**Rosmarinus officinalis** Essential Oil Increases Hair Length and Follicle Diameter of Ultraviolet B-exposed Mice Through VEGF

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**Abstract**

**BACKGROUND:** Ultraviolet (UVB) radiation induces hair photoaging by triggering oxidative stress, causing alterations in hair diameter and length, which contribute to the development of senile alopecia. Development of alternative anti-photoaging agents to prevent hair loss from various materials, including natural products, is currently being explored. *Rosmarinus officinalis* essential oil (ROEO) has been known to have antioxidant and vasodilation properties. However, the effect and mechanism of ROEO on UVB-exposed hair are still unclear. Therefore, this study was conducted to evaluate the effects of ROEO on the hair length and follicle diameter in UVB-exposed mice as well as the skin vascular endothelial growth factor (VEGF) level.

**METHODS:** Thirty male Swiss mice were treated topically with/without paraffin oil, 2% minoxidil, or various concentrations of ROEO, every day for 21 days. Meanwhile, UVB exposure was performed 3×/week. On day 21, the hair length was measured, the skin tissue was collected for hair follicle diameter and VEGF measurements.

**RESULTS:** ROEO contained phenolic, including flavonoids and tannins, as well as non-phenolic antioxidants, including 1.8-cineole, α-pinene, and camphor. The IC₅₀ value of ROEO was 15.977 ppm. Significant higher hair length, follicle diameter, and VEGF level of 10% ROEO+UVB-treated mice were observed, compared with the ones of mice exposed with UVB merely (LSD test, \( p<0.05 \)).

**CONCLUSION:** Since 10% ROEO could significantly increase hair length, follicle diameter and VEGF level, and contained antioxidant compounds, it can be suggested that ROEO might increase hair length, follicle diameter, and VEGF level through its antioxidant component.

**KEYWORDS:** *Rosmarinus officinalis*, hair follicle diameter, hair length, VEGF, ultraviolet B, antioxidant

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**Introduction**

Hair plays an essential role in an individual’s feeling of attractiveness and appearance.(1) However, like other organs, the hair also aged.(2) UVB exposure is one of the factors that causes hair aging. UVB induces reactive oxygen species (ROS) formation, causing photo-oxidation of lipid and aromatic amino acids, which leads to photochemical degradation of hair proteins, thereby accelerating hair aging.(3,4) This UVB-induced hair photoaging, a premature and accelerated aging process (5), causes alterations in hair diameter and length, which contribute to the development of senile alopecia.(6)

A decrease in dermal vasculature is one of phenomena occurring in photoaged skin.(7) This phenomenon reduces blood supply to the hair follicle, which in turn affects the hair size and contributes to hair loss.(8) Vascular endothelial
growth factor (VEGF), a crucial mediator in angiogenesis, plays an important role in controlling hair growth and follicle size by improving follicle vascularization. VEGF overexpression has been reported to increase hair growth and follicle size in mice.\(^9\)

Synthetic drugs, such as minoxidil, are commonly used to promote hair growth and manage hair loss. Despite being approved by the Food and Drug Administration (FDA), minoxidil causes various adverse effects, such as irritant and allergic contact dermatitis, as well as hypertrichosis.\(^10\) Another technique used to treat hair loss is scalp microneedling. However, this minimally invasive technique could also cause brief discomfort and moderate erythema.\(^11\)

Development of alternative anti-photoaging agents to prevent hair loss from various materials, including natural products, is still currently being explored. Natural products have been known to have antioxidant properties and fewer adverse effects.\(^12\) Many products derived from natural sources, including Pandanus conoideus (13) and Lactobacillus reuteri (14) have received attention for its abilities in attenuating UVB-induced photoaging. Rosmarinus officinalis essential oil (ROEO) exhibits antioxidant properties by absorbing UVB radiation and neutralizing reactive oxygen species (ROS), which prevents lipids and amino acid peroxidation, thus protecting cells from oxidative damage.\(^15\) In addition, due to its vasodilation properties, ROEO has been suggested to enhance blood circulation in the hair follicle (16,17). Hence, ROEO has the potential to be used as an anti-photoaging agent. However, the effect and mechanism of ROEO on UVB-exposed hair are still unclear. This study was conducted to evaluate the effects of ROEO on the hair length and follicle diameter in UVB-exposed mice as well as the skin VEGF level.

### Methods

#### Phytochemical analyses of ROEO

ROEO was obtained from PT. Sare Sumber Rezeki, Jakarta, Indonesia. According to the Certificate of Analysis (CoA) provided by the company, ROEO had a specific gravity, refractive index, and optical rotation of 0.914, 1.466, and -2.23\(^\circ\), respectively. Gas chromatography–mass spectrometry results in the CoA showed that 1.8-cineole, alpha (\(\alpha\))-pinene, and camphor content of ROEO were 43.84%, 13.25%, and 13.06%, respectively.

Antioxidant activity, half-maximal inhibitory concentration (IC\(_{50}\)), and antioxidant activity index (AAI) were determined with 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Meanwhile, total phenolic content (TPC), total flavonoid content (TFC), and total tannin content (TTC) were determined with Folin-Ciocalteu, AlCl3, and Folin-Denis methods, respectively.

### Animals and Treatment Groups

Thirty male Swiss mice aged 2.5–3 months, weighing approximately 20–25 g, and free from hair and skin diseases were selected in this study. The mice were acclimated for seven days. During acclimatization, the mice were given standard food and water ad libitum, housed in 12 h light/dark cycles, temperature range of 23±2°C, and humidity level of 35-60%. Ethical clearance for this study has been obtained from the Animal Ethics Committee of Universitas Udayana (No.: B/174/UN14.2.9/PT.01.04/2022).

The mice were divided into six groups with five mice in each group. Mice in the sham group were not exposed to UVB, while those in the negative control (NC) group were exposed to UVB. Mice in both sham and NC groups were given paraffin oil merely. Meanwhile, mice in the positive control (PC) group were exposed to UVB and treated topically with 2% minoxidil (Surya Dermo Medica Laboratories, Surabaya, Indonesia). The other groups were exposed to UVB and treated topically with either 3, 5, or 10% ROEO in paraffin oil. One hundred \(\mu\)L of the topical treatment was applied once daily for 21 days.

### UVB Exposure

The hairs in the dorsal area (3 cm \(\times\) 2 cm) of the mice were shaved. Paraffin oil, 2% minoxidil, or 3, 5, or 10% ROEO was applied topically to the shaved areas 30 minutes before UVB exposure. Each mouse in all groups, except the sham group, were exposed to UVB three times per week at a dose of 65 mJ/cm\(^2\) for 65 seconds. Therefore, the total radiation given was 585 mJ/cm\(^2\). UVB lamp (Kernel Medical Equipment, Xuzhou, China) was positioned 15 cm above the shaved area. On day 21, the mice were euthanized with intramuscular injection of 20 mg/kg of body weight (BW) xylazine (Interchemie, CG Venray, Netherlands) and 50 mg/kg BW ketamine (Guardian Pharmatama, Bogor, Indonesia). The hair length was then measured and the dorsal skin tissues at the shaved area were collected.

### Measurement of Hair Length

Ten dorsal hair shafts were plucked from the shaved area in each mouse. Hair length was measured from the hair follicle base to the hair shaft tip using a digital caliper with a sensitivity of up to 0.1 mm.
Histopathological Examination
Mice's skin tissues were fixed in formalin, dehydrated with graded ethanol and xylol, and embedded in paraffin block. The blocks were sliced into 6-8 μm-thick histological sections. These sections were then stained with hematoxylin (Catalog No.: #3801570, Leica, Deer Park, IL, USA) and eosin (Catalog No.: #3801616, Leica) (H&E), and observed under 100× magnification using an Olympus CX41 microscope (Olympus, Tokyo, Japan). Photomicrography was done using an Optilab Pro camera (Miconos, Sleman, Indonesia). Three fields of view were examined for each sample. The diameter of the largest hair follicle in each field was measured using OptiLab Viewer Plus 2.2 (Miconos) and Image Raster (Miconos) software.

Measurement of VEGF Level
Freshly collected mice skin tissues were stored in phosphate buffer saline at -20°C for 24 hours. Skin tissues were then homogenized with an ultrasonic homogenizer, and the homogenate was centrifuged for 10 minutes at 12,000 rpm. VEGF level in the resulting supernatant was measured using the BT LAB Mouse Vascular Endothelial Cell Growth Factor A (VEGF-A) ELISA Kit (Catalog No.: E1184Mo, Shanghai Korain Biotech, Jiaxing, China) according to the manufacturer’s instructions. Absorbance of the samples was read at 450 nm. The sensitivity and detection range of the kit were 1.32 ng/L and 2–600 ng/L, respectively.

Statistical Analysis
Statistical analysis was performed using SPSS for Windows version 23.0 (IBM, Armonk, NY, USA). Shapiro-Wilk test was used to determine data distribution, while one-way analysis of variance (ANOVA) followed by least significant difference (LSD) post hoc test was employed to determine statistical significance, which was defined as \( p \leq 0.05 \).

Results of Phytochemical Analyses
Based on the results of DPPH assay, the antioxidant activity, IC\(_{50}\), and AAI of ROEO were 42.46 mg of gallic acid equivalent antioxidant capacity (GAEAC)/L, 15.977 ppm, and 0.0025, respectively. TPC, TFC, and TTC of ROEO...
were 90.42 mg of gallic acid equivalent (GAE)/100 g, 256.30 mg of quercetin equivalent (QE)/100 g, and 53.09 mg of tannic acid equivalent (TAE)/100 g, respectively.

**ROEO Increased Hair Length of UVB-exposed Mice**

When compared with the sham group (Figure 1A), NC group showed delayed hair growth upon UVB exposure (Figure 1B). Treatment of minoxidil (Figure 1C) or ROEO in various concentrations (Figure 1D-F) promoted hair growth in PC and ROEO groups, respectively. The 10% ROEO groups showed better hair growth compared with the sham group (Figure 1F).

The average hair length of the NC group (5.64±0.66 mm) was significantly lower than the one of the sham group (7.57±0.42 mm). No significant difference was observed between the average hair length of the 3% ROEO group (5.88±0.40 mm) and the one of the NC group. The average hair length of the 5 (7.13±1.42 mm) and 10% ROEO (10.50±1.23 mm) groups were significantly higher than the one of the NC group. In addition, the average hair length of the 10% ROEO group was significantly higher when compared with the one of the PC group (7.15±1.42 mm) (Figure 1G).

**ROEO Increased Hair Follicle Diameter of UVB-exposed Mice**

The average hair follicle diameter of the NC group (66.72±3.08 μm) was significantly lower than the one of the sham group (74.35±4.93 μm). No significant difference was observed between the average hair follicle diameter of the 3% ROEO group (69.01±4.74 μm) and the one of the NC group. The average hair follicle diameter of the 5 (74.61±4.86 μm) and 10% ROEO (82.41±9.20 μm) groups were significantly higher than the one of the NC group. No significant difference was observed between the average hair follicle diameter of the 5% ROEO group and the one of the PC group (74.26±5.10 μm). Moreover, the average hair follicle diameter of the 10% ROEO group was significantly higher when compared with the one of the PC group (Figure 2).

*Figure 2. Skin histopathological appearance of UVB-exposed mice.* Mice were treated topically with/without paraffin oil, 2% minoxidil, or various concentrations of ROEO, and exposed with UVB in the timeframe as described in Methods. Mice skin tissues were collected, stained with H&E staining, and observed under a microscope. A: sham group; B: NC group; C: PC group; D: 3% ROEO group; E: 5% ROEO group; F: 10% ROEO group. Yellow bar: 75 μm, red arrow: hair follicle. G: Average hair follicle diameter of each group. *p<0.05 vs. sham group; †p<0.05 vs. NC group; ‡p<0.05 vs. PC group, LSD test. NC: Negative control; PC: Positive control.
ROEO Increased Skin VEGF Level of UVB-exposed Mice

The average VEGF level of the NC group (9.22±0.54 ng/L) was significantly lower than the one of the sham group (11.20±0.71 ng/L). No significant difference was observed between the average VEGF level of the 5% ROEO group (9.89±0.71 ng/L) and the one of the NC group. Furthermore, the average VEGF level of the 10% ROEO group (15.48±2.05 ng/L) was significantly higher than those of the NC and PC (12.21±0.73 ng/L) groups (Figure 3).

Figure 3. VEGF levels of UVB-exposed mice. Mice were treated topically with/without paraffin oil, 2% minoxidil, or various concentrations of ROEO, and exposed with UVB in the timeframe as described in Methods. Mice skin tissues were collected, processed, and the VEGF levels were measured with ELISA. *p<0.05 vs. sham group; †p<0.05 vs. NC group; ‡p<0.05 vs. PC group, LSD test. NC: Negative control; PC: Positive control.

Discussion

Essential oils derived from plant species have attracted attention for their beneficial effect on human health. (18,19) In the present study, ROEO exhibited a very strong antioxidant activity (IC$_{50}$<50 ppm).(20) The IC$_{50}$ value of ROEO in the present study was lower than those in the previous studies, which was about 20-22 ppm.(21-23) Antioxidant activity of ROEO can be primarily attributed to the presence of phenolics, including flavonoids and tannins (15,24), as shown by the results of TPC, TFC, and TTC analyses. Phenolics counteract lipid peroxidation and reactive oxygen species (ROS) by stabilizing free electrons released by the oxidants through conversion to ketones.(15) Phenolic compounds that might be related to antioxidant properties of ROEO are rosmarinic acid, carnosic acid, and camphor. Combinations of α-pinene and cineole, cineole and camphor, or α-pinene, cineole, and camphor have been reported to increase plasma VEGF levels in rats underwent random-patterned skin flaps surgery procedure, and thereby improving the survivability of the skin flaps.(30)

UVB has been known to causes alterations in hair size, leading to hair loss.(6) In the present study, UVB exposure markedly reduced the hair length and follicle diameter. Topical application of ROEO could increase the length of and follicle diameter in UVB-exposed hair. Positive effects of ROEO toward hair growth could be due to its antioxidant activity and VEGF-inducing ability. Phenolic compounds in ROEO might be involved in scavenging ROS and inhibiting lipid peroxidation (15), thus protecting the hair from UVB-induced oxidative damage. Furthermore, ROEO-induced VEGF production might increase blood vessel formation around the hair follicles. This phenomenon may enhance blood supply to the follicles, facilitating delivery of oxygen and essential nutrients required for optimal follicle function, which may significantly impact the biological processes within the hair follicles.(17)

While the findings of the present animal study support the conclusion within the setting of the study, more research is required to verify these findings in human subjects. This study can be used as a guide for future human research, highlighting the necessity for more investigations to confirm the potential advantages of ROEO in preventing senile alopecia. ROEO is expected to be used as a potential anti-hair aging agent in humans, so psychological well-being and quality of life could be maintained.(31)
Conclusion

Since 10% ROEO could significantly increase hair length, follicle diameter, and VEGF level, and contained antioxidant compounds, it can be suggested that ROEO might increase hair length, follicle diameter, and VEGF level through its antioxidant component. Taken together, ROEO treatment could prevent UVB-induced hair photaging contributing to senile alopecia.

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Authors Contribution

SL, AED, and IGP were involved in the study conceptization, methodology, formal analysis, and investigation. SL, AED, IGP, and VOW prepared the original draft the manuscript and data visualization. SL, AED, IGP, and VOW were also involved in the validation process, review and editing of the manuscript. All authors read and approved the final manuscript.

References


