the treatment groups. This shows that sea hibiscus leaf extract have activity against hair loss and promoting hair growth.

CONCLUSSION

Based on the research that has been done, sea hibiscus leaf extract (*Hibiscus tileaceus* L.) can be formulated in the form of hair tonic preparations that are stable during 12 weeks of storage in room temperature. Sea Hibiscus leaf extract (*Hibiscus tileaceus* L.) at concentrations of 20%, 25%, 30% can trigger hair growth in guinea pigs, but the most effective concentration in accelerating hair growth in guinea pigs is 30%.

CONFLICT OF INTEREST

All authors have nothing to declare.

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