EFFECTIVENESS OF ETHANOLIC EXTRACT OF AVOCADO LEAVES (PERSEA AMERICANA MILL.) AS A HAIR TONIC ON HAIR GROWTH IN MALE RABBITS (ORYCTOLAGUS CUNICULUS)

Indri Dwi Rahasasti[1*], Rosellynia Calyptranti[2], Triani Kurniawati[3], Erik Subastian Hermawan S[4]

[1,2,3,4]*Pharmacy Study Program, University of An Nasher Cirebon (INDONESIA)*

**Corresponding author: rahasasti@gmail.com*

Abstract

Avocado (*Persea Americana Mill*.) is one of the plants that can increase hair growth. Secondary metabolite compounds contained in avocado leaves are flavonoids, alkaloids, saponins, tannins, terpenoids which act as antioxidants that can protect hair from chemical care products, pollution, and damaged hair due to lack of nutrition. This study aims to determine the secondary metabolite compounds contained in avocado leaves (*Persea Americana Mill*.), to determine the difference in hair growth in hair tonic preparations of avocado leaf ethanol extract (*Persea Americana Mill*.) at concentrations of 2%, 4%, 6%, 8% for male rabbits and to determine the effectiveness of hair tonic avocado leaf ethanol extract (Persea Americana) on hair growth in male rabbits (*Oryctolagus cuniculus*). This study is included in experimental research using the Completely Randomized Design (CRD) method. The results showed that there are secondary metabolite compounds contained in avocado leaves, namely flavonoids, saponins, tannin and terpenoid. The experimental animals used in this study consisted of six male rabbits and hair growth measurements were conducted on the 14th day. Statistical analysis showed differences in hair growth in male rabbits (*Oryctolagus cuniculus*) at each concentration of the ethanolic avocado leaf extract hair tonic, namely 6,60mm (2%), 5,90mm (4%), 5,31mm (6%), 5,25mm (8%), 2,50mm (K+) and 6,51mm (K-). The results indicate that the most effective concentration of the ethanolic avocado leaf extract hair tonic for promoting hair growth is 2%.

Keywords: Avocado leaves, secondary metabolites, hair growth.

1. INTRODUCTION

Maintaining healthy and beautiful hair is not easy, as hair often encounters various problems. One of the most common issues is dandruff and hair loss. These problems can be very disruptive and significantly reduce an individual's self-confidence [4]. Hair loss is a condition in which the amount of hair becomes noticeably less due to excessive shedding beyond the normal range, even though thinning may not be immediately visible. Normally, hair sheds at a rate of around 80–120 strands per day [7]. Excessive hair loss beyond this normal range can lead to baldness. A simple way to address hair loss is by treating the hair using hair tonic, which serves as a nutrient source for the hair [1]. Hair tonic is a liquid cosmetic product composed of chemical substances or other ingredients that help strengthen the hair, promote growth, and maintain hair condition. Hair tonic functions to improve blood circulation in the scalp, thereby preventing hair loss, stimulating hair growth, preventing dandruff and itching, and refreshing the scalp [17].

Natural ingredients derived from plants can be used as active compounds in hair tonic formulations, with avocado leaves being one such option due to their potential to nourish the hair and prevent hair

loss. Phytochemical screening has shown that the ethanolic extract of avocado leaves contains flavonoids, alkaloids, saponins, and tannins [14]. Previous studies have mostly focused on avocado fruit and seeds; however, the leaves also contain bioactive compounds with antioxidant, anti-inflammatory, and antimicrobial properties that may contribute to maintaining scalp health and stimulating hair follicle activity [16][18].

The concentration levels of 2%, 4%, 6%, and 8% were selected to observe the dose-dependent response of the extract in promoting hair growth while ensuring safety and optimal efficacy. The mechanism of action is thought to involve flavonoids acting as antioxidants that protect follicular cells from oxidative stress, saponins improving scalp circulation and facilitating nutrient delivery, and tannins providing astringent effects that strengthen hair roots and reduce excessive sebum production [18].

Based on this explanation, the researcher aims to innovate by using avocado leaf extract as an active ingredient in hair tonic for hair growth. This idea is inspired by previous research such as that by Diana & Wahini [5], which used avocado fruit extract and honey as active ingredients in hair tonic for hair loss, and by Pradiningsih & Risnawati [13], who tested the effectiveness of hair tonic made from avocado seed extract (*Persea Americana Mill)* on hair growth in male rabbits. However, research specifically focusing on the use of avocado leaf extract in hair tonic formulations is still very limited and has not yet been explored.

The hypothesis of this study is as follows:

- **(H_0):** There is no difference in hair growth in male rabbits with the administration of hair tonic containing ethanolic extract of avocado leaves (*Persea americana*).
- **(H_1):** There is a difference in hair growth in male rabbits with the administration of hair tonic containing ethanolic extract of avocado leaves (*Persea americana*).

2. METHODOLOGY

This research is a quantitative study with an experimental design using a Completely Randomized Design (CRD) method. The study tests hair tonic formulated with avocado leaf extract (*Persea Americana Mill*) at various concentrations (2%, 4%, 6%, and 8%) to evaluate its effect on hair growth in male rabbits. Two types of control treatments were used: a positive control and a negative control, along with the four concentrations of the avocado leaf extract hair tonic.

This study was conducted in accordance with the ethical guidelines for the use of experimental animals, with consideration for animal welfare principles, and received approval from the Health Research Ethics Committee of the Faculty of Pharmacy, YPIB University, as evidenced by Ethical Approval Number: 173/KEPK/EC/V/2024.

The inclusion criteria for this study were fresh avocado leaves with a green coloration. The exclusion criteria included avocado leaves that were damaged, infested by pests, or brown in color. For the test animals, the inclusion criteria were healthy male rabbits aged 6–7 months, with a body weight of approximately 1 kg. The exclusion criteria for the test animals included rabbits that were ill, younger than 6 months or older than 7 months, weighing less than 1 kg, or that became sick or died during the study.

The initial preparation involved the collection of avocado leaves (*Persea Americana Mill*) from Gelok Mulya Village, Sumberjaya District, Majalengka Regency. The leaves were washed clean, roughly chopped, and air-dried at room temperature until completely dry. The dried leaves were then ground using a blender until a fine powder was obtained [3]. A total of 1000 g of avocado leaf powder

was weighed and placed in a beaker, then soaked in 96% ethanol until completely submerged. The maceration process was carried out for 3 x 24 hours in a closed container, with a ratio of simplicia and 96% ethanol solvent of 1:10, then the mixture was stirred periodically. After the extraction period, the mixture was filtered. To obtain a concentrated extract, the filtrate was evaporated using a rotary evaporator at a temperature of 50°C.

The resulting avocado leaf extract was then subjected to phytochemical screening to identify the presence of chemical constituents such as alkaloids, phenols, flavonoids, saponins, tannins, and steroids or triterpenoids [6].

The next step involved the formulation of a basic hair tonic with avocado leaf extract concentrations of 2%, 4%, 6%, and 8%. The formulation of the avocado leaf extract hair tonic was adapted from the study on ethanol extract of green tea leaves [12].

Table 1. Hair Tonic Formulation Composition

No.	Ingredients	K–	F1	F2	F3	F4
1	Avocado leaf extract	–	2%	4%	6%	8%
2	Propylene glycol	10%	10%	10%	10%	10%
3	Propyl paraben	0.01%	0.01%	0.01%	0.01%	0.01%
4	Methyl paraben	0.02%	0.02%	0.02%	0.02%	0.02%
5	Menthol	0.1%	0.1%	0.1%	0.1%	0.1%
6	Sodium metabisulfite	0.01%	0.01%	0.01%	0.01%	0.01%
7	Ethanol	60 mL	60 mL	60 mL	60 mL	60 mL
8	*Oleum rosae*	q.s.	q.s.	q.s.	q.s.	q.s.
9	Distilled water (*Aqua dest*)	ad 100 mL	ad 100 mL	ad 100 mL	ad 100 mL	ad 100 mL

Notes:

- K– = Negative control (without avocado leaf extract)
- q.s. = *quantum satis* (as much as sufficient)
- ad = to make up to the final volume

The method for making hair tonic with avocado (Persea americana) leaf ethanol extract according to the formulation is as follows:

1. Preparation of Preservative and Solvent Solution: Dissolve propyl paraben and methyl paraben in a small amount of propylene glycol or ethanol for easier dissolution. And then add sodium metabisulfite
2. Dissolve menthol and oleum rosae in ethanol or propylene glycol to ensure proper mixing of water-insoluble components.
3. Hair Tonic Base Preparation: Mix distilled water and propylene glycol in a clean container. Add the preservative, menthol and *Oleum rosae* solutions while stirring gently.
4. Addition of Avocado Leaf Extract: Add the avocado leaf extract according to the formulation concentration. Stir until homogeneous, ensuring no sediment or layer separation occurs. Final adjustment, adjust the final volume with distilled water to reach 100 mL.

The resulting formulations underwent characteristic evaluations, including organoleptic testing, homogeneity, pH stability, specific gravity, and viscosity.

After formulation, a hair growth activity test was conducted using the ethanol extract of avocado leaves on 6 rabbits over a 14-day period [11]. The experimental animals used were rabbits, with their dorsal fur shaved using scissors and a razor. The shaved area was divided into multiple test zones, each measuring approximately 2.5 cm, with a spacing of about 0.5 cm between zones.

Hair tonic was applied once daily to each zone, with a dosage of approximately 1 mL per zone for 14 days. On day 14, hair length measured. Hair weight was determined by shaving the regrown hair from each test zone and weighing it. For hair length measurement, the three longest strands from each zone were selected, placed on black fabric, secured with adhesive tape, and measured from base to tip using a caliper.

3. RESULTS

The study aimed to investigate the activity of hair tonic formulated with ethanol extract of avocado leaves (*Persea Americana Mill*) in male rabbits (*Oryctolagus cuniculus*). The research was conducted through several stages, including plant identification, extraction of avocado leaves (*Persea Americana Mill*), phytochemical screening, formulation of hair tonic, evaluation of the formulation, and assessment of hair growth effectiveness in male rabbits (*Oryctolagus cuniculus*).

3.1 Plant Identification

The identification of avocado leaves was carried out at the MIPA Laboratory, Faculty of Biology, IAIN Syekh Nurjati Cirebon. The results of the identification confirmed that the plant sample used was *Persea Americana Mill.*

3.2 Extraction of Avocado Leaves

Extraction was carried out using the maceration method, with 96% ethanol as the solvent. From 1 kg of powdered avocado leaf (*Persea Americana Mill*) simplicia, 265.23 grams of extract were obtained, yielding an extract percentage of 26.52%.

3.3 Phytochemical Screening of Avocado Leaves

Phytochemical screening of avocado leaf extract (*Persea Americana Mill*) included tests for flavonoids, alkaloids, saponins, tannins, and terpenoids. The results of the phytochemical screening of avocado leaf extract (*Persea Americana Mill*) are presented in Table 2.

Table 2. Results of Phytochemical Screening of Avocado Leaf Extract (*Persea Americana Mill*)

Phytochemical Test	Reagent(s)	Observation	Reference [8]	Result
Flavonoid	Ethanol, Mg, concentrated HCl	Orange-black coloration	Red, yellow, or orange coloration	+
Alkaloid (Mayer)	Mayer reagent	No white precipitate	Formation of white precipitate	–
Alkaloid (Bouchardat)	Bouchardat reagent	No reddish-brown precipitate	Formation of reddish-brown precipitate	–
Alkaloid (Dragendorff)	Dragendorff reagent	No yellow precipitate	Formation of yellow precipitate	–
Saponin	Distilled water, 2N HCl	Formation of stable foam	Formation of stable foam	+

Phytochemical Test	Reagent(s)	Observation	Reference [8]	Result
Tannin	Distilled water, 5% $FeCl_3$	Dark green coloration	Blue or dark green coloration	+
Terpenoid	Chloroform, concentrated H_2SO_4	Dark brown coloration	Reddish-brown coloration	+

Phytochemical screening revealed the presence of flavonoids, saponins, tannins, and terpenoids. In contrast, alkaloid tests using Mayer, Bouchardat, and Dragendorff reagents were negative, as no characteristic precipitates were observed. Literature and current findings suggest several points:

a. Alkaloid production varies among plant species and plant parts. In this study, alkaloids were detected in seeds, while only trace amounts—or none—were found in pulp or leaves [8].
b. Total alkaloid content was 41.5 ± 1.8 mg/g extract, substantially lower than phenolics (184.1 ± 0.6 mg/g) and flavonoids (115.8 ± 2.1 mg/g), indicating a minor contribution of alkaloids to the overall phytochemical profile [10].
c. Secondary metabolite production, including alkaloids, is influenced by factors such as leaf age, growth conditions, season, light, soil, and environmental stress. Plants grown under optimal conditions or without environmental pressure often produce lower amounts of alkaloids, as their biosynthesis is typically an adaptive response to stress [19].

3.4 Evaluation of the Hair Tonic Formulation Containing Ethanol Extract of Avocado Leaves (*Persea Americana*)

The evaluation of the hair tonic formulation included organoleptic testing, specific gravity, pH testing, and viscosity testing.

Figure 1. *Hair Tonic Preparation at concentrations of 8%, 6%, 4%, and 2%*

3.4.1 Organoleptic Test

The results of the organoleptic evaluation of the hair tonic formulation containing ethanol extract of avocado leaves (*Persea Americana*) are presented in Table 3 below:

Table 3. Organoleptic Test Results

Formula	Color	Odor
K+	Clear green	Characteristic of green tea
K–	Clear white	Characteristic of ethanol
F1	Clear orange	Characteristic of *oleum rosae*
F2	Brownish orange	Characteristic of *oleum rosae*
F3	Brown	Characteristic of *oleum rosae* and avocado leaf
F4	Black	Characteristic of avocado leaf

3.4.2 Specific Gravity

The specific gravity values of the preparations obtained ranged from 0.930 to 0.947, which comply with the Indonesian National Standard (SNI) 16-4955-1998. The specific gravity of the hair tonic preparations is less than 1, which corresponds to the specific gravity of water [2]. The data from the specific gravity measurements are presented in Table 4 below:

Table 4. Specific Gravity Test Results

No.	Formula	Specific Gravity (g/mL)
1	K+	0.994
2	K–	0.920
3	F1	0.930
4	F2	0.935
5	F3	0.942
6	F4	0.947

3.4.3 pH Test

The pH test results of the hair tonic preparation containing avocado leaf extract for all formulas showed a pH of 5. This complies with the Indonesian National Standard (SNI) 16-4955-1998, which specifies a pH range of 3 to 7. The pH measurement results of the hair tonic formulations containing avocado leaf (*Persea Americana Mill*) extract are presented in Table 5 below:

Table 5. pH Test Results

No.	Concentration	pH
1	K+	5
2	K–	5
3	F1	5
4	F2	5
5	F3	5
6	F4	5

3.4.4 Viscosity Test

The viscosity measurements of the hair tonic formulations containing ethanol extract of avocado leaves (*Persea Americana*) are shown in Table 6 below:

Table 6. Viscosity Test Results

Formula	Viscosity (cPs)
K+	0.934
K–	1.242
F1	1.207
F2	1.224
F3	1.249
F4	1.448

It can be concluded that the ethanol extract hair tonic preparation from avocado leaves is suitable for use on the scalp, as it falls within an acceptable range and complies with the Indonesian National Standard (SNI) 16-4955-1998, which stipulates that the viscosity value of hair tonic preparations should be less than 5 cPs.

3.4.5 Hair Growth Effectiveness Test

Table 7. Rabbit hair length during treatment

Treatment	Hair Length (mm)			Average (mm)
	1	2	3	
K+	2,90	2,05	2,56	2,50
K-	6,11	6,98	6,43	6,51
F1	6,62	6,20	6,97	6,60
F2	5,98	6,19	5,54	5,90
F3	5,11	5,40	5,43	5,31
F4	5,21	5,32	5,22	5,25

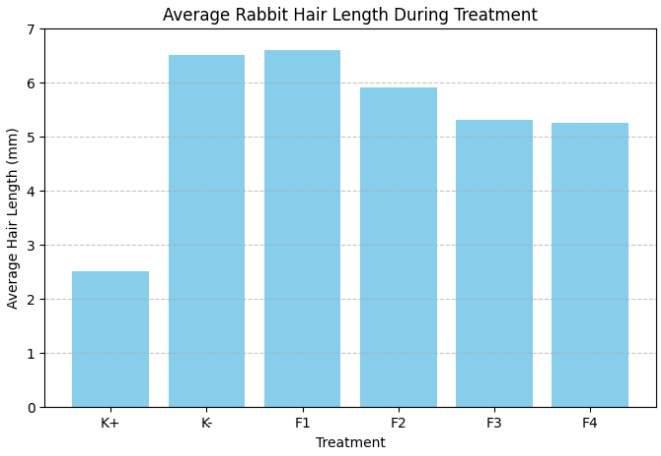

Figure 2. *Average Rabbit Hair Length During Treatment*

Table 8. Data Analysis Results of Hair Growth Effectiveness Test

Statistical Test	Statistic (stat)	p-value	Conclusion
Normality Test (Shapiro-Wilk)			
K+	0.9868	0.7804	Data is normally distributed
K-	0.9772	0.7107	Data is normally distributed
F1	0.9973	0.8999	Data is normally distributed
F2	0.9599	0.6151	Data is normally distributed
F3	0.8196	0.1623	Data is normally distributed
F4	0.8176	0.1572	Data is normally distributed
Homogeneity Test (Levene)	0.7154	0.6239	Variances are homogeneous
One-Way ANOVA	60.7619	0.0000	Significant difference in means

The results of the statistical analysis showed that all treatment groups (K+, K−, F1, F2, F3, F4) met the assumption of normality, as indicated by Shapiro-Wilk test p-values greater than 0.05. Levene's test for homogeneity of variances also yielded a p-value of 0.6239, which is above 0.05, indicating that the variances among groups were homogeneous. Furthermore, the One-Way ANOVA test revealed a highly significant result with a p-value of 0.0000, suggesting that there were statistically significant differences in the mean values between the treatment groups.

Table 9. Tukey HSD Post-hoc Test

Treatment 1	Treatment 2	p-value (p-adj)	Conclusion
F1	F2	0.1867	Not significantly different
F1	F3	0.0053	Significantly different
F1	F4	0.0036	Significantly different
F1	K+	0.0000	Significantly different
F1	K-	0.9993	Not significantly different
F2	F3	0.3208	Not significantly different
F2	F4	0.2320	Not significantly different
F2	K+	0.0000	Significantly different
F2	K-	0.3003	Not significantly different
F3	F4	0.9999	Not significantly different
F3	K+	0.0000	Significantly different
F3	K-	0.0091	Significantly different
F4	K+	0.0000	Significantly different
F4	K-	0.0062	Significantly different
K+	K-	0.0000	Significantly different

These findings indicate that certain treatments, particularly those involving K+ and K-, show distinct effects compared to some fertilizer treatments (F1-F4), while other pairs exhibit similar effects with no significant difference.

The data on rabbit hair length measured over 14 days showed that the negative control exhibited greater hair growth compared to the positive control and the highest extract concentration of 2%. High concentrations of active compounds in the hair tonic may lead to excessive accumulation of secondary metabolites, which can inhibit growth by reducing the optimal absorption of compounds by the hair or follicles, or even cause side effects. In contrast, low concentrations can stimulate the production of secondary metabolites at an optimal level without negative effects [15]. Low concentrations also allow compounds such as flavonoids, saponins, and essential fatty acids from avocado leaves to be absorbed more evenly into the scalp, thereby supporting hair growth.

4. CONCLUSIONS

Based on the results of the study, it can be concluded that avocado leaves (*Persea Americana Mill*) contain secondary metabolites, including flavonoids, saponins, tannins and terpenoid. The evaluation of the hair tonic formulation included organoleptic assessment, specific gravity, pH, and viscosity tests, all of which yielded results that met the required standards. The hypotheisis (H_1) can be accepted, which is there is a difference in hair growth in male rabbits with the administration of hair tonic containing ethanolic extract of avocado leaves (*Persea americana*). Differences in hair growth were observed among male rabbits (*Oryctolagus cuniculus*) treated with various concentrations of ethanol extract-based avocado leaf hair tonic. Hair growth occurred across all treatment groups, with a continuous increase observed up to day 14. The 2% concentration showed the highest average hair growth, reaching 6.60 mm or 0.66 cm.

5. SUGGESTION

a. Recommended for future researchers to conduct further qualitative analysis on avocado leaves, such as thin layer chromatography (TLC) or column chromatography, to detect alkaloids that may be present in very small amounts.
b. Recommended that future researchers perform quantitative analyses on avocado leaf extract to more precisely determine the concentrations of the compounds it contains.
c. Recommended that future researchers combine avocado leaf extract with other natural ingredients that can accelerate hair growth.
d. Recommended for future researchers to create additional concentration variations for each formulation and to extend the treatment duration

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