
Hair Tonic Formulation of Green Spinach Leaf Extract (*Amaranthus viridis* L.) as a Hair Growth Agent for New Zealand Rabbits (*Oryctolagus cuniculus*)

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Abstract

*Hair loss can lead to baldness, and many commercial hair tonics contain synthetic chemicals with potential side effects. This study explores the use of natural ingredients, specifically green spinach leaf (*Amaranthus viridis*) extract, as an alternative. Green spinach leaves are rich in flavonoids, tannins, saponins, and antioxidants, which are known to stimulate hair growth. The research aims to evaluate the hair growth activity of green spinach leaf extract on New Zealand rabbits (*Oryctolagus cuniculus*), assess the physical quality of hair tonic formulations at three concentrations (5%, 10%, 15%), and determine the most effective formula. The study used a quantitative, descriptive, and experimental approach. Extracts were obtained via maceration with 96% ethanol and formulated into hair tonics. Rabbits were divided into six groups: untreated, positive control, negative control, and three treatment groups (F1, F2, F3). Phytochemical screening confirmed the presence of flavonoids, saponins, and tannins. Physical stability tests showed all formulations were stable. Statistical analysis (One-Way ANOVA and Post Hoc Tukey) revealed significant differences in hair growth between treatment and negative control groups, with the 15% formula (F3) yielding the highest average hair growth (10.67 mm).*

Keywords: *Amaranthus Viridis, Hair Growth, Hair Tonic, Natural Extract, New Zealand Rabbit.*

INTRODUCTION

Hair loss is a common problem experienced by many individuals and can lead to baldness, thus triggering the need for effective hair care products (Krisnawati, 2020; Fitria, 2023). Hair tonic products on the market generally contain synthetic chemicals such as minoxidil, which are known to cause side effects such as scalp irritation and other health problems (Hayati et al., 2024; Hindun et al., 2023). Therefore, finding natural alternatives is important to reduce the risk of side effects and increase the safety of hair care products (Fitria, 2023; Sihombing, 2018).

The main problem with using synthetic hair tonics is the potential for side effects that can harm scalp health, such as irritation and local erythema (Hayati et al., 2024; Hindun et al., 2023). Furthermore, the effectiveness of natural products still needs to be scientifically proven, particularly in stimulating hair growth and maintaining healthy hair follicles (Krisnawati, 2020; Fitria, 2023). Green spinach leaves (*Amaranthus viridis*) are known to be rich in vitamins A, K, C, E, B1, and B6, as well as antioxidants that play a role in accelerating hair growth and protecting the hair structure from damage (Krisnawati, 2020; Fitria, 2023). The flavonoid and antioxidant content in green spinach leaves also functions to increase blood flow to hair follicles, thereby supporting healthy hair growth (Muliani et al., 2022; Raharjo et al., 2023).

Previous research has shown that green spinach leaf extract has potential as an active ingredient in hair tonic preparations, with good organoleptic and absorption test results, as well as vitamin B and C content that supports hair growth (Fitria, 2023; Sihombing, 2018). However, the optimal formulation and evaluation of the physical quality of hair tonics based on green spinach leaf extract still require further study to ensure product effectiveness and stability (Krisnawati, 2020; Fitria, 2023).

The main objectives of this study were to evaluate the hair growth activity of green spinach leaf extract in New Zealand rabbits, assess the physical quality of hair tonic at three different concentrations (5%, 10%, and 15%), and determine the optimal formulation as a hair growth aid. The urgency of this research lies in the effort to provide a safe and effective natural-based solution for hair loss treatment, while also providing novelty in the use of green spinach leaf extract as an alternative active ingredient in hair tonic (Krisnawati, 2020; Fitria, 2023). This research is expected to provide a

scientific contribution to the development of natural-based cosmetic products that are safer and more user-friendly.

RESEARCH METHODS

Determination of green spinach plants (*Amaranthus viridis*)

Determination of green spinach (*Amaranthus viridis*) plants was carried out at the Tawangmangu Traditional Health Service Unit of Dr. Sardjito Tawangmangu Karanganyar General Hospital. Based on the determination results, it shows that the plants used are indeed green spinach (*Amaranthus viridis*). The certificate of determination results can be seen in the attachment.

Making Simple Powder

Green Spinach Leaves are obtained from the home garden taken in the morning, 5 kg of spinach leaves that have been picked are put into a container and then sorted dry to remove dirt or unwanted dry leaves, then washed with running water to remove dirt and then drained, the next process the leaves are sliced thinly so that the drying process is faster and even then the leaves are dried in the sun by covering a black cloth so that they are not contaminated with dirt or dust, the next process is the dry *simplicia* is sorted again after the dry *simplicia* is blended into a fine powder and after that it is sieved using a mesh sieve no. 40, then the *simplicia* powder is stored in a well-closed container.

Table 1. Results of Spinach Leaf Simplex Yield

Wet Weight	Dry Weight	Yield Value	Condition	Reference
6000 grams	1300 grams	21.6%	>10%	(Ramdhini, nd.)

Standardization of Simple Green Spinach Leaves (*Amaranthus viridis*)

The results of the green spinach leaf (*Amaranthus viridis*) *simplicia* powder obtained a green color with a distinctive odor of green spinach leaves. Standardization of the quality of *simplicia* is very important before starting the extraction process. Standardization of the quality of *simplicia* aims to ensure the quality of the research sample, so that drying loss and water content tests can be carried out using a moisture balance tool, and for ash content using a furnace. Determination of drying loss is carried out three times. Good requirements for testing drying loss, water content, and ash content are less than 10%.

Table 2. Results of Standardization of Simple Drugs

Parameter Test	Replication I	Replication II	Replication III	Average	Condition	Reference
Drying shrinkage	1.93%	1.90%	3.34%	2.39%	<10%	Ministry of Health of the Republic of Indonesia 2017
Water content	5.16%	4.66%	5.05%	4.93%	<10%	Ministry of Health of the Republic of Indonesia 2017
Ash content	7.0%	3.3%	4.1%	4.8%	<10%	Ministry of Health of the

Yield and Characteristics of Green Spinach Extract (*Amaranthus viridis*)

500 grams of finely ground green spinach leaves were extracted using the maceration method using 96% ethanol solvent, with the first soaking using a ratio of 1:10. From the maceration results, the macerate was obtained and then put into a rotary evaporator at a temperature of 50°C. Next, the yield of the simplicia was calculated, where the yield is the ratio between the dry weight of the sample obtained and the initial weight of the sample. A good yield value is >10% because the higher the yield, the higher the content of substances that will be attracted to the raw material.

Table 3. Extract Redemption Results

Powder Weight (g)	Extract Weight (g)	Yield (%)	Form	Color	Aroma
500	62.5	12.5%	Thick	Blackish Green	Typical

Standardization of Green Spinach Extract (*Amaranthus viridis*)

Standardization of spinach leaf extract included water content testing, drying loss determination, and ethanol-free testing. The results of the extract standardization tests are shown in Tables 8 and 9.

Table 4. Results of extract standardization

Parameter Test	Replication I	Replication II	Replication III	Average	Condition	Reference
Drying shrinkage	0.41%	0.21%	0.43%	0.35%	10%	FHI 2017
Water content	2.37%	1.73%	2.19%	2.09%	10%	FHI 2017

Table 5. Results of ethanol-free testing of green spinach leaf extract

Reagent	Results	Reference
Acetic Acid + Concentrated Sulfuric Acid	No ester odor	(Cendana, 2021).

Phytochemical Screening of Green Spinach (*Amaranthus Viridis*) Leaf Extract

Based on the results of phytochemical screening tests, green spinach leaf extract (*Amaranthus viridis*) contains several secondary metabolite compounds, namely flavonoids, saponins, and tannins.

Table 6. Phytochemical Screening Results of Green Spinach Leaf Extract

Compound	Reagent	Results	Information	Conclusion
Flavonoid	Magnesium powder (mg) + 5 drops of concentrated hydrochloric acid (HCL)	There is an orange layer	There is an orange layer	+
Tannin	3 drops of 1% FeCl3 solution	Blackish Green	There is a color change from blackish green	+

Saponin	Aquadest	Foam Formed	Foam Formed	+
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Based on Table 6, it can be seen that the results of the phytochemical screening test of green spinach leaf extract contain secondary metabolite compounds, namely flavonoids, saponins, and tannins. The results of the qualitative flavonoid test were carried out by adding the sample solution with Magnesium powder, and then adding 5 drops of concentrated HCl. The addition of concentrated HCl was used to hydrolyze flavonoids into their aglycones, namely by hydrolyzing the O-glycosyl. Glycosyl was replaced by H⁺ from the acid due to its electrophilic nature. Reduction with Mg and concentrated HCl can produce complex compounds that are red or orange.

A saponin test is considered positive if foam appears in a vigorously shaken sample solution. The appearance of foam indicates the presence of glycosides, which can form foam in water, which hydrolyzes into glucose and other compounds. Glycosides are complex substances between reducing sugars (glycones) and non-sugars (aglycones). Many saponins have up to 5 sugar units, and the common component is glucuronic acid. The presence of saponins in plants is indicated by the presence of foam. The characteristic properties of saponins are their bitter taste and the presence of foam in water. The results of the saponin compound test in green spinach leaf extract (*Amaranthus viridis*) showed a positive result with the presence of foam.

Tannins have natural properties that can dissolve in water and produce various colors, ranging from light to dark red or brown, because each tannin derivative has a different color depending on its source. Tannins can react with iron to produce a dark color. The formation of a dark color is caused by the formation of a complex compound by tannin with Fe³⁺ ions (Putri et al., 2023). Phytochemical tests of tannin compounds are carried out by dissolving the extract in distilled water and then adding 3-5 drops of FeCl₃ reagent. A positive result indicates a color change to blackish green. The purpose of adding FeCl₃ is to determine the presence of phenol groups in the sample. The results of the test of green spinach leaf extract compounds (*Amaranthus viridis*) showed a positive result with a color change to blackish green.

Evaluation of the Physical Properties of Hair Tonic Preparations Made from Green Spinach Leaf Extract (*Amaranthus Viridis*)

Organoleptic Test Results

Table 7. Organoleptic Test Results and Hair Tonic Extract Preparation

Hair Tonic Preparation Formulation	Form	Color	Smell
F0	Liquid	Colorless	Menthol
F1	Liquid	Dark green	The distinctive smell of spinach leaves
F2	Liquid	Dark green	The distinctive smell of spinach leaves
F3	Liquid	Dark green	The distinctive smell of spinach leaves

Information :

F0: hair tonic base without green spinach leaf extract

F1: hair tonic formulation with a concentration of 5%

F2: hair tonic formulation with a concentration of 10%

F3: hair tonic formulation with a concentration of 15%

Based on the results of organoleptic tests on the hair tonic base, it is liquid, colorless, and has a menthol odor, while in the hair tonic preparation of green spinach leaf extract (*Amaranthus viridis*) in formulations 1, 2 and 3, it is liquid, dark green in color and has a distinctive odor of green spinach leaves (*Amaranthus viridis*). There is a slight difference in color in the three formulations due to differences in the concentration of the extract used. Homogeneity examination of the three formulas shows that the three formulas are physically homogeneous.

pH Test Results

The pH of the preparation was measured using a digital pH meter. The meter was first calibrated using neutral and acidic pH values, then washed with distilled water and dried with a tissue. The pH value of the hair tonic preparation was measured by dipping the meter into the preparation and waiting for a few moments until it showed a constant value.

A pH test is a test to determine whether a product falls within the scalp's pH range, which is between 4.5 and 6.5. A product's pH can affect scalp absorption, as a pH that is too acidic can easily irritate the scalp. A pH that is too alkaline can cause flaking.

Table 8 Results of pH Testing of Hair Tonic Preparation with Green Spinach Leaf Extract

pH testing				
Formula	Replication I	Replication II	Replication III	Average
F0	4.64	4.64	4.71	4.66
F1	4.74	4.84	4.86	4.81
F2	5.12	5.18	5.28	5.19
F3	5.34	5.37	5.42	5.37

The pH test on formulation 0 without green spinach leaf extract had a pH of 4.66. Formulation 1 contained 5% green spinach leaf extract with a pH of 4.81. Formulation 2 contained 10% green spinach leaf extract with a pH of 5.19, and formulation 3 contained 15% green spinach leaf extract with a pH of 5.37, where the pH was the highest when compared to formulations 0, 1, and 2 using green spinach leaf extract. These results are in accordance with the scalp pH range of 4.5-6.5. The difference in pH values of the four hair tonic formulations can be influenced by the addition of the active ingredient green spinach leaf extract.(Amin et al., 2014).

Viscosity Test Results
 This test was conducted to determine the consistency of the preparation. Viscosity measurements can affect the concentration of green spinach leaf extract in the hair tonic. The higher the viscosity, the higher the concentration of extract contained in the preparation. The test results can be seen in Table 9.

Table 9. Viscosity Test Results of Green Spinach Leaf Extract Hair Tonic Formula

Formulation	Replication 1	Replication 2	Replication 3	Average
F0	0.65	0.65	0.67	0.65
F1	0.70	0.74	0.80	0.74
F2	1.03	1.10	1.12	1.08
F3	1.28	1.32	1.39	1.33

Based on the results of the viscosity test on Hair Tonic spinach leaf extract, it shows that the four preparation formulas still meet the viscosity range requirements for Hair Tonic preparations according to SNI, namely below 5 cPs.(Darajati & Ambari, 2021)

Homogeneity Test

The homogeneity test is performed by examining the hair tonic preparation to see whether or not there are any unevenly dispersed particles. It is considered homogeneous if the particles from all the ingredients used are evenly mixed in the hair tonic preparation.

Table 10. Results of Homogeneity Test

Formulation	Results
F0	Homogeneous
F1	Homogeneous
F2	Homogeneous
F3	Homogeneous

Rabbit Hair Growth Activity Test

The hair growth activity test on male rabbits (New Zealand) was measured based on the results of the average hair length to determine the effectiveness of negative control, positive control, formula

1, formula 2, and formula 3 in accelerating hair growth. Observation of treatment in the negative control group was carried out by providing a base formula of Hair Tonic preparation to determine whether it affected hair growth in rabbits. Observation of treatment in the positive control group was carried out by providing Mustika Ratu Hair Tonic preparation because Mustika Ratu is a Hair Tonic that has been widely used in the market as a hair growth agent. The difference in administering F1, F2, and F3 lies in the comparison of the concentration of active substances (5%, 10% and 15%) to determine which of the formulas has the most optimal or good effect on hair growth in test animals.

Testing the activity of hair tonic using a rabbit's back cleaned of hair by shaving it until clean, then divided into 6 parts, each in the form of a 2x2 cm rectangle, with a distance between areas of 1 cm.

Hair Tonic, according to the group, is as much as ± 1 ml for 21 days and is done once a day. Measurement of rabbit hair length was done on days 7, 14, and 21. Measurement of hair length was done by plucking 3 strands of the longest rabbit hair using tweezers, then measuring the length with a caliper with a black plastic lining underneath.

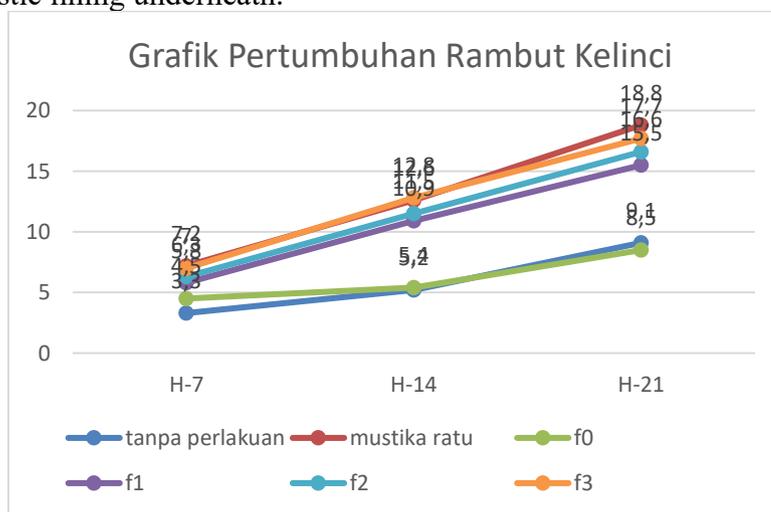


Figure 1. Graph of average rabbit hair growth
 Table 11 Rabbit Hair Growth

No	Treatment Group	Hair Growth (mm)			Hair Length Gain (mm)
		H-7	H- 14	H-21	
1	No Treatment	3.3	5.2	9.1	5.8
2	Queen's Gemstone	7.2	12.6	18.8	11.6
3	F0	4.5	5.4	8.5	4.0
4	F1	5.8	10.9	15.5	9.6
5	F2	6.3	11.5	16.6	10
6	F3	7.0	12.8	17.7	10.6

The hair growth data in rabbits was then analyzed using SPSS version 27. The normality test of the rabbit hair growth data was carried out to determine whether the data were normally distributed or not using the Shapiro-Wilk method because the number of samples to be tested was below 100. The criteria in the Shapiro-Wilk test are that if the p -value > 0.05 , then the data is said to be normally distributed; if the p -value < 0.05 , then the data is said to be not normally distributed. The test was continued with a homogeneity test using the Levene method to determine whether the data were homogeneously distributed and whether the variations of several populations were the same or not. The criteria in the Levene test are that if the p value > 0.05 , then the data are homogeneous; if the p value < 0.05 , then the data are not homogeneous. The test was continued with the One-Criterion test in the One-Way ANOVA test. If the p -value < 0.05 , then it is said that there is a difference in each

group; if the p -value > 0.05 , then it is said that there is no difference in each group. If the average data on hair growth in rabbits is different, it is continued with the Tukey Post Hoc test to observe which variables have differences. The criteria in the Tukey Post Hoc test are that if the p -value is < 0.05 , then it is said there is a difference; if $p > 0.05$, it is said there is no difference. Test results of rabbit hair growth data analysis with Hair Tonic can be seen in Table 16

Table 12. SPSS Analysis Results of Rabbit Hair Growth with Hair Tonic

Treatment (Hair Tonic)	Normality Shapiro-Wilk	Homogeneity Levene	One-Way Anova
No Treatment(K-)	0.935		
Mustika Ratu (K+)	0.554		
F1	0.235	0.211	0,000
F2	0,433		
F3	0.643		
F0	0.363		

The results of the normality test in the negative control group or without treatment showed a significance value of 0.935, the positive treatment group or mustika ratu showed a significance value of 0.554, the formula 1 group showed a significance value of 0.235, the formula 2 group showed a significance value of 0.433, and the formula 3 group showed a significance value of 0.643, the formula 0 group showed a significance value of 0.363. The results of the normality test data showed that the data were normally distributed with a p -value > 0.05 . The test was continued with a homogeneity test, which obtained a significance value of 0.211. It can be concluded that the data were homogeneously distributed with a p -value > 0.05 . The test was continued with a one-way ANOVA test with a significance value of 0.000.

It can be concluded that the data shows a difference, as evidenced by a significance value of < 0.05 . Therefore, the test was continued with a Tukey post hoc test to determine which variables showed differences. The results of the Tukey post hoc test can be seen in Table 13.

Table 13. Tukey Post Hoc Test Results

Tukey HSD^a

Perlakuan	N	Subset for alpha = 0.05		
		1	2	3
f0	3	4.0000		
tanpa perlakuan	3	5.8667		
f1	3		9.6333	
f2	3		10.0000	10.0000
f3	3		10.6667	10.6667
mustika ratu	3			11.6667
Sig.		.065	.514	.113

The results of the Tukey Post Hoc analysis showed significant differences between the treatment groups. The f0 and untreated groups were in the same subset with average values of 4.00 and 5.86, respectively, so there was no significant difference. Meanwhile, the f1, f2, and f3 treatment groups formed a different subset with average values of 9.63, 10.00, and 10.67, respectively, which showed higher results than the negative control, so it can be concluded that the treatment given to these groups had a significant effect. In addition, the queen's mustika group used as a comparison had the highest average of 11.67 and was in the same subset as f2 and f3, which means its effectiveness was not significantly different from the two formulas. Thus, it can be concluded that the f2 and f3 formulations had an effectiveness close to the commercial comparator (Queen's Mustika), while f0 and untreated did not have a significant effect on the parameters tested.

Based on research tests that have been conducted on green spinach leaf extract (*Amaranthus Viridis*) on hair growth in male New Zealand White rabbits, the flavonoid, tannin, and saponin content in the plant extracts used has been proven to have activities that support hair growth. The mechanism

of flavonoids in growing hair increases blood circulation in the scalp. Flavonoids stimulate blood vessel dilation (vasodilation) by increasing the production of nitric oxide (NO). Smoother blood flow ensures optimal oxygen and nutrient supply to hair follicles, which is important for triggering the anagen phase (growth). Hair follicles are susceptible to oxidative stress caused by free radicals. Flavonoids work as free radical scavengers, thereby preventing damage to follicular cell DNA and maintaining cell proliferation activity. (Arifin et al., 2017).

Tannins contain compounds that act as hair nutrients, and tannins also have astringent, antioxidant, and antimicrobial properties, all of which contribute to hair growth. Tannins play a role through several functions, namely, strengthening the roots and hair follicles. The astringent properties of tannins cause contraction of the scalp tissue around the hair follicles. Tannins contain components such as gallic acid, ellagic acid, and proanthocyanidins, which are strong antioxidants that protect follicles from oxidative damage. These antioxidants ward off free radicals that can damage follicle cells and accelerate hair aging. (Wijaya et al., 2024).

CONCLUSIONS

The main conclusion of this study shows that the hair tonic formulation made from green spinach leaf extract (*Amaranthus viridis*) with a concentration of 15% (F3) provides the most optimal effect in stimulating hair growth in New Zealand rabbits, with a significant average hair growth compared to negative controls and approaching the effectiveness of commercial products such as Mustika Ratu. The flavonoid, tannin, and saponin content in green spinach leaf extract plays an important role in supporting hair growth activity through the mechanism of increasing blood circulation, protecting follicles from oxidative stress, and strengthening hair roots. The results of physical tests, such as organoleptic, pH, viscosity, and homogeneity, also show that all hair tonic formulations are stable and comply with applicable cosmetic standards, making them suitable for use as a safer and more natural alternative hair loss treatment (Krisnawati, 2020; Fitria, 2023; Sjamsiah, 2024).

However, this study has several limitations, including the limited use of New Zealand rabbits as test animals and the lack of long-term safety testing in humans. Furthermore, the concentration variation of the extract tested was limited to three formulas. Therefore, further research is recommended to explore other concentrations, conduct toxicity tests, and test the effectiveness in human subjects to ensure the product's safety and clinical efficacy. The practical implication of this study is the potential development of a hair tonic based on green spinach leaf extract as a skin-friendly cosmetic product with minimal side effects, and can be an alternative for people seeking natural hair loss treatment solutions (Fitria, 2023; Krisnawati, 2020; Sjamsiah, 2024).

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