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Physicochemical and in Vivo Evaluation of Rosemary (*Rosmarinus officinalis* L.) Extract Hair Tonic as a Natural Alternative for Hair Growth

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ABSTRACT

Hair loss is a common condition affecting both men and women and often diminishes quality of life. While synthetic agents like minoxidil are widely used to promote hair growth, their application is frequently associated with adverse side effects, highlighting the need for safer natural alternatives. This study aimed to evaluate the effectiveness of rosemary (*Rosmarinus officinalis* L.) extract in hair tonic preparations on hair growth and the product's physicochemical characteristics. Rosemary essential oil was extracted using steam distillation, then formulated into hair tonic at various concentrations (0%, 5%, 10%, and 15%), and compared with 2% minoxidil as a positive control. Physicochemical characterization included pH measurement, viscosity, and compound analysis using GC-MS. Hair growth activity was tested in vivo using male Wistar rats for 4 weeks, with hair length and weight as parameters. The results showed that all formulations had a pH of 4.18–4.75, corresponding to the physiological pH of the scalp. Viscosity increased with increasing rosemary extract concentration. GC-MS analysis revealed key compounds such as β -myrcene, 1,8-cineole, and geraniol, which contribute to biological activity. In vivo testing showed that the 15% formulation produced the highest hair length (16.73 ± 1.97 mm), surpassing 2% minoxidil (10.02 ± 1.83 mm), while the 10% concentration produced the highest hair weight (87.083 ± 30.870 mg/cm²). No signs of irritation were observed in the rosemary group, whereas the minoxidil group showed skin side effects. It can be concluded that rosemary extract has the potential to be a natural active ingredient in an effective and safe hair tonic preparation to stimulate hair growth. A 10% concentration is recommended as the optimal formulation based on the balance between hair length and quality produced.

Contribution to Sustainable Development Goals (SDGs):

SDG 3 – Good Health and Well-being

SDG 12 – Responsible Consumption and Production

1. INTRODUCTION

Hair is an important component of the human body from both aesthetic and physiological perspectives. Healthy, strong, and well-maintained hair contributes to an individual's appearance while also protecting the scalp from environmental exposure.



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Structurally, hair is a protein filament primarily composed of keratin produced by keratinocytes within the hair follicle [1]. Hair growth follows a dynamic biological cycle comprising the anagen (active growth), catagen (transition), telogen (resting), and exogen (shedding) phases. The balance among these phases plays a critical role in maintaining normal hair growth, whereas disruption of the cycle may result in excessive hair loss, progressive thinning, and, in severe cases, baldness. Multiple

factors influence hair loss, including genetic predisposition, hormonal changes, nutritional status, psychological stress, inappropriate use of hair-care products, and certain medical conditions [2]. Beyond its physical manifestations, hair loss may also negatively affect psychological well-being and self-confidence [3-5].

Topical hair-growth preparations, including hair tonics, are commonly used to manage hair loss and support hair growth. Hair tonics are generally formulated as liquid preparations that are easy to apply, leave minimal residue, and help distribute active ingredients across the scalp. Among the pharmacological agents used for hair-growth stimulation, minoxidil is one of the most widely recognized. Despite its demonstrated effectiveness, prolonged topical minoxidil use may be associated with adverse effects, including scalp irritation, allergic reactions, and contact dermatitis [6]. These limitations have increased interest in plant-derived active ingredients that may offer effective and potentially safer alternatives for supporting hair growth.

Rosemary (*Rosmarinus officinalis* L.) has emerged as a promising botanical source for hair-care applications because of its diverse bioactive constituents. Rosemary contains compounds such as 1,8-cineole, camphor, α -pinene, verbenone, and borneol, which have been associated with antioxidant and antimicrobial activities and may contribute to biological mechanisms involved in hair-growth stimulation [7]. Previous studies have reported the potential of rosemary-derived preparations to promote hair growth and influence hair-follicle activity [8]. These findings provide a scientific basis for further investigation of rosemary as an active ingredient in topical formulations intended to support hair growth.

However, the biological activity of a plant extract alone does not necessarily ensure the effectiveness of the final cosmetic formulation. The performance of a topical preparation is also influenced by its physicochemical characteristics, including pH, viscosity, homogeneity, organoleptic properties, and physical stability. These parameters may affect product stability, application characteristics, active-ingredient delivery, and ultimately biological performance. Despite the growing interest in rosemary for hair-care applications, studies integrating the physicochemical properties of rosemary-containing hair tonic formulations with their *in vivo* hair-growth activity remain limited. Consequently, a comprehensive evaluation that links formulation quality with biological effectiveness is needed to establish the potential of rosemary extract as an active ingredient in hair-growth products.

Therefore, this study aimed to formulate a hair tonic containing rosemary extract and comprehensively evaluate its physicochemical characteristics and *in vivo* hair-growth activity using Wistar rats. The physicochemical evaluation included relevant quality and stability parameters, while the *in vivo* assessment examined hair-growth responses using parameters such as hair length, hair weight, and hair-follicle characteristics. We evaluated different concentrations of rosemary extract to determine whether the biological response was concentration-dependent, and compared its effectiveness with minoxidil as a reference treatment. By integrating formulation development, physicochemical characterization, and *in vivo* biological evaluation within a single experimental framework, this study provides a more comprehensive assessment of rosemary extract hair tonic and its potential as a natural alternative for hair-growth stimulation.

2. RESEARCH METHOD

2.1. Materials and Equipment

The main materials used in this study were rosemary leaves and twigs (*Rosmarinus officinalis* L.) obtained from Gelagah, Alahan Panjang, Solok Regency, West Sumatra. The chemicals used included 96% ethanol, propylene glycol, ascorbic acid, menthol, and distilled water. Minoxidil 2% served as a positive control, while depilatory cream was used to prepare the application area on the test animals. The test animals were 15 male Wistar rats (*Rattus norvegicus*), approximately two months old and weighing 150–200 g. The animals were kept on standard commercial feed (Rat Bio®) and provided with drinking water *ad libitum*.

The equipment used included a steam distillation apparatus, GC-MS (Gas Chromatography–Mass Spectrometry), a digital rotational viscometer, a digital pH meter, an analytical balance, and standard laboratory glassware.

2.2. Research Design and Data Analysis

This study used a completely randomized design with five treatments and three replications. The treatments varied the concentration of rosemary extract in the hair tonic: 0%, 5%, 10%, and 15%, with 2% minoxidil as a positive control. Physicochemical characteristics, including pH and viscosity, were analyzed descriptively. Meanwhile, hair growth data, including hair length and weight, were analyzed using analysis of variance (ANOVA) at a 95% confidence level. Further testing was conducted when significant differences were found between treatments.

2.3. Ethical Approval

All experimental procedures involving animals were reviewed and approved by the Health Research Ethics Committee (KEPK) of Universitas Perintis Indonesia (Ethical Approval No. 1656/KEPK.F1/ETIK/2026; Protocol No. 26-05-2035). All animal handling and experimental procedures were conducted in accordance with the committee's ethical standards and the CIOMS-WHO 2016 guidelines for ethical conduct in health research.

2.4. Experimental Procedures

Fresh rosemary (*Rosmarinus officinalis* L.) leaves and twigs were sorted to remove dirt and damaged parts. They were then washed under running water and drained until no free water remained on the surface [9]. The samples were cut into approximately 2–3 cm pieces to increase the contact surface area during extraction. We then placed approximately 2 kg of fresh material in a distillation kettle.

We extracted essential oil by steam distillation for approximately 3–4 hours, starting when the first distillate appeared, while maintaining the temperature near 100°C. During the process, steam released and transported volatile compounds, which were then condensed into liquid form. The collected distillate, consisting of essential oil and hydrosol, was separated using a separatory funnel to obtain the oil fraction. The essential oil was dried over anhydrous sodium sulfate to remove residual water. The final product was weighed to determine the extraction

yield (% w/w based on the initial material) and stored in a dark glass vial at 4–10°C until further analysis.

The hair tonic formulation was prepared at a total volume of 100 mL for each treatment. Ascorbic acid (0.1 g) was dissolved in 5 mL of distilled water using magnetic stirring for approximately 5 minutes until completely dissolved. Next, 25 mL of 96% ethanol was added, followed by 0.2 g of menthol, and the mixture was stirred until the components fully dissolved. Propylene glycol (15 mL) was then incorporated as a cosolvent and humectant. Rosemary essential oil was added according to the treatment concentrations (0%, 5%, 10%, and 15% v/v), followed by distilled water to a final volume of 100 mL. The mixture was homogenized using a magnetic stirrer for approximately 10 minutes at room temperature ($27 \pm 2^\circ\text{C}$). The resulting preparation was filtered through a cotton cloth to remove insoluble particles, packaged in dark glass bottles, and stored at room temperature under light-protected conditions [10].

We evaluated the physicochemical properties immediately after formulation. We determined pH using a digital pH meter calibrated with standard buffer solutions at pH 4.0 and 7.0. We immersed the electrode in 20 mL of the sample and recorded the measurement after obtaining a stable reading. Viscosity was determined using a rotational viscometer equipped with a suitable spindle (e.g., spindle no. 2) operating at 60 rpm under controlled temperature conditions ($25 \pm 1^\circ\text{C}$). The results were expressed in centipoise (cP) [11].

The chemical profile of the rosemary essential oil was analyzed using gas chromatography–mass spectrometry (GC–MS). Before analysis, the sample was diluted with n-hexane at a 1:10 ratio, and 1 μL of the diluted solution was injected into the instrument. We used a capillary column (e.g., HP-5MS) for separation. The oven temperature was initially set to 50°C and held for 2 minutes, then increased gradually to 250°C at $5^\circ\text{C}/\text{min}$. Helium was used as the carrier gas at a constant flow rate of 1 mL/min. Compounds were identified by comparing their mass spectra with those in the NIST library and matching their retention times [12].

Hair growth activity was evaluated in vivo using male Wistar rats approximately 2 months old and weighing 150–200 g. The animals were acclimatized for 7 days under laboratory conditions and provided with food and water ad libitum. Following acclimatization, the rats were randomly assigned to five treatment groups, with three rats in each group. Before treatment, the dorsal area of each rat was shaved to approximately $4 \times 4 \text{ cm}^2$. Remaining hair in the test area was removed with a depilatory cream for 3–5 minutes and then rinsed until the skin was hair-free. We marked the test area to ensure consistent application throughout the study. Animals rested for 24 hours before treatment began.

We applied the rosemary essential oil hair tonic topically at 1 mL/day for 28 days. The treatment groups consisted of a negative control (0% rosemary essential oil) and formulations containing 5%, 10%, and 15% rosemary essential oil. We included a 2% minoxidil formulation as a positive control. Minoxidil is a topical agent widely used in hair-growth therapy and is reported to promote hair growth by shortening the telogen phase, prolonging the anagen phase, and increasing hair follicle size. In the present study, we applied minoxidil at the same dose and frequency as the test formulations, namely 1 mL/day.

We evaluated hair growth on days 7, 14, 21, and 28. Hair length was determined by measuring the 10 longest hairs

collected from the test area using a caliper, with the mean value used for analysis. On day 28, hair weight was determined by shaving the hair within the test area, weighing the collected hair, and expressing the result as mg/cm^2 . Throughout the observation period, the skin condition at the application site was visually examined for signs of irritation, including redness, dryness, and peeling. This assessment was included because topical minoxidil has been reported to cause local adverse effects [13].

3. RESULT AND DISCUSSION

3.1. Physico-Chemical Characteristics of Rosemary-Based Hair Tonic

Physico-chemical characterisation was conducted to evaluate the quality of the resulting hair tonic preparations, including pH, viscosity, and active compound profiles using GC–MS. These parameters play a crucial role in determining the stability, safety, and effectiveness of the preparations on the scalp. The pH measurement results presented in Table 1 show that all hair tonic formulations with added rosemary extract had pH values in the range of 4.18–4.75, while the positive control (minoxidil 2%) had a pH of 7.73. The pH values of the rosemary-based formulations were within the physiological scalp pH range (4.0–5.5), making them safe for topical use.

Table 1. pH Measurement Results of Hair Tonic

Treatments	pH value $\pm$ SD
A	4.75 ± 0.080
B	4.44 ± 0.072
C	4.25 ± 0.025
D	4.18 ± 0.012
E	7.73 ± 0.064

pH decreased as rosemary extract concentration increased. The formulation with the highest extract concentration (15%) showed the lowest pH. This is related to the phenolic compounds and organic acids in rosemary, such as rosmarinic acid and caffeic acid. These are acidic and contribute to a decrease in the preparation's pH. This pattern is consistent with other herbal extract-based formulations, where increasing the extract concentration leads to an increase in the acidity of the system [14].

Compared to commercial products, the pH value of the preparation remains within the acceptable range, indicating that the addition of rosemary extract does not cause extreme changes to the system's pH balance. This is important to maintain compatibility with the scalp and minimize potential irritation. The viscosity measurements presented in Table 2 show that viscosity increases with increasing rosemary extract concentration. Viscosity increased from 2.63 cP in the formulation without extract to 8.63 cP in the formulation with a 15% concentration. The positive control had a lower viscosity of 1.23 cP.

Table 2. Viscosity Measurement Results of Hair Tonic

Treatments	Viscosity (cP) $\pm$ SD
A	2.63 ± 0.153
B	6.77 ± 0.115
C	7.00 ± 0.173
D	8.63 ± 0.058
E	1.23 ± 0.152

This increase in viscosity indicates an interaction between the active components in the rosemary extract and the system's liquid phase. Compounds such as rosmarinic acid and carnosic acid can interact with water molecules and other formulation components, increasing flow resistance and viscosity. Functionally, increasing viscosity can extend the contact time of the preparation with the scalp, potentially enhancing the effectiveness of the active ingredients. However, excessively high viscosity must also be controlled to avoid compromising application comfort [15].

The active compound profile obtained through GC-MS analysis is shown in Table 3, which shows that rosemary essential oil contains several main components, namely β -myrcene (17.18%), 1,8-cineole (16.58%), and geraniol/nerol (6.43%). Additionally, other compounds such as linalool, camphene, camphor, borneol, and α -terpineol were identified. The composition of these compounds is consistent with the chemical profile of rosemary reported in the literature. β -myrcene and geraniol act as anti-inflammatory agents that help maintain healthy hair follicles, while 1,8-cineole contributes to improving blood circulation in the scalp. Linalool and camphene exhibit antimicrobial activity that can inhibit the growth of microorganisms that cause scalp disorders. Furthermore, α -terpineol acts as an antioxidant, protecting follicles from free radical damage [16].

Table 3. GC-MS Analysis Results of Rosemary Essential Oil as Hair

Tonic Active Ingredient		
Compounds	Ret. Time	Area %
β -Myrcene	7.982	17.18
Camphene	8.349	3.88
Sabinene	9.213	3.35
1,8-Cineole	11.168	16.58
γ -Terpinene	11.848	1.45
α -Carene	12.734	1.33
Linalool	13.302	3.98
Tetramethylcyclopentadiene	13.924	1.20
Camphor	14.758	3.57
Isoborneol / Fenchol	15.681	5.71
α -Terpineol	16.471	2.11
Borneol	16.754	4.30
Pinocarvone / Camphorone	16.871	4.82
Geraniol / Nerol	18.137	6.43
Isobornyl acetate	19.018	3.69
Geranyl acetate	21.591	1.03
β -Caryophyllene	22.907	4.54
Caryophyllene oxide	27.103	1.06
Patchouli alcohol (Pogostol)	29.306	1.04

The combination of these compounds' biological activities suggests a potential synergistic effect in supporting hair growth. Thus, the GC-MS analysis results not only confirm the quality of the essential oils used but also strengthen the scientific basis for using rosemary extract as an active ingredient in hair tonic preparations. Overall, the physicochemical characterisation results indicate that the rosemary extract-based hair tonic formulation has properties suitable for topical application and has the potential to provide functional effects in supporting hair health and growth.

3.2. Rosemary Extract Hair Tonic Activity Test on Hair Growth (In Vivo)

Hair growth activity was evaluated in vivo using mouse hair length and weight parameters. The results of hair length measurements over 4 weeks are presented graphically in Figure 4, while hair weight at the end of the observation period is presented in Table 4.

According to Figure 4 (a-d), all treatment groups showed an increase in hair length over time, with clear differences between treatments. The negative control group (0%) showed the lowest growth throughout the observation period, while the rosemary extract treatment group showed a more rapid increase in hair length from the first week. The 15% concentration produced the highest hair growth through the end of the observation period, followed by the 10% and 5% concentrations. These results indicate that increasing the concentration of rosemary extract is directly proportional to increased hair growth. The positive control group (2% minoxidil) also showed increased hair length, but the value was still lower than that of the 15% rosemary formulation. Furthermore, changes in skin condition were observed in the minoxidil group at the application site, including dry, scaly skin and the formation of a hard crust, as shown in Figure 5. These conditions began to appear at the end of the second week and became more pronounced in the third week, prompting minoxidil application to be temporarily discontinued to prevent further damage to the test animals.

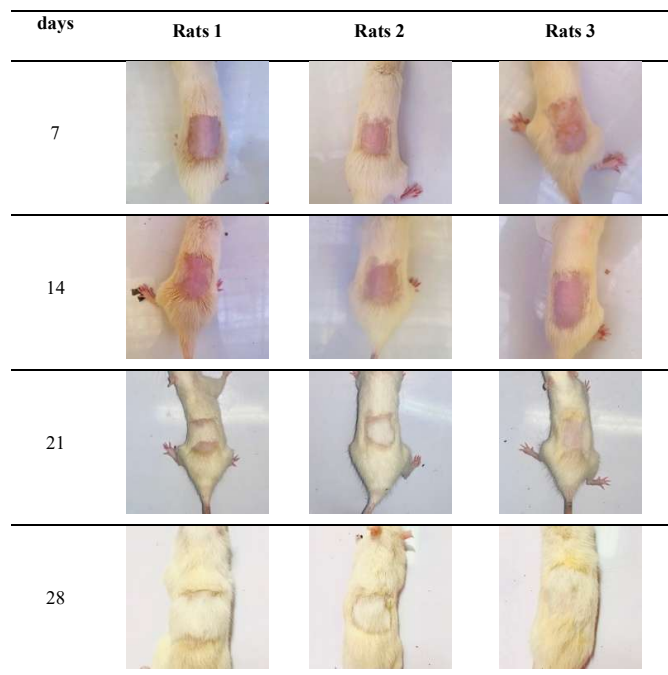


Figure 4a. Photograph of Hair Growth in Rats Treated with 0% of rosemary

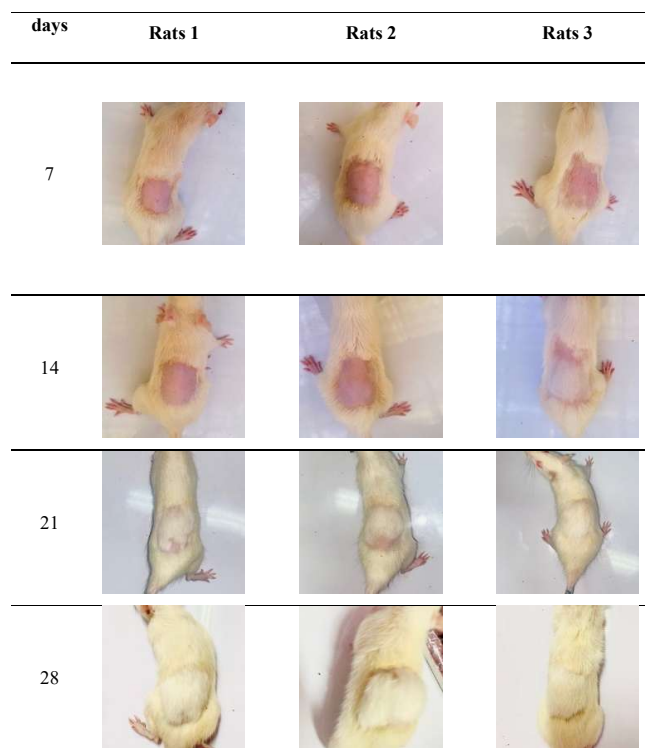


Figure 4b. Photograph of Hair Growth in Rats Treated with 5% of rosemary

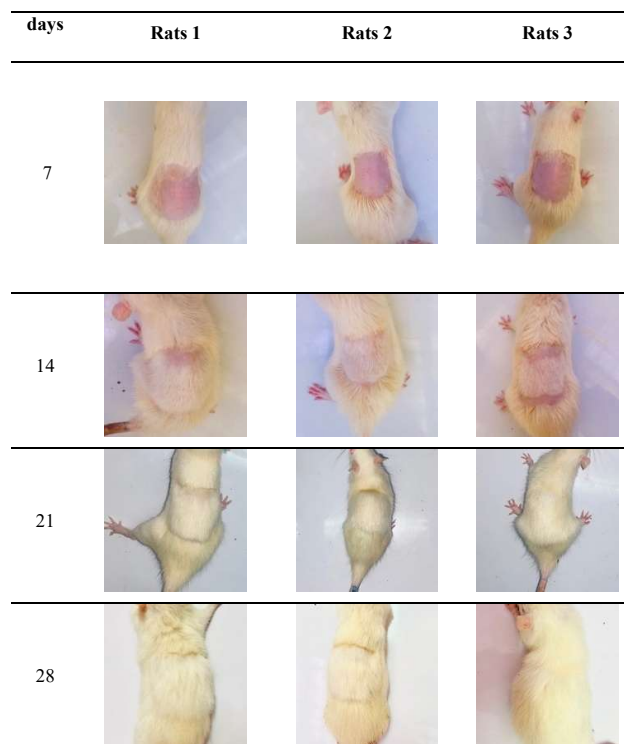


Figure 4c. Photograph of Hair Growth in Rats Treated with 10% of rosemary

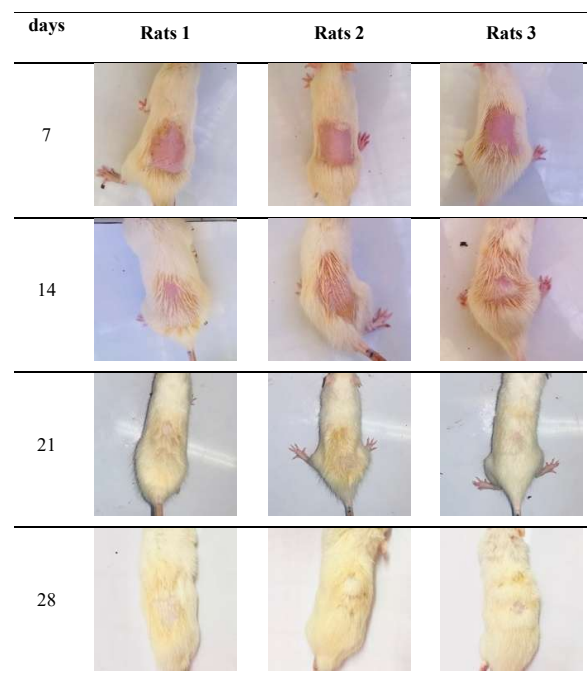


Figure 4d. Photograph of Hair Growth in Rats Treated with 15% Rosemary

The visual findings in Figure 5 indicate that topical minoxidil application can trigger local skin irritation, characterized by dryness and peeling at the treatment site. This reaction likely results from the irritating effects of the active ingredients and solvents in the minoxidil formulation. This can disrupt the epidermal layer. This condition aligns with previous reports that topical minoxidil can cause irritation, redness, dry skin, and peeling at the application site. These side effects can reduce comfort and are a drawback of minoxidil as a hair growth agent. Conversely, no signs of skin irritation were observed in the rosemary extract treatment group during the observation period. The absence of changes in skin morphology indicates that the rosemary-based hair tonic preparation is well tolerated by the test animals' skin. This is thought to be related to the active compounds in rosemary, such as rosmarinic acid and carnosic acid. These have anti-inflammatory and antioxidant activities. Thus protecting skin tissue from oxidative stress and local inflammation [17].

In addition to being safe, rosemary extract also demonstrates good effectiveness in stimulating hair growth. Active compounds such as flavonoids and essential oils are thought to increase blood circulation around hair follicles, accelerating the transition from the telogen to anagen phase, and stimulating follicular activity. Therefore, the increased hair growth at 10% and 15% concentrations indicates that rosemary extract has strong biological potential as a natural hair growth agent.

In addition to hair length, the hair weight results in Table 4 show that the 10% concentration produced the highest hair weight. The 15% concentration produced the highest hair length. This difference indicates that hair length does not always correlate with hair weight, as weight is also influenced by hair shaft thickness and hair density in the growing area. Shorter but thicker hair can produce greater weight than longer but finer hair [18-21].

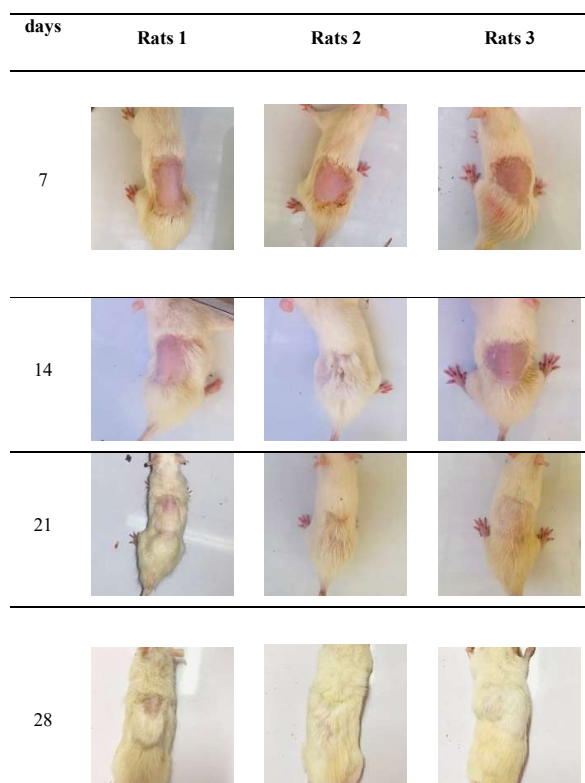


Figure 5. Photographs of Hair Growth in Rats Treated with 2% Minoxidil

Table 4. Average Hair Length of Rats				
Treatments	Average Hair Length (mm) $\pm$ SD			
	Week 1	Week 2	Week 3	Week 4
A (0%)	0 ± 0	0.463 ± 0.803	2.363 ± 0.623	4.250 ± 0.954
B (5%)	2.173 ± 0.512	3.177 ± 0.685	6.533 ± 0.859	8.357 ± 1.359
C (10%)	2.987 ± 0.851	4.443 ± 0.655	9.723 ± 0.964	13.253 ± 2.125
D (15%)	7.520 ± 2.215	9.857 ± 0.931	14.300 ± 2.004	16.737 ± 1.975
Minoxidil 2%	3.023 ± 1.391	5.170 ± 1.481	6.017 ± 2.603	10.027 ± 1.832

Overall, the in vivo test results showed that rosemary extract in hair tonic preparations significantly increased hair growth with a good safety profile. A 15% concentration effectively increased hair length, while a 10% concentration was more optimal in increasing hair weight. Furthermore, the absence of irritation in the rosemary group compared to the minoxidil group suggests that rosemary extract has the potential to be a safer alternative natural active ingredient for hair growth preparations.

4. CONCLUSION

Based on the results of the research, it can be concluded that adding rosemary extract to hair tonic preparations significantly affected hair growth in mice in vivo. Increasing the rosemary extract concentration from 0% to 15% showed a trend toward increased hair length. The 15% concentration produced the highest hair length in the fourth week. These results even surpassed the positive control of 2% minoxidil, demonstrating the effectiveness of rosemary extract as a hair growth agent. However, the 10% formulation performed optimally in terms of

hair quality. This was demonstrated by the highest hair weight value, indicating thicker and stronger hair compared to the other treatments. The difference between hair length and weight indicates that hair growth parameters are determined not only by length but also by hair thickness and density. In terms of physicochemical characteristics, all rosemary-based hair tonic formulations had pH values within the physiological scalp pH range, making them safe for topical use. Furthermore, no signs of irritation were observed in the rosemary treatment group during in vivo testing. Unlike the positive control, which showed signs of skin side effects. Overall, rosemary extract has the potential to be a natural active ingredient in an effective and safe hair tonic formulation to support hair growth.

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