

COMPARISON STUDY OF HAIR TONIC AND GEL FORMULATION OF *ANGIOPTERIS EVECTA* AS A HAIR GROWTH STIMULANT

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ABSTRACT

Objective: Previous research has proven that the water fraction of the *Angiopteris evecta* root has hair growth activities. The objective of this research was to determine the formula of gel and hair tonic preparations of pakis munding (*Angiopteris evecta* L.) which met the requirements as a pharmaceutical preparation, good and effective in stimulating hair growth.

Methods: Formulation of hair tonic and gel preparations were made using 7.5, 10.0, and 12.5 % of *A. Evecta* root water extracts as active compounds. The formulation of the two preparations was based on the standard formulation method. The evaluation and formulation test were carried out by organoleptic examination and homogeneity, pH, viscosity, scattering power test, stability test, and safety test based on the standard preparations including Indonesian pharmacopeia. Statistical tests were carried out on both formulas against rabbits based on the modification of the Tanaka method. Comparison of statistics on both formulas was also carried out.

Results: It was found both formulas fulfill standard requirements as pharmaceutical preparations. Statistically, the best activity in hair tonic preparations was at a concentration of 12.5% and for gels at a concentration of 10.0% ethanol extract seen from measurements of hair length. Statistically, using the Independent T-test to find out the significant differences in the average hair length on hair tonic and gel, it was found there was no significant difference between the two formulas. It was found that the best formula for hair tonic and gel was in the addition of 10.0% and 12.5% extracts, respectively.

Conclusion: The results obtained in this research work clearly indicated that both hair tonic and gel formulas of *A. evecta* root water extract may be used as stimulating hair growth.

Keywords: Hair growth stimulant, *Angiopteris evecta*, Hair tonic, Gel, Pharmaceutical preparations

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INTRODUCTION

To overcome baldness, various methods are carried out, one of which is the innovation of synthetic hair growth formula and herbal products. The use of these types of material has been widely produced, so in the market today there are many hair growth drugs. The use of synthetic materials in cosmetics has been greatly reduced because there are dangerous side effects. For example, minoxidil which is used topically on the scalp usually causes itching on the scalp, irritation, and allergies [1]. The examples of herbal plants that have hair growth activities are algae extract, ginkgo biloba, the green, saw palmetto, stinging nettle [2] as well as *Angiopteris evecta* [3] and *Sauropus androgynous* [4].

Based on the research conducted Mustarichie *et al.* [5] pakis munding (Sundanese) roots have activities to grow hair. Judging from the ethanol extract with a concentration of 10%; 20%; 40% and 20% water extract; 40% have better results in growing rabbit hair compared to positive controls (Minoxidil). Tresnawati [6] mentioned that pakis munding roots contain flavonoids, saponins, polyphenols, tannins, quinones, monoterpenes and sesquiterpenes and water fraction with a concentration of 7.5%, 10%, and 12.5% having the good hair growth activity.

In practice, people tend to use cosmetic products that are easy to use, such as gel and hair tonic. Hair tonic has been used by Native Americans to overcome baldness using several species of plants [7]. The use of hair tonic has advantages as easy and suitable for use on the scalp, both in trans-appendageal absorption, easily spread, not greasy, and does not leave residues on the scalp that can cause irritation [8]. A gel is a preparation that is easier to formulate than other semisolid preparations and has good dispersion [9]. The gel is also easy to wash and not sticky. Besides this gel preparation is not easy to cause irritation and allergies [10].

This research reports on the hair tonic formula and gel of the *A. evecta* water fraction and their activity as a stimulator of hair growth

as well as statistically the most effective preparation between gel and hair tonic in stimulating hair growth.

MATERIALS AND METHODS

Materials

The materials used in this study were pakis munding roots obtained from Mount Galunggung Tasikmalaya and determined at the Laboratory of Taxonomy, Biology Department, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran.

Methods

The research methods included material collection, determination, material processing, ethanol extract quality test, making water fraction from ethanol extract, phytochemical screening, gel preposition and hair tonic from water fraction, fraction gel and hair tonic formulations from fractions water, evaluation of gel and hair tonic preparations, testing of the most active hair growth stimulant activity from gel and hair tonic preparations, gel irritation tests and hair tonic preparations, and analysis using statistical methods

Plant material processing

After the pakis munding root part was separated, then washed by brushing thoroughly. Then drain so that the remaining washing water was lost. After drying, the root was then cut, until a small size was obtained and dried by air at room temperature referred to How to make a simplicial method [11].

Extractions

The simplicial was extracted using the maceration method using 70% ethanol as its solvent based on modification method of Kusuma *et al.* [12]. The extract was concentrated using a rotary evaporator at a temperature of 40-50 °C and continued by using a water bath at a temperature of 40-50 °C to obtain a dried extract with a constant

weight. The parameter extract was analyzed its organoleptic including shape, smell, color, and taste and water content referring to Indonesian Pharmacopeia [13].

Phytochemical screening

Phytochemical screening was carried out to determine the content of secondary metabolites guidance by Farnsworth method [14]. This test included: Flavonoid Test, the 0.5 g fraction was added to a cup and then added with 2 ml of 70% ethanol then stirred. Then added a number of 0.5 g magnesium powder and also dripped 3 drops of concentrated HCl. The appearance of red color to purplish red is a sign of flavone; Alkaloid Test, a number of fractions of 0.5 g were carried out by 2 N HCl. Then the acid layer was separated into three parts. The first part was used for blank, the second part was added dragendorff reagent and observed whether there was a deposit that has a brown-orange color. The third part was added to the major reagent and the presence of white deposits became a positive sign of the alkaloid compound; Saponin Test, a number of fractions of 0.5 g were dissolved using distilled water and then left for fifteen minutes and then shaken for ten seconds. If there was a stable froth for about 10 min then the froth remains when adding 2N HCl, then a positive sample contains saponin; Tanin Test, a number of fractions of 0.5 g were dissolved in 50 ml hot water and then boiled in 15 min, then cooled and filtered. The filtrate was given 1% gelatin. Signs of tannins when white deposits are formed; Polyphenol Test, a number

of 0.5 g fractions were first dissolved in some water in the test tube. The test tube was then heated, then the solution was filtered while still hot. The filtrate that had been obtained then drops a number of solutions of iron (III) chloride. A sign of the presence of polyphenol compounds when black blue color was formed; Test Monoterpenes and Sesquiterpenes, some 0.5 g fractions were extracted with ether, then evaporation was carried out using a vaporizer dish until the fraction dried. The resulting residue was then dripped with vanillin sulfate reagent. Signs of the presence of monoterpenoid and sesquiterpenoid compounds in the sample when colors were formed; Steroid and Triterpenic Tests, a number of fractions of 0.5 g were searched using ether, then evaporation was carried out using a vaporizer plate until the fraction dried. The residue was then given a few drops of Liebermann Burchard reagent. The presence of purple indicated that the sample contains compounds from the triterpenoid group, whereas if there was a blue-green color it was a sign of compounds from the steroid group; Quinone Test, a number of 50 mg fractions are dissolved in 50 ml of hot water, then simmer for about 15 min, then cooled and filtered. The filtrate was given a number of NaOH, a sign of quinone compounds when yellow to red was formed.

Hair tonic and gel formulation

Two types of formulas were made using the different variation composition of the water extract of pakis munding, as shown in table 1 and 2. Formulation referred to cosmetic textbook [10, 15, 16]

Table 1: Pakis munding water fraction gel formula

Materials	Quantity (% w/w)			
	I	II	III	IV
Pakis munding water fraction	7.5	10.0	12.5	-
Carbopol 940	1.0	1.0	1.0	1.0
Methylparaben	0.2	0.2	0.2	0.2
Propylparaben	0.02	0.02	0.02	0.02
Glycerin	10.0	10.0	10.0	10.0
Na2EDTA	0.03	0.03	0.03	0.03
Triethanolamine	qs	qs	qs	qs
Aquadest ad100	qs	qs	qs	qs

Notes: qs = quantum satis (Latin, a pharmaceutical term for sufficiently)

Table 2: Pakis munding water fraction Hair tonic formula

Materials	Quantity (% w/w)			
	I	II	III	IV
Pakis munding water fraction	7.5	10.0	12.5	-
Ethanol 96 %	40.0	40.0	40.0	40.0
Propyleneglycol	2.0	2.0	2.0	2.0
Methylparaben	0.075	0.075	0.075	0.075
propylparaben	0.02	0.02	0.02	0.02
Sodium metabisulphite	0.2	0.2	0.2	0.2
Aquadest ad 100	qs	qs	qs	qs

Notes: qs = quantum satis (Latin, a pharmaceutical term for sufficiently)

Evaluation of gel and hair tonic formula

Gel Evaluations included Organoleptic examination and homogeneity, pH, viscosity, spreadability, and stability. For the Hair tonic formula, evaluations included Organoleptic examination and homogeneity, pH, viscosity, and stability.

Test hair growth activity of gel and hair tonic

Testing of hair growth stimulant activity for gel and hair tonic preparations for pakis munding roots was carried out on 8 male rabbits aged 4-5 mo, healthy without defects and having a body weight of 2-2.5 Kg. Ethical approval no 1401/UN6. KEP/PEC/2018 was issued by the Research Ethics Committee-Universitas Padjadjaran for this work. This test followed the modification of Tanaka *et al.* methods [17].

Dermal acute irritation test gel and hair tonic preparations

The dermal acute irritation test was a test on animals (albino rabbits) with the aim of detecting toxic effects that appear after

exposure to the preparation for 3 min to 4 h. The principle of this method was that the preparations be exposed to a single dose test preparation on animal skin with areas of skin that were not treated as controls. The degree of irritation was assessed at 1, 24, 48, and 72-time intervals after exposure to the test preparation [18].

Statistical analysis

The results of measurements of rabbit hair length obtained were then averaged. Normality test using the Shapiro Wilk method and the Levene method's homogeneity and then the data is processed using the statistical method Kruskal Wallis [19, 20].

RESULTS AND DISCUSSION

Plant determination

Plant determination was carried out at the Laboratory of Plant Taxonomy, Department of Biology, Faculty of Mathematics and Natural Sciences. The results of plant determination, Plant Identification sheet

no. 499/HB/02/0-17 showed that the Family was Marattiaceae, genus Angiopteris, and species *Angiopteris evecta* (G. Forst.) Hoffm.

Extraction results

The extracted results obtained a dry extract with a yield of 12.6% w/w and the results of observations of organoleptic were in the form of dry powder, blackish brown color, and distinctive smelling simplicia.

Phytochemical screening

Phytochemical screening results can be seen in table 3.

Examination of moisture content

The measurement of the water content has the purpose of providing a range of the amount of water contained in the material and knowing the quality of the extract. The results of the water content, which was 0.2 ml from 2 grams of extract, meant that the value of the moisture content was 10% and still met the requirements of water content which was below 10% [13]. It meant that the extracts of pakis munding obtained met the specified standards so that the storage period will be relatively stable. If the extract had a low water content, it would minimize the risk of developing microorganisms in the extract and avoid the degradation of the active compound metabolism of microorganisms.

Table 3: Secondary metabolites of *A. evecta*

No	Secondary metabolites	Extract
1	Alkaloids	-
2	Flavonoids	+
3	Quinone	+
4	Monoterpenoids and Sesquiterpenoids	+
5	Polyphenol	+
6	Saponins	+
7	Steroids and Triterpenoid	-
8	Tannin	+

Notes: += detected; -= not detected, this result similar to our previous report [3] in which we reported the secondary metabolites of Pakis munding simplicia and its ethanol extract.

Fractionation of ethanol extract

The fractionation process was a process carried out with the aim of separating compound components based on certain properties, namely based on their polarity. The fractionation method used is by the liquid-liquid extraction method (LLE) [21]. The principle of separation of compounds with liquid-liquid extraction was like dissolve like where compounds that had the same polarity would be attracted or carried away with compounds that had the same level of polarity. The solvent used was a solvent that has a different level of polarity so it would not be mixed, namely water, n-hexane, and ethyl acetate. Non-polar compounds would be attracted to n-hexane, semipolar compounds would be attracted to ethyl acetate, and polar compounds would be attracted to water. In this study 315 grams of a thick extract of pakis munding roots were fractionated with a ratio of extract: solvent (1:10) to obtain a water fraction.

Gel and hair tonic formulation

The hair tonic formula can be seen in table 2 and 3. For Gel preparations, first Carbopol 940, Methylparaben, Propylparaben, and EDTA were dispersed on some distilled water 40 °C with constant stirring for 30 min then this dispersion was stored first for 24 h to fully expand [22]. Furthermore, the water fraction of pakis munding was dissolved in some distilled water and then added with glycerin. The two mixtures above were then mixed in beaker glass and TEA was added to stabilize the pH [23]. Carbopol® 940 polymer was a white powder, crosslinked polyacrylic acid polymer. It was an extremely efficient rheology modifier capable of providing high viscosity [24]. Methylparaben [25] and propylparaben [26] were used as a preservative. Glycerin [27] was used as a moisturizer to treat or prevent dry, rough, scaly, itchy skin and minor skin irritations. The use

of the chelating agent ethylenediaminetetraacetic acid (EDTA) was widespread in the pharmaceutical industry. It was used as a treatment of heavy metal poisoning as well as an antioxidant in many pharmaceutical preparations [28]. Triethanolamine in Gel was used as a buffering agent, masking and fragrance ingredient, and surfactant, in addition to its primary use as a pH adjuster [29].

For Hair tonic preparations, nipagin and nipasol were first dissolved in 50% ethanol, and sodium metabisulphite was dissolved in distilled water. Then the two phases were mixed using a stirrer. Then the fraction of water that had been dissolved with distilled water was entered into the initial mixture slowly while still stirring with the stirrer. Finally, propylene glycol was added and stirred with a stirrer until homogeneous [30].

Evaluation of hair tonic and gel

The results of observations of organoleptic, pH, stability, viscosity and dispersion were shown in table 4. Organoleptic Observation was made on formulas in the form of odor, color, and physical homogeneity [22, 31]. pH testing using universal pH paper or pH meters. 1 gram of gel preparation into 100 ml of distilled water, each formula was done triple and the average was searched. The standard pH of the scalp was around 4.5-6.5 [32]. Stability test using the freeze-thaw method, where the sample underwent a freeze and thaw cycle for 12 d with 6 cycles. Each cycle consisted of storage at 4 °C and 45 °C, 24 h each [22]. The viscosity test of the preparation was determined using a Brookfield viscometer; the test for Gel was carried out using number 4 spindle and 2 rpm speed whereas, for Hair tonic, the test was carried out using number 3 spindle and 200 rpm speed. Testing of gel dispersion was determined by measuring the spread diameter of 1 gram of gel between 2 horizontal plates measuring 20 cm x 20 cm after 1 minute [23].

Table 4: Evaluation of gel and hair tonic formulas

Formula	Organoleptic	Evaluation pH	Tests		
			Viscosity	Homogeneity	Dispersion
Gel: FI	brown, fragrant	6.05±0.02081666	236.495±5.398225 mPas	Homogenous	3.62±0.076376262 cm
FII	brown, fragrant	5.91±0.088881944	257.320±6.633822 mPas	Homogenous	3.98±0.256580072 cm
FIII	brown, fragrant	5.51±0.047258156	144.627± 4.733987 mPas	Homogenous	4.45±0.130766968 cm
Hair tonic: FI	brown, distinctive smell, thick	4.5±0.040414519	140.000±20.000 mPas	Homogenous	-
FII	brown, distinctive smell, thick	4.5±0.075055535	140.000±10.000 mPas	Homogenous	-
FIII	brown, distinctive smell, thick	4.5±0.02081666	125.000±5.773502 mPas	Homogenous	-

Note: Gel FI, FII, FIII consisted of respectively 7.5%, 10.0%, 12.5% water fractions, Hair tonic FI, FII, FIII consisted of respectively 7.5%, 10%, 12.5% water fractions

Hair growth stimulant activity test of gel and hair tonic formula

Testing of hair growth stimulant activity for gel and hair tonic preparations for pakis munding roots was carried out on 8 male rabbits aged 4-5 mo, healthy without defects and having a body weight of 2-2.5 Kg. The study was carried out based on a modification of the Tanaka *et. al* method using 4-5-month-old male rabbits, healthy without defects and having a body weight of 2-2.5 kg. The number of rabbits used was obtained from calculations using the Federer formula [33], namely:

$$(t-1)(n-1) \geq 15$$

Where: (t) number of groups; (n) number of subjects per group.

Before being treated, the test animal was acclimatized for 7 d so that the rabbit could get used to living in the new environment and treatment to be carried out. Next, one day before testing, the rabbit was shaved on the back to clean and also smeared with 70% alcohol as an antiseptic. Then the rabbit was rested for 24 h [4, 17]. Furthermore, on the back of the tested shaved rabbits, 7 boxes of 2x2 cm in size were made as to the resting place. Each box is given the following test materials: Box 1: normal control (without treatment), Box 2: positive control (minoxidil 2%), Box 3: base control (hair tonic or gel bases),

Box 4: hair tonic/gel preparation with 7.5 % water fraction, Box 5: hair tonic/gel preparation with 10 % water fraction, Box 6: hair tonic/gel preparation with 12.5% water fraction, Box 7: 7.5% water fraction. The application of ingredients was given every two times in the morning and evening with the amount of 0.5 g per application. The parameters in the test of hair growth stimulating activity were the length of the test rabbit hair in each box. Tests were carried out for 18 d, with basting on the first day considered to be the 0th day. Hair growth was observed every 3 d, and sampling on the 3rd, 6th, 9th, 12th, 15th, and 18th days. Samples were taken as many as three of the longest hairs in each box. Then the test rabbit hair was taken, then stored on a flat plane, taped to tape and measured using a caliper. In another group of rabbits, a comparison of the gel and hair tonic was carried out in which one more box was added to the rabbit hair tonic, namely the box for a gel with a concentration of 7.5%.

Data obtained from the measurement results were made of tables and diagrams. For the measurement of the effect of the hair tonic formula, for example, data was obtained (table 5) and illustrated in fig. 1 and then calculated statistically. Table 5 was data from the results of rabbit hair length measurements for formula hair tonics whereas fig. 1 and fig. 2 respectively showed hair growth stimulant activity test of Gel and Hair tonic formula.

Table 5: Rabbit hair length measurements for formula hair tonics

Treatments	Measurement of rabbit hair on days (cm)					
	3	6	9	12	15	18
Normal control	0.09	0.11	0.18	0.30	0.48	0.54
	0.09	0.16	0.40	0.45	0.50	0.68
	0.10	0.20	0.36	0.56	0.69	0.70
Mean	0.093333	0.156667	0.313333	0.436667	0.556667	0.64
SD	0.005774	0.045092	0.117189	0.130512	0.115902	0.087178
Negative control	0.30	0.38	0.42	0.55	0.58	0.60
	0.41	0.51	0.61	0.76	0.96	1.05
	0.28	0.58	0.61	0.70	1.00	1.06
Mean	0.33	0.49	0.546667	0.67	0.846667	0.903333
SD	0.07	0.101489	0.109697	0.108167	0.231805	0.262742
Positive control	0.46	0.98	1.10	1.35	1.59	1.675
	0.41	1.17	1.27	1.55	1.99	2.04
	0.19	0.20	0.22	0.38	0.46	0.68
Mean	0.30	0.783333	0.863333	1.093333	1.346667	1.465
SD	0.155563	0.514036	0.56359	0.625806	0.793494	0.7039
7,5% water fraction	0.13	0.30	0.56	0.85	1.00	1.24
	0.47	0.66	0.72	0.75	0.83	1.00
	0.48	0.85	1.14	1.27	1.36	1.44
Mean	0.356667	0.603333	0.806667	0.956667	1.063333	1.226667
SD	0.196299	0.279344	0.299555	0.275923	0.270617	0.220303
Hair tonic (7.5% fraction)	0.15	0.22	0.465	0.58	0.635	0.65
	0.30	0.48	0.93	1.70	2.00	2.13
	0.26	0.39	0.42	0.50	0.57	0.90
Mean	0.236667	0.363333	0.595	0.926667	1.068333	1.226667
SD	0.077675	0.132035	0.265094	0.67092	0.807501	0.792233
Hair tonic (10.0% fraction)	0.34	0.67	1.2	1.22	1.47	1.58
	0.19	0.21	0.40	0.44	0.53	1.18
	0.29	0.30	0.34	0.48	0.53	0.83
Mean	0.273333	0.393333	0.646667	0.713333	0.843333	1.196667
SD	0.076376	0.24379	0.480139	0.439242	0.542709	0.375278
Hair tonic (12.5% fraction)	0.45	0.585	0.61	1.33	1.80	2.27
	0.20	0.33	0.35	0.53	0.68	1.32
	0.26	0.30	0.31	0.50	0.55	0.68
Mean	0.303333	0.405	0.423333	0.786667	1.01	1.423333
SD	0.130512	0.156605	0.162891	0.470779	0.687241	0.800021

Statistical analysis

The following was an example analysis with statistical methods for hair tonic.

Hair tonic**a. Normality test**

Objective: to determine the normality distribution of the average rabbit hair length.

Hypothesis: Ho: the distribution of the average hair length of a normal rabbit

H1: the average distribution of rabbit hair length is not normal

α : 0.05

Example of Test of normality Shapiro Wilk is shown in table 6.

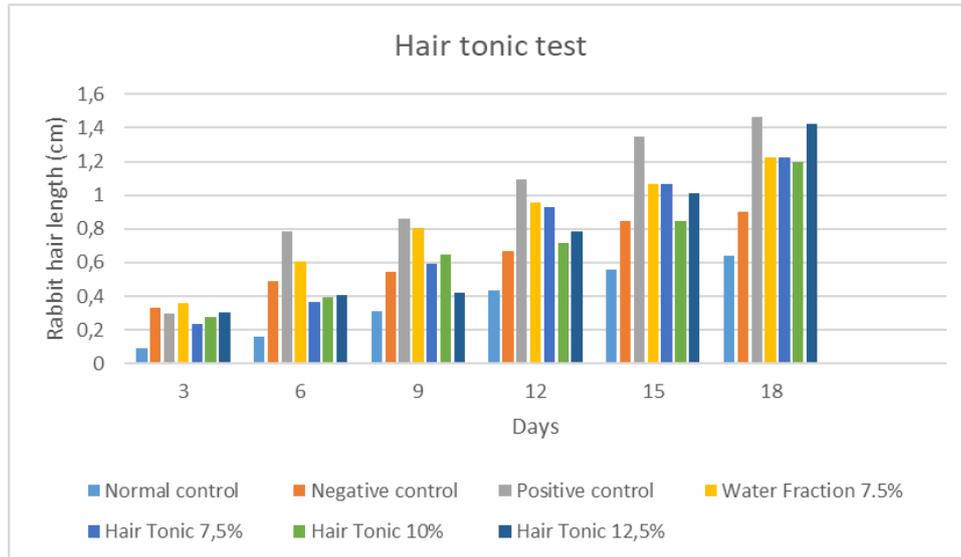


Fig. 1: Hair tonic formula test

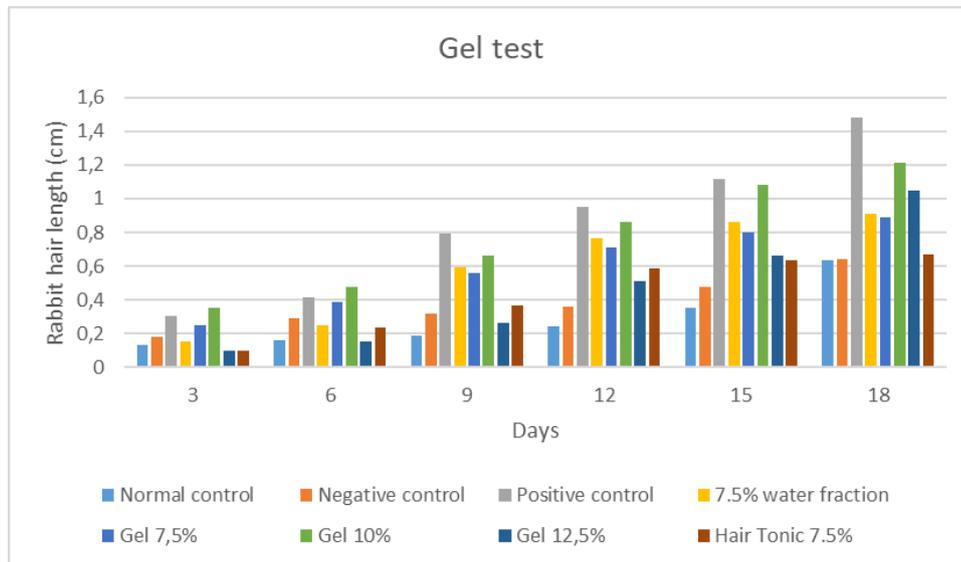


Fig. 2: Gel formula and comparison test

Table 6: Test of normality

		Kolmogorov	-	Smirnov ^a	Shapiro	-	Wilk
Treatment		Statistic	df	Sig.	Statistic	df	Sig.
Hair length	Normal control	.165	6	.200*	.950	6	.740
	Negative control	.171	6	.200*	.959	6	.815
	Positive control	.151	6	.200*	.965	6	.859
	Fraction 7.5%	.148	6	.200*	.978	6	.942
	Hair tonic 7.5%	.206	6	.200*	.928	6	.566
	Hair tonic 10.0%	.141	6	.200*	.970	6	.889
	Hair tonic 12.5%	.256	6	.200*	.901	6	.378

*This is a lower bound of the true significance

^aLilliefors Significance Correction

Criteria: Ho was rejected if the significance value was <0.05

Ho was accepted if the significance value is >0.05

Conclusion: Ho is accepted, so the data on the average rabbit hair length of each group was normally distributed.

b. Homogeneity Test

Objective: to find out the similarity of variants from the data of the average rabbit hair length

Hypothesis: Ho: data on average rabbit hair length varies homogeneously

H1: Data on average rabbit hair length was not homogeneous

α: 0.05

Test statistics: Levene Statistics

Criteria: Ho was rejected if the significance value was <0.05

Ho was accepted if the significance value was >0.05

Conclusion: Data on average rabbit hair length on ethanol extract testing varied homogeneously

c. ANOVA Test

Objective: to find out the significant difference of the average rabbit hair length for each group

Hypothesis: Ho: There is no significant difference from the average rabbit hair length for each group

H1: There was a significant difference from the average rabbit hair length for each group

α : 0.05

Test statistics: ANOVA

Criteria: Ho was rejected if the significance value is <0.05

Ho is accepted if the significance value is >0.05

Conclusion: There was no significant difference from the average rabbit hair length for each group

With the same calculation applied to the gel formulation, it was found statistically that the hair tonic formula with 12.5% water fraction, Gel formula with 10.0% water fraction was the best application and there was no significant difference between the formula of hair tonic and gel.

Irritation test

An irritation test was performed to determine the reaction caused by the preparation after the preparation was applied to the skin. Based on the results of the study, there was no irritation reaction on the skin when the preparation was applied on the first day. However, after the application was applied twice a day until the 6th day, there was a reddish color on the skin part which was given by negative control of gel formula. The reddish color started to slightly disappear after the ninth day. The reddish was thought to be due to the presence of Gel excipients such as triethanolamine which slightly irritate the skin. Erythema was indicated by the appearance of redness on the skin and the appearance of the visible wound. While edema was seen from the swollen surface of the skin compared to normal skin. Irritant materials could cause damage to skin cells through chemical work. These chemicals could damage the keratinocyte lipid membranes and then partially penetrate the cell membrane and damage the lysosomes, mitochondria, and core components. Membrane damage activates several mediators such as phospholipase, arachidonic acid (AA) which was converted into prostaglandin and leukotriene, diacylglyceride, platelet activating factor, and also inositide which induces vasodilation and increased vascular permeability. Vasodilation and increased vascular permeability which then showed symptoms of erythema and edema called irritation [34]. This result was not a dangerous irritation test, because it should be noted that basically, the skin sensitivity of experimental animals was slightly different from human skin. Especially rabbit animals whose irritation levels could be easily seen. Irritation index in humans was still uncertain if the value in experimental animals had a mild irritation index [35].

CONCLUSION

Based on the results of this study, gel and hair tonic formula showed the ability of hair growth. Gel formula containing 12.5 % pakis munding water fraction and hair tonic containing 10.0 % water fraction was the best formula. Statistically, there was no significant difference between hair tonic and gel formulas. Both formulas did not irritate the skin.

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AUTHORS CONTRIBUTIONS

All the author have contributed equally

CONFLICT OF INTERESTS

There is no conflict of interest between authors

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