



Additive effects of clove (*Syzygium aromaticum*) and rosemary (*Rosmarinus officinalis*) essential oils on hair regrowth in a rat model: A comparative study with 2% minoxidil

[Efectos aditivos de los aceites esenciales de clavo (*Syzygium aromaticum*) y romero (*Rosmarinus officinalis*) sobre el crecimiento del cabello en un modelo de rata: un estudio comparativo con minoxidil al 2%]

Osama S. Alrufayyiq¹, Rehab F. Abdel-Rahman², Samah M. El Sohafy³, Gamal A. Soliman⁴, Hisham Y. Elzorba⁴, Maged S. Abdel-Kader^{3*}

¹Jathr Alhayawiya Company, VITAROOT, Almasif Discreet, Riyadh 12467, Kingdom of Saudi Arabia.

²Department of Pharmacology, National Research Centre, Giza 12622, Egypt.

³Department of Pharmacognosy, College of Pharmacy, Alexandria University, Alexandria 21215, Egypt.

⁴Department of Pharmacology, College of Veterinary Medicine, Cairo University, Giza 12613, Egypt.

*E-mail: m.youssef@psau.edu.sa

Abstract

Context: Alopecia affects a large population and current treatments have limited efficacy and tolerability. Essential oils have gained increasing interest as natural, low-risk alternatives.

Aims: To evaluate the effects of *Syzygium aromaticum* and *Rosmarinus officinalis* oils, alone and in combination, on hair regrowth in rats and compared with 2% minoxidil.

Methods: *S. aromaticum* oil was prepared by hydro-distillation, while *R. officinalis* oil was purchased from the local market and analysed by GCMS. Male Wistar rats (n=30) were randomized to negative control (vehicle), 2% minoxidil, *S. aromaticum* oil, *R. officinalis* oil, and a *S. aromaticum*-*R. officinalis* oil mixture groups. Treatments were applied topically on a shaved dorsal skin area once a day for 35 days. The times required for hair growth initiation and completion were recorded, along with hair growth scores on days 7, 21, and 35. On day 35, hair weight, length, and thickness were measured, and skin histology was examined.

Results: All active treatments showed significant improvements in hair regrowth parameters manifested by a significant reduction in the duration of hair initiation and completion and improvement in all the measured parameters. The effects of *S. aromaticum* oil and *R. officinalis* oil on hair re-growth were comparable to that of minoxidil. The oils mixture demonstrated superior performance to minoxidil in terms of faster regrowth initiation (6.5 days), completion (21.83 days), and higher hair weight (22.2 mg/cm²) and length (16.67 mm). Histology indicated a higher follicle density in treated groups.

Conclusions: The oils mixture exhibited an additive effect, outperforming minoxidil in promoting hair regrowth.

Keywords: alopecia; essential oils; hair follicle; hair regeneration; minoxidil; *Rosmarinus officinalis*; *Syzygium aromaticum*.

Resumen

Contexto: La alopecia afecta a una gran parte de la población y los tratamientos actuales presentan una eficacia y tolerabilidad limitadas. Los aceites esenciales han despertado un creciente interés como alternativas naturales y de bajo riesgo.

Objetivos: Evaluar los efectos de los aceites de *Syzygium aromaticum* y *Rosmarinus officinalis*, solos y en combinación, sobre el crecimiento del cabello en ratas y compararlos con minoxidil al 2%.

Métodos: El aceite de *S. aromaticum* se preparó mediante hidrodestilación, mientras que el aceite de *R. officinalis* se adquirió en el mercado local y se analizó mediante cromatografía de gases-espectrometría de masas (GC-MS). Treinta ratas Wistar macho se asignaron aleatoriamente a los grupos de control negativo (vehículo), minoxidil al 2%, aceite de *S. aromaticum*, aceite de *R. officinalis* y una mezcla de aceites de *S. aromaticum* y *R. officinalis*. Los tratamientos se aplicaron tópicamente sobre una zona de piel dorsal rasurada una vez al día durante 35 días. Se registraron los tiempos necesarios para el inicio y la finalización del crecimiento del cabello, junto con las puntuaciones de crecimiento capilar en los días 7, 21 y 35. El día 35, se midieron el peso, la longitud y el grosor del cabello, y se examinó la histología de la piel.

Resultados: Todos los tratamientos activos mostraron mejoras significativas en los parámetros de regeneración capilar, manifestadas por una reducción significativa en la duración del inicio y la finalización del crecimiento, así como una mejora en todos los parámetros medidos. Los efectos del aceite de *S. aromaticum* y del aceite de *R. officinalis* sobre la regeneración capilar fueron comparables a los del minoxidil. La mezcla de aceites demostró un rendimiento superior al del minoxidil en términos de un inicio (6,5 días) y una finalización (21,83 días) más rápidos de la regeneración, y un mayor peso (22,2 mg/cm²) y longitud (16,67 mm) del cabello. La histología indicó una mayor densidad folicular en los grupos tratados.

Conclusiones: La mezcla de aceites mostró un efecto aditivo, superando al minoxidil en la promoción de la regeneración capilar.

Palabras Clave: aceites esenciales; alopecia; folículo piloso; minoxidil; regeneración capilar; *Rosmarinus officinalis*; *Syzygium aromaticum*.

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AUTHOR INFO

ORCID:

[0009-0008-2290-9027](https://orcid.org/0009-0008-2290-9027) (OSA)

[0000-0002-4341-4954](https://orcid.org/0000-0002-4341-4954) (RFAR)

[0000-0003-0444-4963](https://orcid.org/0000-0003-0444-4963) (SMES)

[0000-0003-4622-3501](https://orcid.org/0000-0003-4622-3501) (GAS)

[0009-0005-4330-6311](https://orcid.org/0009-0005-4330-6311) (HYE)

[0000-0002-5128-2656](https://orcid.org/0000-0002-5128-2656) (MSAK)

Abbreviations: GC: Gas Chromatography; GC-MS: Gas Chromatography–Mass Spectrometry; H&E: Hematoxylin and Eosin; IRB: Institutional Review Board; iNOS: Inducible Nitric Oxide Synthase; MS: Mass Spectrometry; NF- κ B: Nuclear Factor kappa-light-chain-enhancer of activated B cells; NIST: National Institute of Standards and Technology; RRI: Relative Retention Index.

INTRODUCTION

Alopecia is a condition characterized by partial or complete loss of scalp hair or failure of hair regrowth (Damodaran & Gupta, 2011). Hair loss is caused by genetic factors and other factors, such as disturbances in hormone secretion (Kwak & Park, 2021). It can also be caused by blood-thinning medications, contraceptives, antidepressants, steroidal anti-inflammatory drugs, beta and calcium-channel blockers, retinoids, and chemotherapeutics (Vicky et al., 2018).

Hair loss interferes with many of the beneficial biological functions of hair, such as sun protection and the dispersion of sweat gland products (Blume-Peytavi et al., 2011). In healthy people, the hair growth cycle consists of anagen (growth), catagen (regression), and telogen (resting) phases; disruption of this cycle contributes to hair thinning and hair loss (Ma et al., 2018). If the cycle is not properly regulated, telogen continues, resulting in hair loss (Stenn & Paus, 2001). Normally, hair follicles are contracted after the anagen phase, and the hair shaft falls out during the catagen or telogen phase. As the follicles begin a new anagen phase, they grow back to their original size and produce new hair of a normal thickness (Paus & Foitzik, 2004; Stenn & Paus, 2001).

Compared to the increasing prevalence of hair loss, current FDA-approved treatment options for hair loss are limited to topical minoxidil, oral finasteride, and low-level light therapy (Ashique et al., 2020; Uronnachi et al., 2022). These approved treatments show variable responses and potential side effects (Mysore & Shashikumar, 2016; Vesoulis et al., 2014), encouraging the evaluation of complementary therapies such as essential oils.

Development of alternative agents derived from natural products for hair loss prevention is being actively explored (Adirajan et al., 2003; Boisvert et al., 2017; Kim et al., 2019; Murata et al., 2013). Natural products are usually less toxic, less expensive, and more available (Kaushik et al., 2011). Essential oils are among the most popular natural products and have significant applications in dermatology. Essential oils are natural, non-toxic, non-polluting, and biodegradable compounds with a wide range of therapeutic benefits and low-risk side effects (Uronnachi et al., 2022).

Syzygium aromaticum (L.) Merr. & L.M.Perry (family *Myrtaceae*), commonly called clove, is a culinary spice with medical uses. *S. aromaticum* essential oil has been revealed to have antibacterial, antioxidant, analgesic,

antifungal, and anticarcinogenic activities (Pandey et al., 2024). At least 30 compounds have been identified in *S. aromaticum* oil (Golmakani et al., 2017); eugenol is the major compound, accounting for at least 50%. Among the reported biological activities of eugenol are insecticidal, antimicrobial, anti-inflammatory, wound-healing, antiviral, antioxidant, and anticancer activities (Haro-González et al., 2021). In addition, Abadi et al. (2020) mentioned that the ethanol extract of *S. aromaticum* leaves can increase rabbit hair growth.

Rosmarinus officinalis L. (synonym of *Salvia rosmarinus* Spenn., family *Lamiaceae*) is currently one of the most promising herbal medicines due to its pharmaceutical properties (Li Pomi et al., 2023). Many biological activities have been reported for *R. officinalis*, including antimicrobial, anti-inflammatory, antitumor, antiapoptotic, antinociceptive, antioxidant, and neuroprotective properties. *R. officinalis* has also been known to have hair growth-stimulating properties (Murata et al., 2013). *In vivo* experiments supported the traditional knowledge regarding *R. officinalis* effect on hair growth. Several studies indicated that *R. officinalis* oil enhances hair growth via inhibiting the enzyme 5- α -reductase, which converts testosterone to dihydrotestosterone, by stimulating blood circulation to the scalp, which enhances the delivery of nutrients and oxygen to the hair follicles (Leite Junior & Bastos, 2024; Panahi et al., 2015) and improving the oxidative phosphorylation and antioxidant defenses of the hair follicle cells (Wang et al., 2023). *R. officinalis* oil also expressed anti-inflammatory effect on the hair follicle cells via inhibiting pro-inflammatory cytokines as well as the expression of nuclear factor kappa B (NF- κ B) and inducible nitric oxide synthase (iNOS) that suppresses the scalp inflammation and stimulates hair growth (Choi et al., 2023; Wang et al., 2023).

Although individual studies have demonstrated the benefits of *S. aromaticum* and *R. officinalis* oils, their combined effect on hair growth remains unexplored. This study aims to evaluate the efficacy of *S. aromaticum* oil and *R. officinalis* oil, alone and in combination, on hair growth in a rat model compared with 2% minoxidil, and to explore potential additive effects.

MATERIAL AND METHODS

Plant material and oils

Dried *Syzygium aromaticum* flower buds were authenticated by Prof Sanyia Kamal, Department of Botany, Faculty of Science, University of Alexandria,

and a voucher specimen (SA2022) was deposited at the Pharmacognosy Department, Faculty of Pharmacy, Alexandria University. The oil was prepared by hydro-distillation using a Clevenger apparatus with a 2 L round-bottom flask for 8 h. The condensate was extracted with ether. The ether extract was dried over anhydrous sodium sulphate and concentrated to yield the *S. aromaticum* oil. *R. officinalis* leaves oil (*Salvia rosmarinus*) was obtained from Neferatri company (Cairo, Egypt, Product number: 6223003832063).

GC and GC-MS analysis

GC Analysis was carried out using a GC HP 5890 Hewlett Packard equipped with FID and Rtx-5MS fused silica capillary column (30 m × 0.25 mm internal diameter (I.D.), film thickness 0.25 µm). Aliquots of diluted oils (1 mL at 5 µg/mL) were used, with an injection volume of 0.03 µL. The oven temperature was programmed to increase from 45°C to 300°C at 5°C/min. the injector temperature was set at 250°C, while the detector temperature was 280°C. Carrier gas, 99.999% pure helium, was used with a constant flow rate of 1.41 mL/min. Automatic sample injection using Autosampler in the split mode of 1: 15. The relative proportions of the active constituents were expressed as percentages obtained by peak area normalization. Relative retention indexes were obtained using a series of hydrocarbons C7-C40 (Figs. S1-S3). GC-MS analysis was performed under the same conditions and using a PerkinElmer quadrupole MS system (Model 5). The MS operating parameters were as follows: interface temperature: 280°C, ion source temperature: 200°C, EI mode: 70 eV, scan range: 35–500 amu (Abdelhalim et al., 2023).

Experimental animals

A total of 30 male Wistar albino rats at 5 weeks of age, weighing between 120 and 140 g, were purchased from the animal facility at the National Research Center, Egypt. Animals were kept in standard cages, under pathogen-free conditions, and maintained under controlled room temperature and under normal dark – light cycles. Animals were provided with standard food and water *ad libitum*. Rats were allowed to adapt to these conditions for one week before beginning the experimental protocol. Cages were cleaned regularly, and bedding materials were changed to maintain cleanliness and prevent the buildup of ammonia and other contaminants.

Chemicals and drugs

Minoxidil 2% solution (Avogain, Avalon Pharma, Riyadh, Saudi Arabia) and CeraVe moisturizing lotion (CeraVe LLC, New York, USA) were purchased from a

local pharmacy in Alexandria city and used as reference and base for the oils, respectively.

Animal ethics statement

All procedures were conducted in accordance with the National Regulations on Animal Welfare and the study protocol number SCBR-183/2023, approved by the PSAU Bio-Ethical Research Committee.

Experimental design

The experiment was conducted on 30 male Wistar albino rats, which were randomly assigned to treatment groups by simple random draw. Each rat was numbered, and numbered tokens were drawn from an opaque container by a person not involved in data collection. Cages were labelled only with anonymized codes to conceal allocation. A group size of six animals was selected based on comparable earlier studies investigating hair growth in rats and mice (Baek et al., 2023; Park et al., 2024; Rathie et al., 2017; Roy et al., 2007). The rats were acclimated to stable conditions for one week before experimentation. After acclimatization, anesthesia was induced using ketamine hydrochloride (50 mg/kg) and xylazine hydrochloride (20 mg/kg) administered intraperitoneally (Jalilzadeh et al., 2025; Orasan et al., 2016). A 6 cm² area of the dorsal side of all the rats was shaved using an electric shaver and wiped with alcohol 24 hours before starting the experiment. Subsequently, depilatory cream (Dwight Co., Inc.) was applied to the shaved area to remove residual hair completely. The skin was examined for any sign of injury or irritation before applying the tested material. The rats of the first group received CeraVe moisturizing lotion, representing the control group, and the second received minoxidil (2%). The animals in the 3rd–5th groups were given topical applications of 5% *S. aromaticum* oil, 5% *R. officinalis* oil, and their combination (5% each), each loaded into CeraVe moisturizing lotion. The test materials were applied topically once daily to the test areas for 35 days. Animals were monitored daily for signs of dermal irritation, erythema, and ulceration at the application site, as well as other adverse events. Qualitative hair growth, hair weight, length, and thickness, and histopathological examination were evaluated by investigators blinded to treatment allocation to minimize observer bias.

Qualitative evaluation of hair growth

Qualitative hair growth was evaluated by visual observation of hair regrowth initiation time, hair regrowth completion time, and hair regrowth score. Hair regrowth initiation time refers to the minimum time for noticeable hair growth to begin, while hair regrowth completion time refers to the minimum time

taken for a bare skin area to be completely covered with new hair (Rambwawasvika et al., 2019).

The hair growth score (hair density) was estimated following de Lacharri re et al. (2001): 0 = no hair growth, 1 = less than 20% of hair growth, 2 = 20–39% of hair growth, 3 = 40–59% of hair regrowth, 4 = 60–79% of hair regrowth, 5 = 80–100% of hair regrowth. The average score was calculated and determined by skilled researchers through blinded testing.

Quantitative assessment of hair growth

At the end of the experimental period, rats were anesthetized with ketamine (50 mg/kg) and xylazine (20 mg/kg, i.p.). Regrown hair samples were obtained by shaving the treated skin area of each rat (6 cm²) again using an electric shaver. Regrown hair was weighed using a precision balance (Premium Microbalance (Cubis2); Sartorius, Seoul, Republic of Korea). Randomly selected 20 strands of hair from each rat were placed on glass slides and securely fixed. The length of hair was measured by using a digital vernier caliper, while the hair shaft thickness was measured using an optical microscope (BX43F; Olympus, Tokyo, Japan) at 40× magnification (Anjum et al., 2024).

Histopathological examination (Anjum et al., 2024)

After confirming surgical depth of anesthesia (absence of pedal and corneal reflexes), animals were humanely euthanized by cervical dislocation in accordance with the AVMA Guidelines (2020). A full-thickness skin sample was immediately excised from the treated dorsal area of each rat, fixed in 10% neutral buffered formalin for 24 h, and then embedded in paraffin blocks. Longitudinal and horizontal sections of 5.0- m-thick skin were prepared and stained with hematoxylin and eosin (H&E). Hair follicle diameter ( m), hair follicle density (%), dermal thickness ( m), and % ratio of the anagen and telogen of hair follicles were calculated. All scores were determined in 5 microscopic fields of view using an Olympus BX63 microscope and the hair analysis system (ImageJ 1.53t, Wayne Rasband and contributors, National Institutes of Health, USA).

Statistical analysis

Data are expressed as mean ± standard error of the mean (SEM). For comparisons among multiple groups, one-way analysis of variance (ANOVA) was performed, followed by Tukey's post hoc test (SPSS for Windows, version 16). A p-value < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

GC-MS analysis of *S. aromaticum* oil indicated that eugenol is the main oil component, followed by  -caryophyllene, then eugenol acetate (Table 1) (Evans & Evans, 2009).

R. officinalis essential oil was obtained from the aerial parts of the plant. Based on taxonomic evidence, the genus *Rosmarinus* (family *Lamiaceae*) was transferred to the genus *Salvia* in 2017. The plant *Rosemarinus officinalis* was then given the name *Salvia rosmarinus* Spenn., traditionally known as "rosemary" (Drew, 2017). Analysis of the oil by GC-MS revealed 21 components. The main three components were 1,8-cineole (21.60%), camphor (17.28 %),  -pinene (16.00%), and borneol (12.70%) (Hussain et al., 2010; Sienkiewicz et al., 2013) (Table 2).

Hair growth occurs in a continuous process characterized by four phases: anagen, catagen, telogen, and exogen. Anagen is a highly mitotic phase characterized by the production of a hair shaft from the hair follicle (Natarelli et al., 2023). Hair loss is an increasingly common condition caused by many factors, such as genetics, hormonal fluctuations, autoimmune diseases, the natural aging process, and severe stress (Xing et al., 2024).

Treatment strategies for hair loss include a range of topical medications. Among these, minoxidil has emerged as the preferred topical treatment for many types of hair loss (Gupta et al., 2022). The use of conventional treatments, such as minoxidil, can have side effects or be less effective for some people. This has led to a growing interest in natural alternatives, particularly essential oils, which are widely used in dermatology due to their antimicrobial, anti-inflammatory, and antioxidant properties. Among these oils, *S. aromaticum* oil (Shahtalebi et al., 2016) and *R. officinalis* oil (Panahi et al., 2015) have been highlighted for their hair-strengthening effects.

The results of the current study clearly show that topical application of *S. aromaticum* oil, *R. officinalis* oil, and, particularly, their combination significantly enhances various hair regrowth parameters in rats compared with the control group. Furthermore, the oil combination outperforms the reference treatment (2% minoxidil) in most aspects (Fig. 1).

Qualitative evaluation of hair growth

The minimum time to visible hair growth on shaven skin (hair growth initiation) and the minimum time to complete hair growth on denuded skin (hair growth completion time) were observed visually, and the results are shown in Table 3.

Table 1. Composition of *S. aromaticum* essential oil.

No.	Name of component	RT (min)	Occurrence (%)	RI Cal.	RI Theo.
1	Eugenol	19.724	79.18	1344	1356
2	β -Caryophyllene	21.860	10.64	1419	1416
3	α -Humulene	22.704	1.37	1440	1449
4	Eugenol acetate	23.820	6.77	1530	1530
5	β -Caryophyllene oxide	25.721	1.21	1579	1581
Total			99.17		

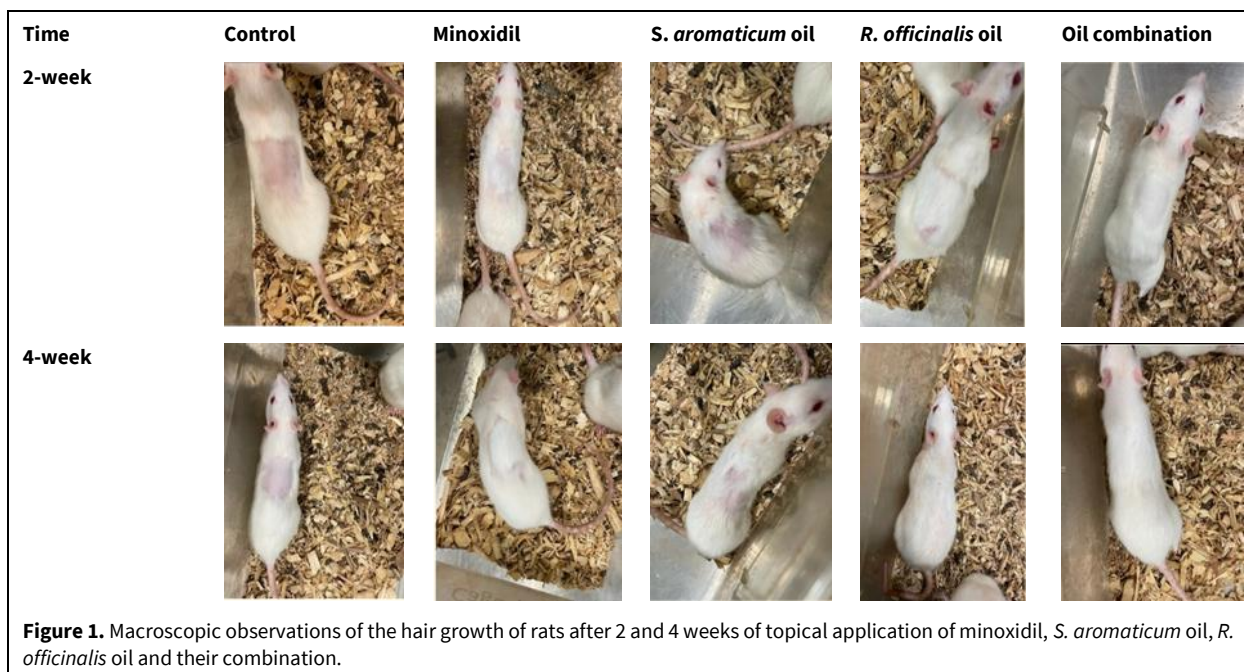
Table 2. Composition of *R. officinalis* essential oil.

No.	Name of component	RT (min)	Occurrence (%)	RI Cal.	RI Theo.
1	Tricyclene	7.165	1.26	919	914
2	α -Thujene	7.330	0.15	922	917
3	α -Pinene	7.516	16.00	931	938
4	Camphene	7.859	6.37	943	941
5	β -Pinene	8.668	5.44	969	970
6	β -Myrcene	9.759	1.82	986	990
7	p -Cymene	10.015	4.92	1020	1022
8	1,8-Cineole	10.216	21.60	1031	1030
9	β -Ocimene	10.290	3.12	1041	1037
10	γ -Terpinene	12.120	0.64	1059	1058
11	Linalool	12.361	2.44	1090	1100
12	Fenchol	12.526	0.29	1109	1110
13	D-Fenchyl alcohol	12.925	0.27	1119	1115
14	Camphor	13.278	17.28	1143	1144
15	Isoborneol	13.393	0.46	1154	1156
16	<i>trans</i> -3-carene-2-ol	13.593	0.45	1158	1160
17	Borneol	13.961	12.70	1164	1163
18	Terpinen-4-ol	14.212	0.51	1167	1177
19	α -Terpineol	14.615	1.95	1191	1191
20	Bornyl acetate	14.885	0.12	1282	1277
21	β -Caryophyllene	14.978	1.82	1440	1434
Total			99.61		

Table 3. Effect of topical application of *S. aromaticum* oil, *R. officinalis* oil and their combination on hair growth initiation time, hair growth completion time and hair growth score of rats.

Treatment	Hair growth initiation time (day)	Hair growth completion time (day)	Hair growth score		
			Day-7	Day-21	Day-35
Control	11.67 $\pm$ 0.49	31.17 $\pm$ 1.25	0.00 $\pm$ 0.00	2.17 $\pm$ 0.17	4.17 $\pm$ 0.31
Minoxidil	8.00 $\pm$ 0.37*	26.17 $\pm$ 1.38*	0.00 $\pm$ 0.00	3.17 $\pm$ 0.31*	5.00 $\pm$ 0.00*
<i>S. aromaticum</i> oil	9.17 $\pm$ 0.40*	27.33 $\pm$ 0.92*	0.00 $\pm$ 0.00	2.83 $\pm$ 0.17*	5.00 $\pm$ 0.00*
<i>R. officinalis</i> oil	8.50 $\pm$ 0.43*	24.17 $\pm$ 1.05*	0.00 $\pm$ 0.00	3.50 $\pm$ 0.22*	5.00 $\pm$ 0.00*
Oil combination	6.50 $\pm$ 0.43*#	21.83 $\pm$ 1.08*#	0.67 $\pm$ 0.21*#	5.00 $\pm$ 0.00*#	5.00 $\pm$ 0.00*

Data are expressed as mean $\pm$ SE of 6 rats. Statistical significance is denoted as *p<0.05 versus the control group; #p<0.05 versus the minoxidil group.



Rats in the control group showed the longest time for hair regrowth initiation (11.67 days) and completion (31.17 days). Minoxidil reduced initiation to 8.00 days and completion to 26.17 days, while *R. officinalis* oil produced a comparable reduction (8.50 days initiation; 24.17 days completion). Furthermore, the use of *S. aromaticum* oil significantly reduced the initiation time (9.17 days) and the completion time (27.33 days). These results demonstrate that *S. aromaticum* oil and *R. officinalis* oil treatments are comparable to 2% minoxidil treatment.

The results also suggest that both essential oils promote early follicular activation, likely due to their vasodilator, antioxidant, and anti-inflammatory properties. *S. aromaticum* oil, rich in eugenol, may protect and/or stimulate hair follicles via anti-inflammatory and antioxidant pathways (Nisar et al., 2021). Regarding *R. officinalis* oil, Panahi et al. (2015) and Uronnachi et al. (2022) reported that the results of *R. officinalis* use for hair growth were not significantly different from those obtained with minoxidil. Dhariwala & Ravikumar (2019) and Maleš et al. (2019) explained that the mechanism of action of *R. officinalis* oil is its ability to improve microcirculation around hair follicles, which helps regenerate hair follicles, an effect similar to that provided by minoxidil.

The combination produced the most pronounced effects, reducing initiation to 6.50 days and completion to 21.83 days, significantly outperforming both individual oils and minoxidil. Interestingly, the combination of *S. aromaticum* oil/*R. officinalis* oil produced the most pronounced effects, reducing initiation to 6.50 days and completion to 21.83 days, outperforming

both minoxidil and individual oils. The superiority of the oil combination over minoxidil and individual oils suggests a potential additive effect between the *S. aromaticum* and *R. officinalis* oils, targeting multiple pathways involved in follicular cycling and hair growth. This combination may also enhance transdermal absorption due to the lipophilic nature of its components, thus improving bioavailability.

The hair regrowth scores for all groups of rats at 7, 21, and 35 days were estimated visually (Table 3). By Day 21, only the combination group achieved a full regrowth score of 5, whereas minoxidil, *S. aromaticum* oil, and *R. officinalis* oil groups required 35 days to reach this score. The hair regrowth scores reflect the superior effect of the oil combination, resulting in improved coverage of the shaved areas on the backs of the rats and denser, more uniform hair.

Measurement of hair weight, length and thickness

Figs. 2-4 show the hair weight, length, and thickness of the experimental animals at the end of the experimental period, respectively. On the 35th day, the control group showed hair weight of 11.47 ± 0.71 mg/cm². Rats treated with *S. aromaticum* oil and *R. officinalis* oil had hair weight of 15.28 ± 0.56 and 18.65 ± 0.71 mg/cm², respectively, while those treated with minoxidil had a weight of 17.82 ± 1.08 mg/cm².

The average hair length measured in control rats was 8.83 ± 0.48 mm, while the average length in the minoxidil group was 12.83 ± 0.31 mm. The average length of hair in the *S. aromaticum* oil and *R. officinalis* oil groups was 11.67 ± 0.49 mm and 14.17 ± 0.60 mm, respectively. No significant difference in the length of

hair was found between the *S. aromaticum* oil, *R. officinalis* oil, and minoxidil groups. The oil combination group again outperformed all other groups in its effect on hair weight (22.20 mg/cm^2) and hair length (16.67 mm). These improvements were statistically significant compared to both the control and minoxidil groups, confirming the superior efficacy of the oil combination treatment.

Although the combination group showed the highest hair weight (22.20 mg/cm^2), this was accompanied by a slight reduction in average hair shaft thickness ($11.00 \pm 0.52 \mu\text{m}$) compared to the control ($12.67 \pm 0.56 \mu\text{m}$). This suggests that the combination treatment may promote activation of a greater number of follicles, producing more numerous but finer hairs, thereby increasing total hair mass without increasing individual hair thickness.

Histopathological examination

Fig. 5B shows the effects of various treatments on the percentage of hair follicle density. Compared to control rats ($17.8 \pm 0.46\%$) and those treated with *S. aromaticum* oil ($26.2 \pm 1.82\%$) and *R. officinalis* oil ($34.6 \pm 1.35\%$), the group treated with the oil combination exhibited a greater hair follicle density ($41.9 \pm 2.87\%$). The *R. officinalis* oil-treated group's hair follicle density ($34.6 \pm 1.35\%$) was similar to the minoxidil group's ($32.4 \pm 2.45\%$). Additionally, the group treated with the oil combination had a higher hair follicle density ($32.4 \pm 2.45\%$) than the minoxidil group, indicating that the oil combination has a strong ability to regenerate hair. An increase in hair follicle density indicates a transition from the telogen to the anagen phase (Shin et al., 2013).

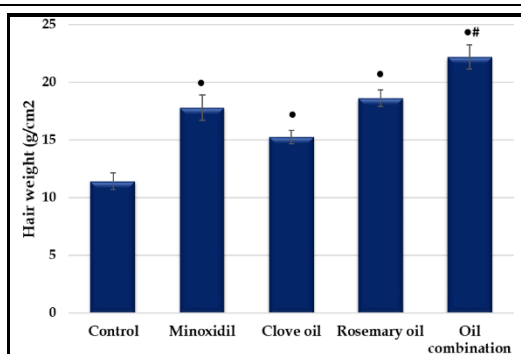


Figure 2. Effect of topical application of minoxidil, *S. aromaticum* oil (clove), *R. officinalis* oil (rosemary) and oil combination on hair weight of rats.

Data are expressed as mean $\pm$ SEM of 6 rats. Statistical significance is denoted as * $p < 0.05$ versus the control group; # $p < 0.05$ versus the minoxidil group.

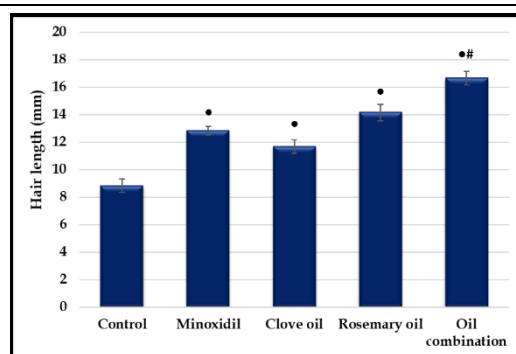


Figure 3. Effect of topical application of minoxidil, *S. aromaticum* oil (clove), *R. officinalis* oil (rosemary) and oil combination on hair length of rats.

Data are expressed as mean $\pm$ SEM of 6 rats. Statistical significance is denoted as * $p < 0.05$ versus the control group; # $p < 0.05$ versus the minoxidil group.

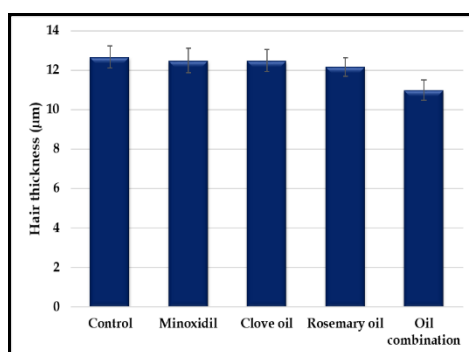
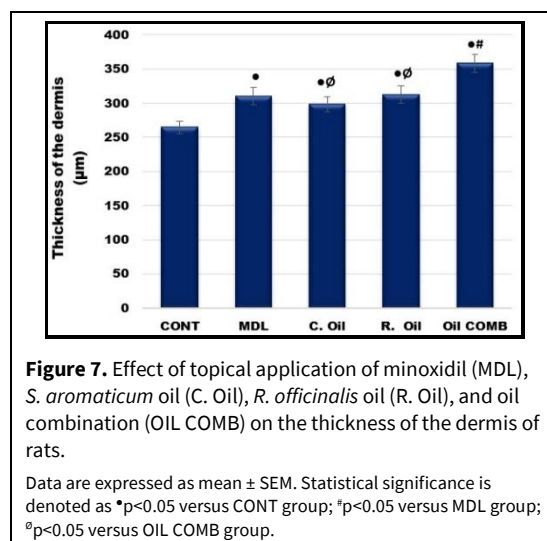
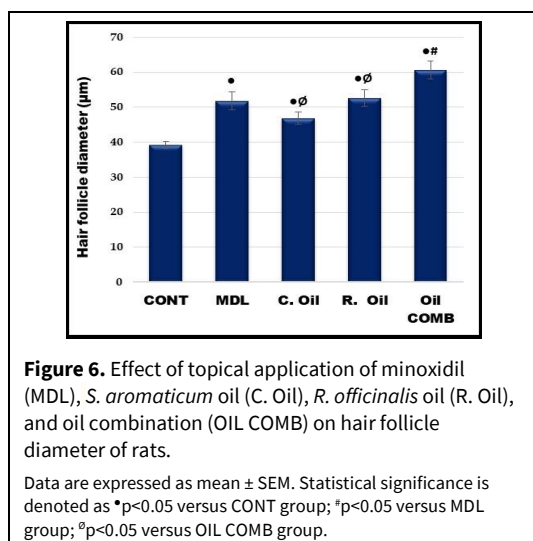
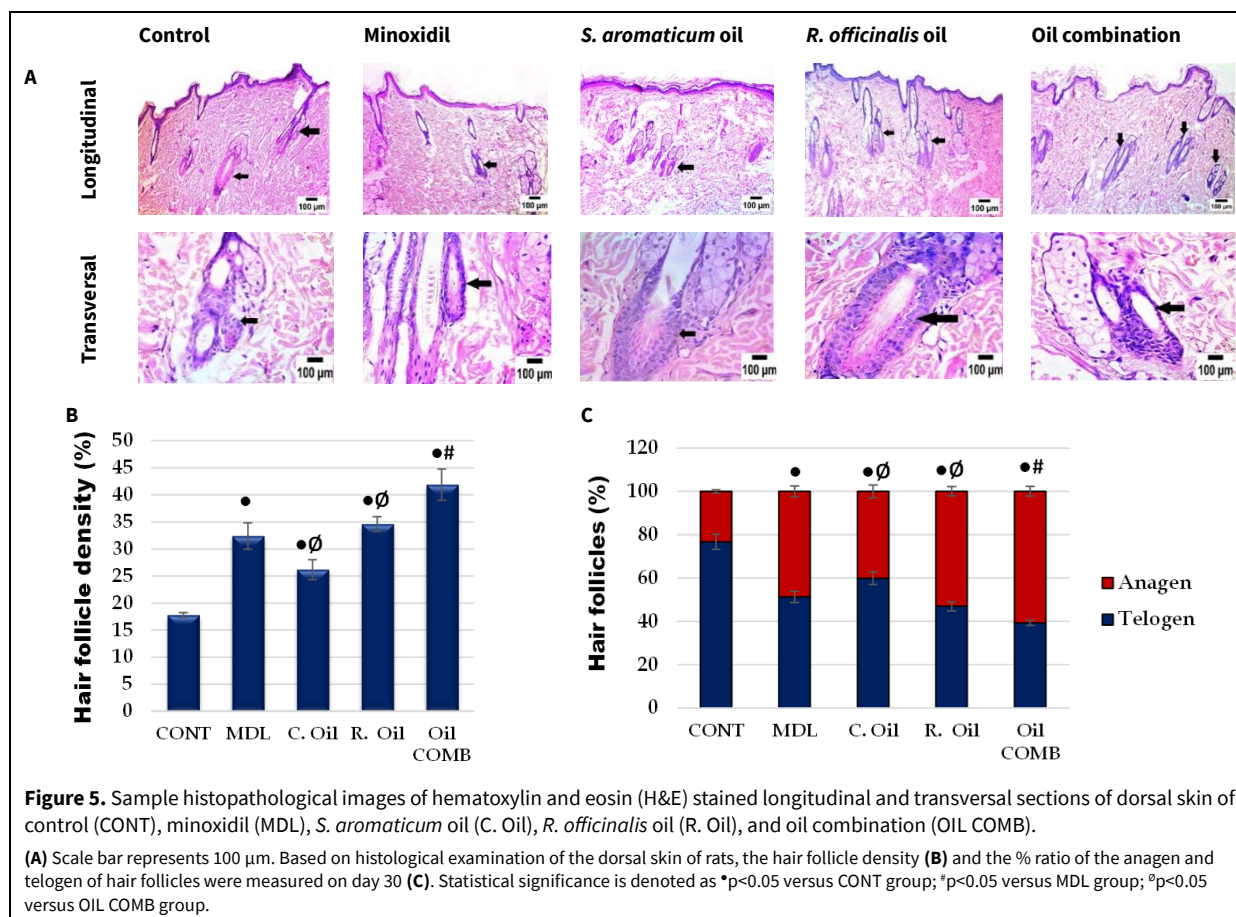


Figure 4. Effect of topical application of minoxidil, *S. aromaticum* oil, *R. officinalis* oil (clove), *R. officinalis* oil (rosemary) and oil combination on hair thickness of rats.

Data are expressed as mean $\pm$ SE of 6 rats. Statistical significance is denoted as * $p < 0.05$ versus control group; # $p < 0.05$ versus minoxidil group.



Alopecia medications have been developed to maintain or induce the anagen stage of the hair cycle (Oh et al., 2014). The more hair follicles in the anagen phase, the better the hair growth (Anjum et al., 2024). The skin samples from the control and treated rats showed a significant variation in the various cyclic phases (anagen and telogen) of hair follicles, as seen in Fig. 5C. Compared with the control and other treatment groups, the oil combination-treated rats

exhibited a high anagen ratio ($60.67 \pm 2.25\%$) on the last day of treatment. The hair follicles of animals treated with *S. aromaticum* oil and *R. officinalis* oil also showed a transition from the telogen to the anagen phase at rates of $40.12 \pm 2.97\%$ and $53.08 \pm 2.14\%$, respectively. The animals treated with minoxidil showed $48.72 \pm 2.54\%$ induction of anagen hair follicles. The animals treated with vehicle showed very little anagen induction ($23.28 \pm 0.82\%$).

Fig. 6 displays the results of the hair follicle diameters. The control, minoxidil, *S. aromaticum* oil, *R. officinalis* oil, and oil combination groups had hair follicle diameters of $39.2 \pm 1.03 \mu\text{m}$, $51.8 \pm 2.47 \mu\text{m}$, $46.9 \pm 1.69 \mu\text{m}$, $52.6 \pm 2.32 \mu\text{m}$, and $60.7 \pm 2.55 \mu\text{m}$, respectively. Compared with the minoxidil treatment group, the oil combination-treated rats had the largest follicle diameters in their regenerated hair. There was no discernible difference between minoxidil and either *S. aromaticum* or *R. officinalis* oil, according to statistical analysis.

Fig. 7 shows the dermal thickness of the experimental animals at the end of the experimental period. For the group treated with *S. aromaticum* oil, *R. officinalis* oil, and the combination of the two oils, the skin thickness was $298.64 \pm 11.14 \mu\text{m}$, $312.62 \pm 12.71 \mu\text{m}$, and $358.26 \pm 13.16 \mu\text{m}$, respectively. In the group treated with minoxidil, the skin thickness was $310.42 \pm 12.28 \mu\text{m}$. In the vehicle-treated control group, skin thickness was lower than in the other groups ($264.5 \pm 9.25 \mu\text{m}$).

CONCLUSION

S. aromaticum oil and *R. officinalis* oil demonstrated significant hair growth stimulation in rats. The combination treatment was particularly effective in accelerating the initiation of hair regrowth and achieving earlier full coverage (score 5 by Day 21), highlighting a potential additive effect. While the combination increased hair weight and length, this was associated with finer hairs rather than uniformly thicker shafts. These findings suggest the *S. aromaticum*-*R. officinalis* oil blend as a promising natural adjunct to current therapies, warranting further mechanistic investigation.

CONFLICT OF INTEREST

The research was conducted entirely free from any involvement in commercial, financial, or personal matters that might be perceived as a possible conflict of interest or that could have influenced the outcomes or interpretation of the study.

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GENERATIVE ARTIFICIAL INTELLIGENCE (AI)

The authors declare that no generative artificial intelligence (AI) or AI-assisted technologies were used in the preparation, writing, or editing of this manuscript. All content was developed solely by the authors, who take full responsibility for its accuracy and integrity.

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AUTHOR CONTRIBUTION:

Contribution	Alrufayyiq OS	Abdel-Rahman RF	El Sohafy SM	Soliman GA	Elzorba HY	Abdel-Kader MS
Concepts or ideas				x		x
Design		x		x	x	x
Definition of intellectual content		x	x		x	
Literature search	x	x	x			
Experimental studies		x	x		x	
Data acquisition		x	x		x	
Data analysis	x		x			x
Statistical analysis	x	x		x		
Manuscript preparation		x	x			
Manuscript editing	x	x			x	
Manuscript review	x	x	x	x	x	x

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Supplementary data

