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Formulation, Physical Evaluation and Hair Growth Activity of White Champaca Flower (*Magnolia alba*) Extract Hair Tonic in Rabbits (*Oryctolagus cuniculus*)

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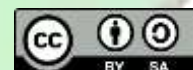
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ABSTRACT

Hair loss has created a demand for natural hair tonic preparations that are safer than synthetic alternatives. White champaca flowers (*Magnolia alba*) contain bioactive compounds specifically flavonoids, phenols, and essential oils that have the potential to stimulate hair follicles. This study aimed to evaluate the effect of an ethanolic extract tonic derived from white champaca flowers on hair growth in rabbits (*Oryctolagus cuniculus*) and to determine the optimal concentration. An experimental method employing a Randomized Block Design (RBD) was used, featuring five treatments: a negative control (distilled water), a positive control (2% minoxidil), and extract formulations at concentrations of 3%, 6%, and 12%, all applied topically over a 28-days period. Test results indicated that all formulations were homogeneous and stable, with an ideal pH range (4.9 – 5.1). By the fourth week, the 3% extract formulation yielded the greatest hair length growth (1.97 ± 0.49 cm), surpassing both the positive control (1.87 ± 0.71 cm) and the negative control (1.17 ± 0.42 cm). Concentration of 6% and 12% showed reduced effectiveness, likely due to high viscosity. Two-way ANOVA analysis revealed that the time factor had a significant effect ($p < 0.05$). Descriptively, the 3% ethanolic extract of white champaca flowers proved to be the optimal formulation as a natural hair tonic alternative compared to the other formulations tested.

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Introduction

Hair is a biological structure that plays a crucial role in protecting the body from environmental exposures, such as ultraviolet radiation and extreme temperature fluctuations. Furthermore, hair functions in thermoregulation through sweat evaporation and acts as a sensitive sensory receptor (Yasir, 2019). Healthy

hair is generally characterized by a thick, strong, manageable texture, and strong roots (Achmadi *et al.*, 2020). Physiologically, hair undergoes a growth cycle consisting of the anagen (growth), catagen (transition), and telogen (resting) phases (Nurbaya *et al.*, 2017). Disruptions to this cycle can lead to hair loss, characterized by a lengthened telogen

phase, a delayed anagen phase, and a shortened anagen phase (Habsari *et al.*, 2019). Hair loss can occur physiologically or pathologically and is influenced by various factors, such as genetics, hormones, nutrition deficiencies, exposure to free radicals, stress, and the use of certain chemicals (Febriani *et al.*, 2016, in Rizka and Yuliana, 2019)

Hair care efforts are necessary to maintain scalp health and prevent further damage. Proper hair care includes selecting products that are suited to your hair condition and environment, thereby minimizing the risk of hair loss (Wijaya *et al.*, 2024). One commonly used treatment method is hair tonic, which offers the advantages of easy application, fast absorption, and no residue on the scalp (Anwar and Darusman, 2022). However, most commercial hair tonics still use synthetic ingredients such as minoxidil, which, while effective in treating alopecia, can potentially cause side effects such as dermatitis, itching, and allergic reactions (Rahmawati, 2022). Several studies have shown that natural ingredients containing secondary metabolites such as flavonoids, saponins, and phenols have the potential to stimulate hair growth. Flavonoids are known to act as antioxidants that can support the anagen phase and reduce hair loss (Anwar and Darusman, 2022). Sativa

(2021) reported on the potential of *Ziziphus nummularia* (bidara) leaf ethanolic extract, showing that a tonic formulation with a 3% extract concentration was the most optimal it was proven to increase hair length by 10.67 ± 1.15 mm over 28 days without causing skin irritation in test animals.

Although various herbal plants have been explored for hair care, the use of white champaca flowers (*Magnolia alba*) has thus far been limited to traditional aromatherapy and topical cosmetics. *Magnolia alba* is rich in bioactive secondary metabolites, specifically flavonoids, tannins, phenols, and essential oils that possess potent antioxidant properties capable of mitigating oxidative stress in hair follicles and stimulating the anagen growth phase (Hayatillah and Hapsari, 2024). However, comprehensive scientific evidence evaluating its specific efficacy and optimal formulation as a hair growth stimulant is currently lacking in the literature. To address this research gap, this study undertook the innovative formulation and evaluation of a hair tonic based on an ethanolic extract of *Magnolia alba* flowers. Furthermore, the study examined the physical stability and concentration dependent hair growth activity of the formulation using rabbits (*Oryctolagus cuniculus*) as test subjects. The findings are expected to provide an empirical and

pharmaceutical foundation for the development of standardized, effective, and safe phytocosmeceutical hair care products.

Materials and Methods

Research Location and Time

This research was conducted at the Multifunction Laboratory of Ar-Raniry State Islamic University Banda Aceh for a period of 4 months starting from December 2025 to March 2026. The object of this research was white champaca flowers (*Magnolia alba*) taken in Kruet Tumpen Village, Glumpang District, Pidie Regency.

Experimental Design

This study used a quantitative method with an experimental approach, as the data collected consisted of measurements of hair growth in rabbits (*Oryctolagus cuniculus*) after administration of a tonic preparation containing white champaca flower extract. These observations were then tested using statistical computations to determine the significance of the variables being tested.

Preparation of Test Materials

The first stage carried out was the preparation of research tools and materials, the tools used were; oven, mortar, glass bottle, hot plate, rotary evaporator, hair clipper, tweezers, ruler and analytical balance. While the materials used were; white champaca flower (*Magnolia alba*), 96% ethanol, distilled water, sodium

metabisulfite, Na₂EDTA, Tween 80, Regrou, methyl paraben, menthol, propylene glycol, 70% alcohol, and test rabbit (*Oryctolagus cuniculus*).

Stages of Extraction of White Champaca Flower (*Magnolia alba*)

The extraction steps in this study refer to the procedure developed by Sulastri *et al.*, (2020) with certain adjustments. The raw material, 1 kg of high quality white champaca flower, was cleaned under running water, then only the petals were removed and dried using tissue paper. The petals were then dehydrated in an oven at 40 °C for 2 x 24 hours before being converted into powdered herbal medicine by grinding process using a mortal. A total of 73 grams of champaca flower powder was extracted using a maceration technique in a glass container with 96% ethanol solvent (ratio 1:10 w/v). The soaking process lasted for 3 days at room temperature with periodic stirring every 24 hours. The maceration solution was then separated using Whatman No. 1 filter paper (diameter 99 mm), then the filtrate was concentrated with a rotary evaporation at 70 °C until a thick extract was formed.

Tonic Formulation Procedure

The steps for making the tonic in this study are modifications of the basic formula according to Hindun *et al.*, (2017).

The process begins by dissolving white champaca flower extract in distilled water to produce various concentrations: 3%, 6%, and 12%. This hydro alcoholic liquid preparation is designed to deliver active ingredients to the scalp to stimulate hair growth through the synergy of various excipients (Hidayah *et al.*, 2020). The first phase (solution 1) was prepared by mixing 0.03 grams of sodium metabisulfite and 0.2 grams of Na₂EDTA into 3.2 ml distilled water, which was then mixed with 1 ml of Tween 80, which acts as a non-ionic surfactant and solvent to prevent the separation of the oil and water phases (Shoviantari *et al.*, 2019) until a

homogeneous mass was formed. Separately, methyl paraben (0.08 grams) was dissolved in 2 ml of ethanol (solution 2). Ethanol 96% and propylene glycol act as consolidants and penetration enhancers to aid the penetration of active ingredients into the follicles while protecting the scalp from evaporation (Darajati and Ambari, 2021). Next, menthol (0.15 grams) was dissolved in 3 ml of ethanol as solution 3, then added to solution 2 with 15 ml of propylene glycol. As a final step, solution 3 and solution 1 were mixed, and the final volume was brought to 100 ml with aquadest.

Table 1. Hair Tonic Formulation of Ethanol Extract of White Champaca Flowers

Composition	Negative control	Formulation (%)			Positive control
		Formula 3%	Formula 6%	Formula 12 %	
White champaca flower extract	-	3	6	12	-
Ethanol	35	35	35	35	35
Propylene glycol	15	15	15	15	15
Tween 80	1	1	1	1	1
Methyl paraben	0.08	0.08	0.08	0.08	0.08
Na ₂ EDTA	0.2	0.2	0.2	0.2	0.2
Menthol	0.15	0.15	0.15	0.15	0.15
Sodium metabisulfite	0.03	0.03	0.03	0.03	0.03
Regrou	-	-	-	-	10
Aquadest (Final formula volume)	100	100	100	100	100

Furthermore, the physical stability of the tonic preparations in 3%, 6%, and 12% formulations was assessed through a homogeneity test, which was visually assessed using a watch glass and cover glass. Organoleptic testing was conducted

visually by observing the color, aroma, texture, and sensation of the tonic from 20 respondents. Furthermore, viscosity was determined using a viscometer, while the acidity of the preparations was monitored using a pH meter. All tests followed the

protocols of Hindun *et al*, (2017) and Sativa *et al*, (2021).

Research Methods and Parameters

All animal handling and experimental procedures were conducted in strict accordance with the ethical standards for animal care set by the Multifunction Laboratorium of UIN Ar-Raniry Banda Aceh. Prior to the experiment, the animals were acclimatized for one week under controlled laboratory conditions to ensure health and stability, with free access to standard feed and water.

The evaluation of hair growth effectiveness following administration of champaca flower extract adopted an experimental procedure developed by Sativa *et al*, (2021). This study involved three 9-month-old male domestic rabbits with an average body weight of 1,5 kg. The dorsal hair was then shaved into five 2 x 2 cm square zones with 1 cm intervals. The treatment groups were divided into the following areas:

Table 2. Division of Treatment Groups and Variations in Test Preparation Concentration

Test Category	Intervention Description	Concentration/Material
Group I	Negative Control	Aquadest
Group II	Test Preparation A	Champaca Flower Extract 3%
Group III	Test Preparation B	Champaca Flower Extract 6%
Group IV	Test Preparation C	Champaca Flower Extract 12%
Group V	Positive Control	Regrou ^R

The tonic was applied routinely every afternoon for 28 days, with two sprays per treatment area. Growth was evaluated by plucking and measuring the maximum length of hair strands from each test box. This observation procedure was carried out periodically at seven-day intervals. The parameters measured included hair growth, in the form of hair length, during the application of the champaca flower extract hair tonic. Hair length was measured by plucking one strand of hair using tweezers in each group area once a week and measuring it using a ruler after the first day of hair tonic

application, for a total of four measurements during the study period (Sativa *et al*., 2021).

Data Analysis

This study used a Randomized Block Design (RBD) as the main experimental design. To determine the effectiveness of various concentrations of champaca flower extract on stimulating rabbit hair growth and the physical characteristics of the tonic, data processing was performed using SPSS version 25.0 software. Prior to hypothesis testing, statistical assumptions were checked using Levene's test for homogeneity and the one-

sample Kolomogorov-Smirnov test for normality. If the data were found to be normally distributed and had uniform variance, testing was continued with two-way ANOVA at the 95% confidence level. If significance was found, the Bonferroni post-hoc test was applied to identify differences in effects between treatment groups in depth (Sativa *et al.* 2021).

Results

Results on Physical Properties and Stability of *Magnolia alba* Flower Ethanol Extract Tonic Formulations

An assessment of the stability and physical properties of a hair tonic

formulation derived from *Magnolia alba* flower extract was conducted to ensure the product possessed good physical quality, was evenly dispersed, and was safe for scalp application. The parameters evaluated include organoleptic properties, homogeneity, viscosity, and pH. All test results were required to meet the Indonesian National Standard (INS) specifications for hair tonic; the SNI 16-4955-1998. Standardization specifications and physical property test results for the tonic preparation are presented in **Table 3**,

Table 3. Quality requirements for hair tonic: Indonesian National Standard (SNI) 16-4955-1998 ad physical properties of a hair tonic formulation containing white champaca (*Magnolia alba*) flower ethanol extract

Testing	Formulation					
	K-	3%	6%	12%	K+	SNI
Organoleptic	Clear, mild aroma, very low viscosity	Turbid, moderate aroma, low viscosity	Light brown, strong aroma, low viscosity	Dark brown, strong aroma, low viscosity	Turbid, very strong aroma, low viscosity	The color and aroma resemble those of the plant extract used
Homogeneity	+	+	+	+	+	+
Viscosity	0.40	0.50	0.60	0.70	0.70	< 5 mPa·s)
pH	4.8	4.9	5.0	5.1	5.4	3.0 – 7.0

Note: K+ : Control Positive (+), K- : Control Negative. SNI : Standard Nasional Indonesia. Homogeneous (no coarse particles or phase separation). Viscosity units are expressed in mPa's (*milliPascal-second*), equivalent to *centiPoise* (cP)

Rabbit Hair Growth Effectiveness Test and ANOVA Statistical Analysis

The results of the observations on rabbit hair length growth over four weeks

across various treatment groups are presented in **Table 4**.

Table 4. Effect of White Champaca Flower Ethanol Extract Tonic on Rabbit Hair Length over 4 Weeks (n=3)

Treatment Groups	Week 0	Week 1	Week 2	Week 3	Week 4
Negative Control	0.00±0.00	0.27±0.06	0.50±0.26	0.90±0.26	1.17±0.42
3% Champaca Extract	0.00±0.00	0.30±0.17	0.63±0.25	1.30±0.10	1.97±0.49
6% Champaca Extract	0.00±0.00	0.27±0.21	0.70±0.36	1.17±0.15	1.57±0.12
12% Champaca Extract	0.00±0.00	0.13±0.06	0.30±0.10	0.87±0.12	1.23±0.25
Positive Control	0.00±0.00	0.40±0.10	0.80±0.26	1.37±0.51	1.87±0.71

Furthermore, the results of the two-way ANOVA test are presented in statistical significance analysis using the **Table 5.**

Table 5. Result of the Two-Way ANOVA Test of the Effect of Tonic Treatment and Observation Time on Rabbit Hair Length.

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F- Score	Significance (p-value)
Treatment Factors	1.663	4	0.416	2.966	0.073
Error (Treatment)	1.388	8	0.139		
Time Factor (Week)	24.009	4	6.002	105.671	< 0.001
Error (Time)	1.342	10	0.134		
Treatment x Time Interaction	1.083	16	0.068	1.192	0.316
Error (Interaction)	2.272	40	0.057		

To further visualize the interaction effect between treatment groups and observation time on rabbit hair growth trends over the 28 day period, the estimated mean growth is presented in the **Figure 1.**

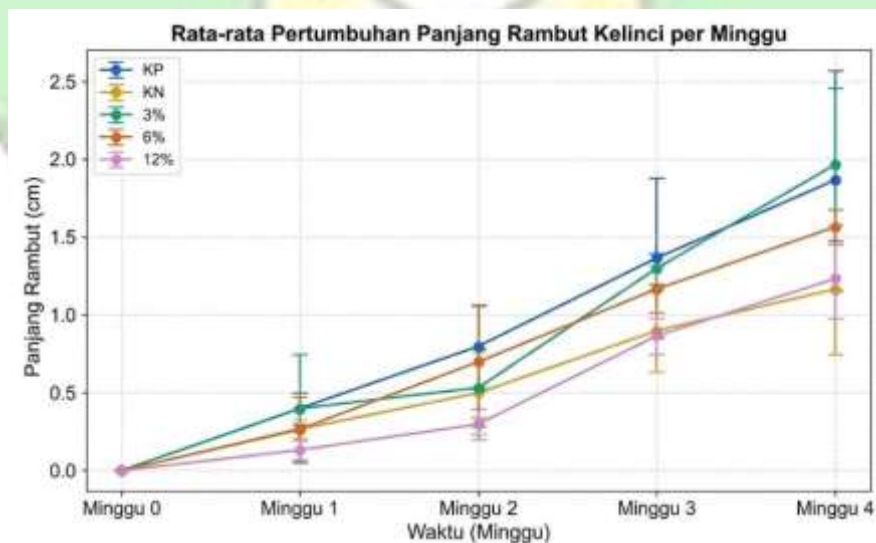


Figure 1. Average Hair Length of Rabbits (*Oryctolagus cuniculus*) in Various Treatment Groups during 28 Days of Observation.

Discussion

Evaluation of Physical Properties and Stability of *Magnolia alba* Flower Ethanol Extract Tonic Formulations

Based on Table 3, increasing the extract concentration influenced the organoleptic properties of each formula. The negative control remained clear, with a mild aroma and a very low viscosity. At the 3% extract concentration, the preparation began to appear turbid, with a moderate aroma and a low viscosity. The 6% formula exhibited a light brown color, a stronger aroma and a low viscosity, while the 12% formula showed a dark brown color, a very pungent extract aroma, and a low viscosity. Meanwhile, the positive control displayed a turbid appearance and a very strong aroma, resulting from the commercial active ingredient it contained along with a low viscosity.

Homogeneity testing yielded homogeneous (+) results for all formulas. No coarse particles, extract clumps, or phase separation between the aqueous and organic/alcohol phases were observed. Viscosity and pH values showed a pattern directly proportional to the increase in active ingredient concentration. The lowest viscosity was recorded for the negative control (0.40 mPa·s) for the 12% formula and the positive control. The viscosity

values for all formulations met the quality specifications for hair tonics specifically, being less than 5 cP or mPa·s at room temperature (Koralina *et al.*, 2023). Similarly, pH values were lowest for the negative control (4.8) and rose progressively across the 3% formula (4.9), 6% formula (5.0), and 12% formula (5.1), with the highest pH value (5.4) observed in the positive control. The pH test results met the standardization requirements for scalp pH, which range from 4.5 to 6.5 (Muliani *et al.*, 2022).

The Effect of *Magnolia alba* Flower Ethanol Extract Tonic on Rabbit Hair Length

Testing the effectiveness of a tonic made from the ethanolic extract of white champaca flower on rabbit hair growth revealed an increase in hair length across all treatment groups throughout the observation period. Based on descriptive data collected over four weeks, the onset of hair growth was observed in the first week. The positive control group (minoxidil 2%) recorded the greatest increase in length at 0.40 ± 0.10 cm, followed by the 3% champaca extract group and the 6% champaca extract group showed identical figures of 0.27 cm (specifically the 0.27 ± 0.06 cm and 0.27 ± 0.21 cm, respectively), while the 12% champaca extract group

exhibited the slowest growth at 0.13 ± 0.06 cm.

By the second week, the positive control group still led with a length of 0.80 ± 0.26 cm, followed by the 6% champaca extract (0.70 ± 0.36 cm) and the 3% champaca extract (0.63 ± 0.25 cm). The negative control reached 0.50 ± 0.26 cm, while the 12% champaca extract continued to record the lowest figure at 0.30 ± 0.10 cm. Entering the third week, significant acceleration occurred in the 3% champaca extract group, reaching 1.30 ± 0.10 cm nearly matching the positive control group, which measured 1.37 ± 0.51 cm. Meanwhile, the 6% champaca extract reached 1.17 ± 0.15 cm, the negative control reached 0.90 ± 0.26 cm, and the 12% champaca extract measured 0.87 ± 0.12 cm.

At the end of the observation period, the application of champaca extract tonic at a 3% concentration yielded the highest average hair growth by the fourth week (1.97 ± 0.49 cm), a figure that tended to be higher than those of the positive control (1.87 ± 0.71 cm) and the negative control (1.17 ± 0.42 cm). Conversely, higher extract concentrations specifically 6% and 12% showed a trend of reduced effectiveness regarding hair length growth, resulting in averages of 1.57 ± 0.12 cm and 1.23 ± 0.25 cm, respectively. These

findings indicate that the optimal concentration of champaca extract for stimulating hair follicles lies at the lower level (3%).

The Effect of Tonic Treatment and Observation Time on The Hair Length of Rabbits (*Oryctolagus cuniculus*)

The results presented in Table 6, show an F-value of 105.671 with a significance level of $p < 0.001$ ($p < 0.05$). This indicates that the time factor has a highly significant influence on the increase in rabbit hair length. In other words, a statistically significant change in hair length occurred during the observation period from week 0 to week 4 across all test groups. The tonic treatment factor yielded an F-value of 2.966 with a significance value of $p = 0.073$. Since $p > 0.05$, it can be concluded that, regarding the overall main effect, the differences in the administered tonic formulas did not result in statistically significant differences in mean hair length at a 95% confidence level.

Although the descriptive data in Table 5 shows that the 3% extract group exhibited the highest nominal mean value (1.97 cm), inter-subject data variation meant that the differences among the five treatment groups did not reach statistical significance, largely due to the limited number of replicates or test animals used. Statistically, this non-significant result ($p >$

0.05) was influenced by low statistical power resulting from the limited sample size (n). Charan and Kantharia (2013) explain, via the Resource Equation method ($E = N - k$), that when the degrees of freedom for error (E) fall below the minimum value of 10 (indicating the study is underpowered), statistical tests become less sensitive; this creates a risk of False Negatives (Type II Errors) a situation where the actual biological effect of the 3% extract fails to be detected statistically due to high data variance and an insufficient sample size to achieve significance.

The interaction test results between the tonic treatment type and the observation time yielded an F-value of 1.192 with $p > 0.05$ ($p = 0.318$). This indicates the absence of a significant interaction effect between the tonic formula and the duration of observation. The rate of pattern of rabbit hair length increase from week to week showed relatively parallel or similar trends across the treatment groups, with no striking difference in growth patterns at specific time intervals.

Based on the curve patterns in **Figure 1**, all test groups exhibited consistent trends in hair length from the initial observation point (week 0) to the final point (week 4). An analysis of cumulative growth between week 1 and week 4 revealed that the 3% extract group

experienced the most rapid increase in length, with a difference of 1.67 cm between week 1 (0.30 cm) and week 4 (1.97 cm) resulting in a total growth of 1.97 cm from week 0. The positive control group recorded an increase of 1.47 cm (from 0.40 cm at week 1 to 1.87 cm at week 4), its growth rate was stable initially but was surpassed by the 3% extract group in the final week. Meanwhile, the 6% extract group showed an increase of 1.30 cm (from 0.27 cm at week 1 to 1.57 cm at week 4). The 12% extract group recorded an increase of 1.10 cm (from 0.13 cm at week 1 to 1.23 cm at week 4), although growth occurred, the rate of increase was the slowest among the extract treatment groups. Finally, the negative control group showed an increase of only 0.90 cm (from 0.27 cm at week 1 to 1.17 cm at week 4), reflecting the natural physiological elongation rate of rabbit hair in the absence of active substance stimulation.

The observed decrease in stimulatory activity at higher concentrations (6% and 12%) can be explained by several physiological and pharmaceutical mechanism. From a pharmaceutical perspective, the high content of dissolved solids in the extract at a 12% concentration increase the formulation's viscosity and density as shown in the physical evaluation table

thereby hindering the absorption and penetration of active secondary metabolites into the hair follicles. Meanwhile, from a physiological standpoint, excessively high concentrations of bioactive compounds such as flavonoids, phenols, and tannins may trigger receptor saturation in hair matrix cells or cause irritation and local toxicity, ultimately suppressing cell division during the anagen phase (Indrawati, 2025). Thus, the observed trends in Figure 1 reinforce the finding that the administration of white champaca flower ethanol extract does not follow a linear dose-dependent pattern; instead, it exhibits an optimal concentration of 3% for achieving maximum and safe hair growth stimulation (Onikola *et al.*, 2025).

Conclusions

In conclusion, the hair tonic formulation containing 3% ethanol extract of white cempaka flowers (*Magnolia alba*) exhibited satisfactory physical stability, suitable viscosity, and a scalp-compatible pH. In the preclinical rabbit model, the 3% formulation demonstrated optimal hair growth-stimulating efficacy comparable to the positive control, whereas higher extract concentrations showed diminished effectiveness. While these preliminary findings suggest that *Magnolia alba* extract holds promise as a natural hair care constituent, its practical application

remains limited by the scope of this animal study. Consequently, further comprehensive evaluations—including long-term cutaneous safety assessments and human clinical trials—are essential before concluding its therapeutic efficacy and safety for clinical or commercial use.

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