

## Development of Hair Tonic with a Combination of Bitter Melon Leaves Extract (*Momordica charantia* L) with Stringbean Leaves Extract (*Vigna sinensis* L)

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### ABSTRACT

Beauty long hair is each women dream, many Indonesian natural plants can make healthy hair, one of them is Bitter melon leaves extract (*Momordica charantia* L) and Stringbean leaves extract (*Vigna sinensis* L). Bitter melon leaves extract contains flavonoids and long bean leaves extract contains saponin and flavonoids as hair growth in hair tonic and preparations for the stability testing of 4% Bitter melon leaves extract and long bean leaves extract with variant ethanol 96% knowing the activity hair tonic of hair growth in New Zealand's White rabbits. The results show that hair tonic activity F2 show long maximals New Zealand's White rabbits hair growth (225,67±3,40 mg is longer than positive control. Physical stability test was carried out for 3 months at 25±2 0C dan 40±2 0C. Dermal acute irritation test is known that the single extract of the irritant index pare leaves is very mild 0-0,4).

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## 1. INTRODUCTION

In humans, hair also has a function to improve appearance and increase self-confidence (Harahap M, 2000). Each strand of hair has a different cycle of growth and hair loss. The frequency of hair loss may increase resulting in baldness on the head. Hair loss is influenced by several factors such as age, heredity, hormones, immunity, malnutrition, psychological stress, physical trauma, certain skin problems and other unknown factors (Mitsui, 1996). The use of shampoos and conditioners is not enough to keep hair healthy. Hair as a living cell so it needs to be cared for and maintained and given hair nutrition so as not to cause high frequency loss (Wasitaatmadja, 1997). Hair tonic in the form of a mixture of cosmetics commonly used for hair care and contains substances needed by hair, hair roots and scalp (Latifah, 2007). The composition of the hair tonic mixture consists of solvents, vasolidator carriers as hair growth stimulants, conditioners and perfumes (Azis., et al, 1999). The resulting effect of hair tonic preparations is to stimulate hair growth, thickening or fertilizing.

In line with the development of technology, many people like natural herbal products because they are often used as hair treatments with a low risk of irritation, compared to minoxydil active ingredients sometimes give side effects such as sensitivity or soreness on the scalp (Dalimartha, 1999). Bitter melon leaf plant (*Momordica charantia* L) and Long bean leaves (*Vigna sinensis* L) are native Indonesian plants that are often used by the public as a hair tonic. Flavonoids contained in bitter melon leaves and bitter melon leaves have the ability as bactericides and long beans have the ability as antivirals so that they can maintain the growth of bacteria and viruses on the scalp so that the scalp is healthier and accelerates hair growth and prevents hair loss (Subahar, 2004).

## 2. METHODS

### Tools and Materials

Bitter melon leaves, long bean leaves taken from vegetable plantations in Curug Cikupa-Tangerang Village. Other ingredients are Propilenglikol (DOW), Phenoxyethanol (Salicylates &

*Development of Hair Tonic with a Combination of Bitter Melon Leaves Extract (*Momordica charantia* L) with Stringbean Leaves Extract (*Vigna sinensis* L). Ike Setiawan, et.al*

Chemicals), PEG-40 Dehydrogenated Castrol Oil (Clariant), Na<sub>2</sub>EDTA (Akzo Nobel), water, Sodium Metabisulfite (Chengxin), Minoxidil (Regrou 5%), Ethanol 96% (Food Grade), reagents for phytochemical screening: Mercury Chloride (HgCl<sub>2</sub>), Potassium Iodide (KI), Bismuth sub nitrate, Glacial acetic acid, toluene, magnesium metal powder.

Analytical balances (AND), pH meters (Ohaus), Brookfield LV viscometers, glassware (Pyrex), spatulas, refrigerators (Sharp), mesh sieves 30, ovens (Mettler), filter paper, dies and stampers, micro-calipers, razors, scissors, black tape, healthy New Zealand White Rabbits weighing around 2.5-3.5 kg are known grooves.

### **Stages of Research**

#### **Material Preparation**

Ingredients in the form of simplisia of long bean leaves and bitter melon leaves were obtained from vegetable plantations in Curug Cikupa-Tangerang Village

#### **Plant Determination**

Before processing, plants are determined first at the Biology Research Center-LIPI, Cibinong, Botanical Section (Herbarium Bogoriense).

#### **Simplisia Powder Manufacturing**

Bitter melon leaves and long bean leaves are obtained from vegetable plantations in Curug Cikupa-Tangerang Village, the leaves are collected and sorted from branches and dirt mixed and washed using running water until clean, then drained. The leaves that have been drained then continue the drying process with an oven at 400C, after drying re-sort after drying to ensure there are no more foreign objects or unwanted impurities if they are still present or left on dry simplisia. Next, dry simplisia is mashed using a blender until dry simplisia can pass the degree of fineness of the mesh 30, simplisia powder is stored in a dry and clean container.

#### **Pare Leaf Extract Processing as well as Long Bean Extract**

Extract processing is carried out by maceration, namely: by soaking each bitter melon leaf powder and long bean leaf powder with 70% ethanol solvent, then separate the solvent and filtrate using a Whatman 40 sieve until finally obtained filtrate which will be concentrated with a *rotary evaporator* to produce a thick extract.

#### **Raw Material Quality Test**

Viscous extracts obtained in quality tests include: organoleptic tests such as color, odor, taste, drying shrinkage tests, ash content determination tests, water content determination tests, acid insoluble ash content determination tests, phytochemical tests and total flavonoids in the extract, based on morphology, microscopic examination is a simplisia examination seen with the help of a microscope.

#### **The contents of water-soluble compounds**

Testing the content of water-soluble compounds with samples of 1 g extract (W1) in a 25 ml measuring flask then soak by maceration in chloroform solvent 25 ml leave for 24 hours, then repeated shaking, assisted by shaking for the first six hours, after that stay for 24 hours and strain. Testing, test 5 ml of filtrate formed and then evaporate in a steamer cup that has been tare (W0) with the procedure of evaporating until the solvent runs out and remains the residue, after which heat the residue at a temperature of 1050 C to a constant weight (W2).

% Water-soluble compound content:

$$= \frac{W2 - W0}{W1} \times 100\%$$

Information:

W0 = Empty cup weight

W1 = Initial extract weight

W2 = Cup weight + oven residue

#### **The content of soluble compounds in ethanol**

Testing of compounds in the form of ethanol soluble compounds was carried out with samples of 1 g of extract (W1) macerated in a 25 ml measuring flask corked with 96% ethanol solvent 12 ml for 24 hours, assisted by shaking for the first six hours, after which it was kept for 24 hours and

*Development of Hair Tonic with a Combination of Bitter Melon Leaves Extract (Momordica charantia L) with Stringbean Leaves Extract (Vigna sinensis L). Ike Setiawan, et.al*

filtered. Evaporate 5 mL of filtrate in a steamer cup that has been intermediate (W<sub>0</sub>) by aerating method until the solvent runs out and the residue is left, then dry the residue at 1050 C to constant weight (W<sub>2</sub>).

% The content of ethanol-soluble compounds:

$$= \frac{W_2 - W_0}{W_1} \times 100\%$$

Information:

W<sub>0</sub> = mass of empty cups

W<sub>1</sub> = initial extract mass

W<sub>2</sub> = massa cangkir + residu dioven

### **Simplisia Drying Shrinkage**

In a constant porcelain cup put 1 gram of simplisia powder and weigh it as weight W<sub>1</sub> then Dry the extract at 1050C for 30 minutes do three repetitions to obtain weight always, weighing is done after the cup and simplisia powder constanted first in excitator W<sub>2</sub>.

$$\% \text{ Shrinkage} = \frac{W_2 - W_1}{W_1} \times 100\%$$

Informati:

W<sub>1</sub> = bobot simplisia before setting

W<sub>2</sub> = simplisia weight after assignment

### **Setting of water rate**

Equipment *Moisture Balance* used in the determination of water content for both simplisia. The working procedure is tare weighing plates on the tool *moisture balance* first, set the drying temperature at 1050C, place on a weighing plate one gram of simplisia powder in a tare weighing plate, close the tool *moisture balance*. The reading process starts until the sign stops and then record the results. The percent of the figure that comes out is the value of moisture content contained in dry simplisia, Repeat the test three times.

### **Designation of ash content**

Determination of ash content was carried out on both simplisia powders by weighing carefully in a constant empty crucible container (W<sub>0</sub>) as much as 2 grams of extract (W<sub>1</sub>). Then put it in the kiln at a temperature of 5000C-6000C until the charcoal runs out and cooled, then weigh it to a constant weight (W<sub>2</sub>). If after testing there is still unwashed residue then rinse with hot water the same cup and strain, dry and incandescent the filtrate to its weight always, weigh the total ash content against the weight of the material that has been dried in the air, the total ash content is expressed in % w/w.

$$\% \text{ Total ash content} = \frac{W_2 - W_0}{W_1} \times 100\%$$

Information:

W<sub>0</sub> = Constant cup weight (grams)

W<sub>1</sub> = Initial extract weight (grams)

W<sub>2</sub> = Cup weight + extract after grinding (grams)

### **Ash content insoluble in acid**

Testing the insoluble ash content in acid by the method of dissolving ash comes from the results of determining the ash content (W<sub>1</sub>) boiling in 0.025 L of dilute sulfuric acid over Bunsen for five minutes, taking the remaining undissolved acid using a 0.45μ whatman paper sieve. The filtrate can be rinsed with hot water. Re-incandescent the phytrate (ash) and filter paper that has been rinsed with a furnace that has been set to a temperature of 600 ±25 degrees Celsius until there is no more charcoal residue (W<sub>3</sub>)

$$\% \text{ Acid insoluble ash content} = \frac{(W_3 - C) - W_0}{W_1} \times 100\%$$

Information:

W<sub>0</sub> = Empty cup weight (grams)

*Development of Hair Tonic with a Combination of Bitter Melon Leaves Extract (Momordica charantia L) with Stringbean Leaves Extract (Vigna sinensis L). Ike Setiawan, et.al*

- C = Filter paper weight (grams)  
 W1 = Initial extract weight (grams)  
 W3 = Cup weight + extract after grinding (grams)

### ***Skринing Phytochemical Extract Ethanol daun pare***

#### **Test flavonoids**

The flavonoid test was carried out on 0.5 g of test illustration then dissolve in water 10 milliliters in a long test tube and put in a water bath then add 100 mg of magnesium powder into the test tube and add 1 milliliter of concentrated hydrochloric acid, 3 milliliters of amyl alcohol, shake vigorously until a separation of red layer, yellow color layer, orange layer in amyl alcohol solvent indicates positive presence of flavonoids in the extract.

#### **Terpenoid/steroid test**

Terpenoid test was tried by macerating 1 gram of simplisia powder illustration in 20 ml of ether for 120 minutes, take the filtrate using a 0.45 $\mu$  whatman sieve for further testing with the following groove: Take 3 drops of illustrative filtrate add 2 drops of Lieberman Bouchard reagent. Test results will be positive if a bright violet or red color is seen that changes to violet blue or blue green indicating the presence of terpenoidal compounds/steroids.

#### **Uji tanin**

Positive tannin testing was carried out by dissolving 0.5 grams of extract illustration, mix with 20 ml of 70% ethanol. As a solvent. Take a soaking solution of 1 ml and then add 1-2 drops of 1% FeCl<sub>3</sub> solution. The color of the sample becomes blue-black or blackish-green, indicating that the simplisia contains tannins.

#### **Water saponin**

Saponin testing begins by dissolving 0.5 grams of illustration per 10 ml of hot water then inserting it in a test glass tube and cooling, and shaking vigorously for 10 seconds. If simplisia contains saponins, the whisk will release a stable foam with a foam height of about 1-10 cm under 10 minutes and the foam lasts if dripped with 1 drop of 2 N hydrochloric acid.

#### **Test Animal Treatment**

Rabbits are acclimatized for 5 days with the aim of getting used to living in the environment and the treatment that

new. The cage used must meet the requirements of temperature, humidity, light, nutrition, sound and hygiene. The rabbit's back is cleaned of hairs and allowed to stand for 24 hours before the treatment is carried out.

After going through safety tests, if there are no harmful effects for rabbits, rabbits will be kept.

#### ***The procedure for manufacturing tonic hair preparations:***

Weigh all ingredients, Put the ingredients Disodium edta and Sodium metabisulfite add the weighed Aquades stir until dissolved (mixture 1), mix the extract, PEG-40 dehydrogenated castor oil, phenoxyethanol and ethanol 96% then stir until dissolved and homogeneous put into mixture 1 stir until homogeneous (mixture 2). Add propilenglikol to the mixture 2 stir until homogeneous. Evaluate the preparation of hair tonic.

**Table 1.** Hair Tonic Preparation Formula

No.	Material	F1 (%)	F2 (%)	F3 (%)	K(+) (%)	K(-) (%)
1 2 3	Etanol 96% Ekstrak daun pare Ekstrak	20 4 15	25 4 15	30 4 15	0 0 0 0	25 0 0
4 5 6	daun kacang panjang PEG-40	1 0,02	1 0,02	1 0,02	0 0 0 0	1 0,02
7 8 9	dehydrogenated castor oil Sodium	0,8 0,1	0,8 0,1	0,8 0,1	100	0,8 0,1
10	metabisulfite phenoxyetanol Disodium EDTA Propilenglikol Aquadest ad Minoxidil (Regrou 5%)	5 100 -	5 100 -	5 100 -	0,02	5 100 -

#### ***Evaluation of Hair Tonic Preparations***

The preparations formed are evaluated preparations include: organoleptic examination, specific weight, pH, besides that hair tonic preparations are also carried out activity tests and irritation tests.

### 3. RESULTS AND DISCUSSION

The bitter melon leaf extract and long bean leaves produced have met the criteria for good extract quality requirements according to MMI. Data from the quality analysis of bitter melon leaf extract and leaf extract string beans can be seen in Table 2. Referring to the results of the test for determining the total ash content of bitter melon leaf extract and long bean leaf extract describe the large amount of minerals contained both internally from the extract and externally derived from impurities, The test results of water-insoluble ash content show a very small number which illustrates that the extract is clean from mineral contamination in the form of sand, silicate or soil. Checking the moisture content of bitter melon leaf extract and long bean leaf extract which is less than 10%, meaning that the extract can be maintained in quality during storage. So that both extracts are ready for research as active ingredients in hair tonic preparations.



**Figure 1.** Dried Simplisia Bitter Gourd Leaves



**Figure 2.** Bitter melon leaf simplisia powder



**Figure 3.** Thick bitter melon leaf extract



**Figure 4.** Dried Simplisia Long Bean Leaves



**Figure 5.** Long bean leaf simplisia powder



**Figure 6.** Thick Long Bean Leaf Extract

**Table 2.** Simplisia and extract parameter check

No.	Testing	Bitter melon leaves (%)		String Bean Leaf (%)	
		average $\pm$ SD		average $\pm$ SD	
		Simplisia	Extract	Simplisia	Extract
1 2	Kandungan air Kandungan abu	8.43 $\pm$ 0.06	7.70 $\pm$ 0.01	9.71 $\pm$ 0.02	8.56 $\pm$ 0.03
3 4	tidak larut air Kandungan abu	TTD 15.19 $\pm$	0.04 $\pm$ 0.01	0.09 $\pm$ 0.01	TTD
	total Kandungan sari alkohol	0.05 17.32 $\pm$	29.31 $\pm$ 0.01	9.57 $\pm$ 0.01	1.38 $\pm$ 0.03
		0.02	1.88 $\pm$ 0.01	15.22 $\pm$ 0.01	27.81 $\pm$ 0.02

Ket : TTD = not detected

**Table 3.** Simplisia and extract parameter check

No.	Golongan senyawa	Daun Pare	String bean leaves
1	Alkaloid	+	-
2	Flavonoid	+	+
3	Phenolic	-	-
4	Saponin	+	+
5	Tannin	+	+
6	Triterpenoid	-	-

Information:

+ = Contains secondary metabolites

- = Does not contain secondary metabolites

*Development of Hair Tonic with a Combination of Bitter Melon Leaves Extract (Momordica charantia L) with Stringbean Leaves Extract (Vigna sinensis L). Ike Setiawan, et.al*

The results of the phytochemical screening test of bitter melon leaves (*Momordica charantia* L) contained secondary metabolite compounds such as alkaloids, flavonoids, saponins, tannins and long bean leaves (*Vigna sinensis* L) containing three secondary metabolite compounds in the form of flavonoids, saponins, tannins.

Flavonoids contained in bitter melon leaves trigger hair growth activity and control the development of bacteria and viruses that can reduce hair loss.<sup>9</sup> Secondary metabolites from long bean leaves in the form of saponins that have foaming or soap characteristics have the effect of stimulating cell division during the anagen phase, while secondary metabolites in the form of flavonoids also function as bactericides, anti-virals in all phases of hair growth and secondary metabolites in the form of polyphenols are antiseptic and keratolytic which prevent the growth of microorganisms and prevent scalp stiffness that inhibits circulation blood in the anagen phase takes place (Siska et al., 2011).

**Table 4.** Characteristics of hair tonic preparations

No.	Observation	F1	F2	F3
1 2 3 4 5	Color Odor	Green-brownish	Green-brownish	Green-brownish
6	Homogeneity	Typical	Typical compliant	Typical
	Clarity pH	compliant 5.28	5.32 0.9953	corresponding
	Specific gravity	0.9970		corresponding 5.37 0.9883

**The results of the Hair Growth Test show the ability of Hair Tonic Hair Preparations, Bitter Melon, Leaf Extract, and Long Bean Leaf Extract.**

There are two test variables observed are the average length of rabbit hair and its weight which is recorded and monitored every week.

**Table 5.** Hair Growth Test

Monitoring	Average rabbit hair length (mm) ± SD			
	Week 1	Week 2	Week 3	Week 4
FORMULA 1	0.83 ±0.07	2.21± 0.06	5.82±0.06	10.15±0.11
FORMULA 2	0.90 ±0.05	3.38± 0.06	6.39±0.20	11.45±0.13
FORMULA 3	1.20 ±0.09	2.66±0.25	5.56±0.20	10.41±0.19
BASE	0.48±0.05	1.58±0.07	3.78±0.14	6.28±0.24
POSITIVE CONTROL	1.75±0.16	3.46±0.26	6.88±0.26	11.74±0.34
CONTROL NORMAL	0.33±0.04	0.59±0.02	3.48±0.17	4.47±0.18

In the initial week measurements, the average development of rabbit hair compared to reasonable control was 0.33 millimeters. Measurements were taken from the ability to grow rabbit hair in each treatment compared to reasonable controls and the comparison of hair development in each formula with ethanol dose variations can be determined by statistical calculations.

Rabbit hair growth can be measured during the first week by plucking rabbit hair, the average rabbit hair length against Normal Control is 0.33 ± 0.04 mm, Formula 1 (20% ethanol content) 0.83 ± 0.07 mm, Formula 2 (25% ethanol content) 0.91 ± 0.05 mm, Formula 3 (30% ethanol content) 1.20 ± 0.09 mm. Based on existing data, it was seen that different hair growth in the Formula 1 treatment group gave the lowest rabbit hair length of 0.83 mm, Formula 2 gave the rabbit hair length longer than Formula 1 which was 0.91 mm, formula 3 gave the longest rabbit hair length of 1.20 mm compared to normal controls. The data above is then statistically displayed to see whether the value is meaningful or not. The Kruskal Walls test provides significant differences between the first Formula, the second Formula, the third Formula (p<0.05) The above results show that all formulas have the ability to grow hair length different, on the contrary, the Mann Whitney statistical test compared to the fair group with each formula has a significant difference (p < 0.05). So it can be concluded that the three formulas have the ability to grow hair that is different from the normal group. Statistical analysis leads to unnaturally distributed and inhomogeneous data.

Measurements in the second week of the average hair length data in the reasonable control were 0.59 ± 0.02mm, Formula 1 (20% ethanol content) 2.21± 0.06mm, Formula 2 (25% ethanol content) 3.38± 0.06 mm, Formula 3 (30% ethanol content) 2.66 ± 0.25 mm. So from this second week data

*Development of Hair Tonic with a Combination of Bitter Melon Leaves Extract (*Momordica charantia* L) with Stringbean Leaves Extract (*Vigna sinensis* L). Ike Setiawan, et.al*

there is an increase in hair length growth in Formula 1 at the lowest which is 2.21 mm, formula 2 gives the longest rabbit hair length of formula 1 and formula 3 which is 3.38 mm, formula 3 gives hair length longer than formula 1 but shorter than formula 2 compared to reasonable controls. Test *Kruskal veggir* Providing significant differences between the three formulas showed different hair length growth from each formula, while in statistical tests *Mann Whitney* We compare the group of each formula with the normal group where the result ( $p < 0.05$ ) means that the first formula, the second formula, the third formula show different hair growth ability from the normal group. The Mann Whitney test on formula 2 with positive control gave a meaningless difference result. Evaluation through statistics can be concluded that data is not reasonably distributed and not homogeneous.

In the third week the average hair length monitoring compared to Reasonable Control was  $3.48 \pm 0.17$  mm, in Formula 1 (20% ethanol content)  $5.82 \pm 0.06$  mm, in Formula 2 (25% ethanol content)  $6.31 \pm 0.20$  mm, in Formula 3 (30% ethanol content)  $5.56 \pm 0.20$  mm. It was seen that the average increase in hair growth in Formula 3 was the lowest at 5.56 mm, formula 2 gave the longest rabbit hair length from formula 1 and formula 3 at 6.31 mm, formula 1 gave a longer hair length than formula 3 but shorter than formula 2 at 5.56 mm compared to normal controls. The *Kruskal Walls test* provides significant differences between the first Formula, the second Formula, the third Formula with an index of ( $p < 0.05$ ) which concludes that hair length growth activity is different in each formula, while the Mann Whitney statistical test is performed on the normal group and the group of each formula, which results in ( $p < 0.05$ ) meaning that there is a significant difference in the normal control of the three formulas. So that the first formula, the second formula, the third formula provide the ability to grow hair that is different from the normal group. His statistical calculations concluded that the data was abnormally distributed and inhomogeneous.

In the fourth week the data rerata the length of rabbit hair on the natural control is  $4.47 \pm 0.18$  mm, Formula 1 (ethanol rate 20%)  $10.15 \pm 0.11$  mm, Formula 2 (ethanol rate 25%)  $11.45 \pm 0.13$  mm, Formula 3 (ethanol rate 30%)  $10.41 \pm 0.93$  mm. The data above shows Hair growth in Formula 1 is lowest at 10.15 mm, Formula 2 gives the longest rabbit hair length of Formula 1 and Formula 3 at 11.45 mm, Formula 3 gives hair length longer than Formula 1 but shorter than Formula 2 at 10.41 mm compared to normal controls. The *Kruskal Walls test* provides significant differences between the first Formula, the second Formula, the third Formula ( $p < 0.05$ ) which means that all formulas have different hair length growth abilities in their respective formulas, while statistical tests with Mann Whitney were tested in a reasonable group compared to the three formulas having a meaningful comparison ( $p < 0.05$ ) in this case showing that the three formulas have different hair growth activities with normal groups. The calculation of statistical results concludes that the data is abnormally distributed and inhomogeneous.

In addition to the increase in hair length, observations were made on hair weight at week four. Before the test, shave rabbit hair in the test area of each formula, then the weight is weighed as the initial weight before the test. This hair weight test aims to see the effect of each formula giving a difference in hair density in rabbits or not. The results of monitoring the weight measurement of rabbit hair are shown in table 6.

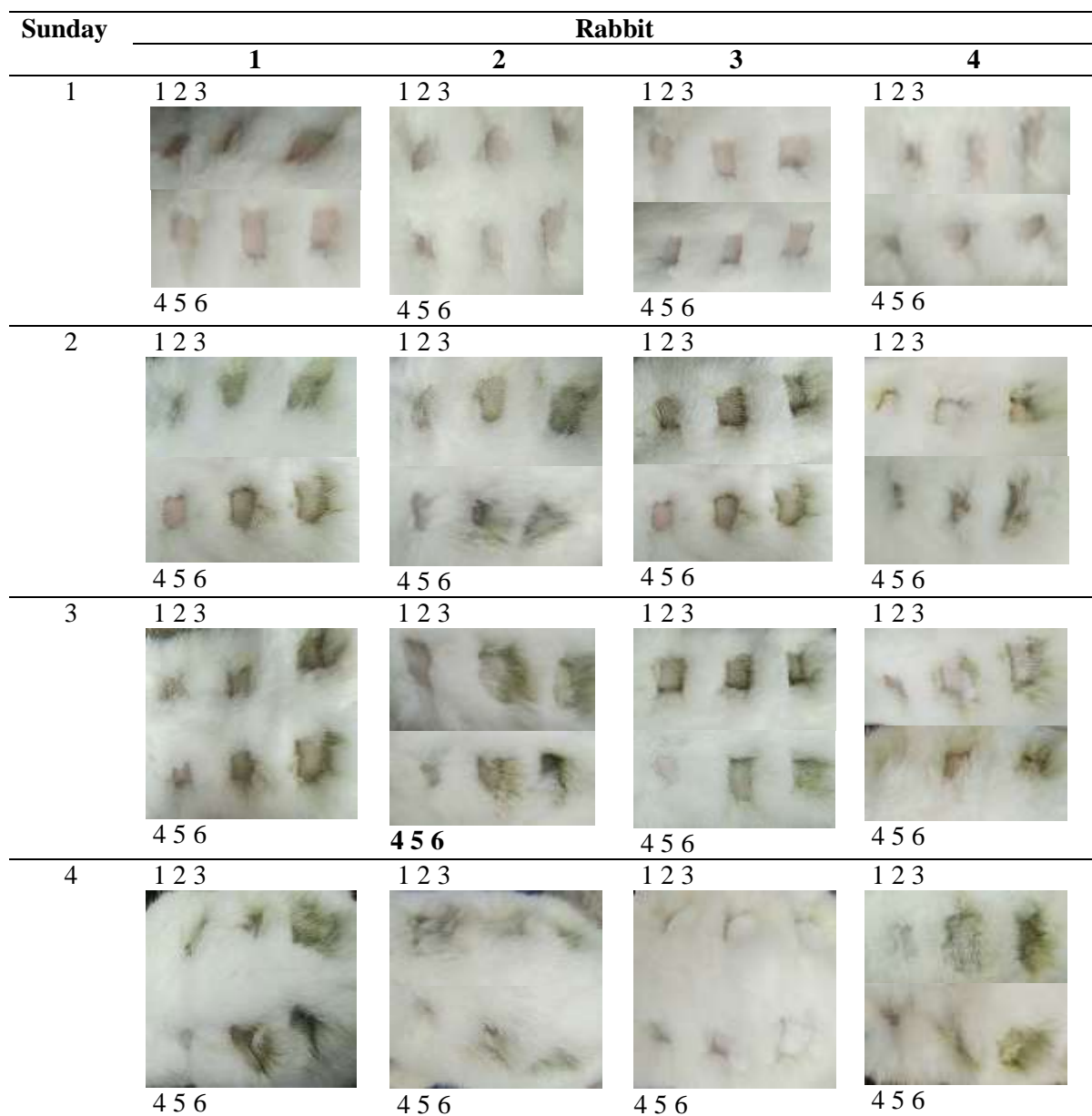
**Table 6.** Results of Monitoring Average Rabbit Hair Weight on Day 28

Formula	Rabbit Hair Weight (mg) Average $\pm$ SD
FORMULA 1	$176.67 \pm 10.72$
FORMULA 2	$225.67 \pm 3.40$
FORMULA 3	$183.00 \pm 3.10$
BASIS	$84.33 \pm 4.39$
POSITIVE CONTROL	$250.00 \pm 3.59$
CONTROL NORMAL	$35.33 \pm 7.36$

The average measurement of rabbit hair weight against Normal Control was  $35.33 \pm 7.36$  mg, Formula 1 (20% ethanol content)  $176.67 \pm 10.72$  mm, Formula 2 (25% ethanol content)  $225.67 \pm 3.40$  mg, Formula 3 (30% ethanol content)  $183.00 \pm 3.10$  mg. From the measurement data above, it can be concluded that Formula 2 provides the heaviest hair weight compared to Formula 1 and Formula 3,

*Development of Hair Tonic with a Combination of Bitter Melon Leaves Extract (Momordica charantia L) with Stringbean Leaves Extract (Vigna sinensis L). Ike Setiawan, et.al*

which is 225.67 mg, so Formula 2 is the best formula for rabbit hair weight gain. To recognize whether the information contained is meaningful or not, then testing continues with the Anova statistical test. *Anova statistical test* averaged the hair weight of each treatment was clearly different ( $p < 0.05$ ). Statistical calculations continue with *the Post Hoc Test* to ensure the test type is based on the results of the Test of Homogeneity of Variances *because the homogeneity test results obtained* ( $p > 0.05$ ) means that the data are homogeneous, the Post Hoc Test uses the Bonferroni test to compare all treatment groups in the study, the results of the *Bonferroni* test Conclude that there is a clear difference ( $P < 0.05$ ) against the three formulas with base groups, positive and normal, but if we compare formula 1 with formula 3 there is no clear difference ( $P > 0.05$ ). The results of the ANOVA statistical test showed that the formula 2 treatment group and the positive control were the treatment groups that provided the best hair density and hair length compared to other treatment groups.



**Figure 7.** Photo of rabbit hair growth First week to fourth week on the activity test of a tonic mixture of hair fertilizer bitter melon leaf extract and string bean leaves.

### Acute Dermal Irritation Test Results

Irritation test on rabbit back skin using Formula 2, single extract, base, normal control and positive control. Based on observations and calculations of the irritation index in the Single extract shows a number (0-0.4) meaning that irritation occurs very mildly. The irritation index result in the combination extract showed the same range of 0-0.4, so single and combined extracts did not cause irritation. Bitter melon leaf extract combination with 25% ethanol solvent concentration topically did not show erythema. The results of observation and calculation of the irritation index in hair nourishing tonic preparations and bases after the preparation stability test, the irritation index value shows (range 0-0.4) meaning that irritation occurs very mildly.

### 4. CONCLUSION

The results of the determination test of bitter melon leaves (*Momordica charantia* L) tribe *Cucurbitaceae* contain secondary metabolite compounds, namely alkaloids, saponins, tannins, flavonoids while long bean leaves contain flavonoid compounds, saponins, tannins. (*Vigna sinensis* L.) tribe *Leguminosae*. The results of the development of a combination formulation of two extracts, namely 4% bitter melon leaf extract (*Momordica charantia*) and 15% long bean leaf extract (*Vigna sinensis* L) with a variation in 96% ethanol concentration of 25% provide activity as the highest hair growth and hair density with the results of hair length:  $11.45\text{mm} \pm 0.13$  and hair weight:  $225.67\text{mg} \pm 3.40$ . the results of the irritation test showed very mild (range 0-0, 4).

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