



# Promising Mediterranean Natural Oil Based Formulation to Enhance Beard Growth and Visual Performance through *In Vitro* and *In Vivo* Studies

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## Abstract

A well-groomed beard allows men to look distinguished as well as boosts self-confidence and self-expression. Many chemical or natural agents are used in male grooming products to improve the growth, health and quality of a man's beard. This study aimed to evaluate the effect of various natural oils in beard oil formulation on beard growth and nourishment. High-antioxidant natural oils including olive, jojoba, apricot, argan, macadamia, soybean, and avocado oils were incorporated in a beard grooming formulation to keep the beard hydrated and hydrophobic. A microbial inhibition test was conducted to assess its ability to prevent microbial growth on the beard. A total of 39 men (aged 20–29 years) participated and they had their beards shaved and underwent a washout period for 14 days without using any beard product. After this period, baseline measurements of the beard were taken before shaving it again, with assessments including length, density, and thickness using Phototrichogram and Caselite Imaging, along with trichologist evaluation and self-assessments. The subjects used the product once a day and the improvement was measured after 2 and 4 weeks. Results indicate that the beard oil formulation significantly improved beard growth rate at W2 (12.9%,  $p < 0.0001$ ) and W4 (24.30%,  $p < 0.0001$ ) compared to baseline. It also increases beard density at W2 (21.93%  $p < 0.0001$ ) and W4 (48.43%,  $p < 0.0001$ ), and improved beard thickness at W2 (9.17%,  $p < 0.0001$ ) and W4 (18.34%,  $p < 0.0001$ ). According to the subject self-assessment and trichologist evaluation, the product significantly improved the overall beard's look and growth, and reduced beard roughness and itchiness. These oils also impart antibacterial activity to inhibit *P. acnes* proliferation. Based on this study, a formulation containing natural antioxidant oils is effective in promoting growth and preserving health by providing nourishment to the beard.

**Keywords:** beard, natural oil, antioxidants, growth, nourish

## 1. INTRODUCTION

Facial hair including beards has gained popularity as a style choice and means of self-expression for men in many cultures. Research indicates that men with facial hair are often seen as more commanding, mature, and assertive compared to those who are clean shaven [1]. The presence of facial hair often carries certain social perceptions, and for some men, having sparse facial hair can be distressing. As a result, facial health care has become a critical aspect of personal grooming and overall health, with significant implications for aesthetic appeal and self-esteem. Most hair care formulations contain minoxidil, testosterone and

retinoids which have been reported to improve facial hair growth. However, the use of certain chemicals has been associated with adverse effects in users. Topical application of minoxidil has been shown to induce allergic contact dermatitis, pruritus, scalp irritation, hypertrichosis, and may exert vascular effect. Topical retinoids such as isotretinoin might increase the anagen phase excessively and induce telogen effluvium [2]. Several natural botanical extracts have also been investigated for their potential benefits in boosting hair performance. *Ficus religiosa*, *Ficus bengalensis*, coriander seeds, and *Chlorophytum borivilianum* were reported to have antioxidant activity and stimulate hair growth [3].

Beard has basically the same structure and growth phase with scalp hair, with some differences. Beard hair is thicker and has a more intricate structure with a larger root compared to scalp hair [4]. Its chemical composition includes lower levels of disulfide, valine, and serine, but higher levels of aspartic acid, lysine, and tyrosine. Beard hairs are often asymmetrical and elongated, whereas scalp hairs are generally more rounded and smaller in cross section. Additionally, beard hair has more cuticle layers and a less uniform scale

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**Table 1.** Schedule of panelist assessment at baseline, week 2, and week 4.

Activity*	Baseline		Week 2		Week 4	
	D0	D2	D14	D16	D28	D30
Acclimatization before evaluation	X	X	X	X	X	
Digital imaging (photograph)	X		X		X	
Trichoscan (Phototrichogram)	X	X		X		X
Caselite imaging	X		X		X	
Dermatologist evaluation	X		X		X	
Trichologist evaluation	X		X		X	
Subject self-assessments	X		X		X	

**Note:** Washout period to check natural beard growth after 14 days.

pattern, with more extensive medullation [5]. In terms of hair growth, facial hair such as beard has much shorter anagen phase than scalp hair and more time in catagen, resulting in shorter length [6].

The maintenance of facial hair poses significant challenges. Beards can serve as reservoirs for sebum, desquamated epithelial cells, and pathogenic microorganisms, potentially leading to the development of acne vulgaris [7]. Therefore, it is imperative for individuals to prioritize meticulous hygiene and adopt evidence-based grooming protocols to minimize adverse effects on the dermal microbiome and overall integumentary health. In addition to microbial concerns, external aggressors such as pollutants and UV irradiation can also compromise the health and growth cycle of hair. The pollutants can migrate into the dermis through the hair follicle, increasing oxidative stress level on the follicles which could lead to hair loss [8]. Elevated oxidative stress may contribute to the fragility and thinning of hair follicles. Skin tissues and hair follicles possess free radical scavenging enzymes that offer strong protection against oxidation, however, if this protection is not adequate the cells can enter a phase of permanent growth arrest and/or programmed cell death (apoptosis). Thus, hair follicle cells may sustain oxidative damage, disrupting the hair growth cycle and leading to pathological hair loss [9][10]. Environmental pollutants and UV irradiation also induce inflammation on skin and scalp that can extend to the hair follicle. Inflammation of the hair follicle can lead to fibrosis, hair shedding and permanent hair loss [11]-[13].

Traditional hair care practices often incorporate natural substances believed to enhance hair health. Among these, natural oils have been used across various cultures for centuries to address a range of hair-related issues, from dryness and breakage to scalp health and shine. Natural oils provide emollient properties by forming a protective layer around the hair shaft, which smooths and flattens the cuticle surface, thereby mitigating hair loss [14]. Additionally, their high antioxidant content is crucial in preventing oxidative damage caused by ultraviolet radiation and other forms of oxidative stress [15].

The activity of various plant oil ingredients in improving hair performance has been reported in several studies, but limited research has focused on how these ingredients specifically influence beard performance. The objective of this study was to evaluate the effect of selected natural oils on improving beard quality and growth as well as the stability and safety of the formulation. Microbial inhibition test using the Kirby-Bauer diffusion method was done to determine beard oil's ability to prevent microbial growth on the beard. Then, the *in vivo* test was conducted to evaluate the efficacy of beard oil formulation by using Phototrichogram and Caselite Imaging.

## 2. MATERIALS AND METHODS

### 2.1. Materials

A natural beard oil was formulated with actives: apricot (*Prunus armeniaca*) kernel oil, olive (*Olea europaea*) oil, macadamia (*Macadamia ternifolia*)

seed oil, argan (*Argania spinosa*) kernel oil, soybean (*Glycine soja*) oil, jojoba (*Simmondsia chinensis*) seed oil, and avocado (*Persea gratissima*) oil. Other ingredients are emollient, preservative, and fragrance.

## 2.2. Methods

### 2.2.1. Stability and Safety Tests

The stability of the formula was tested for 1 month in an oven 50 °C and 3 months in room temperature, oven 45 °C, freezer (-4 °C), display and sun exposure condition. The products were evaluated for performance parameters, including appearance, color, odor and sensory feel. The safety of this product was tested using the Single Patch Test and HRIPT (Human Repeat Insult Patch Test) with reference method IS 4011:2018, Methods of test for safety evaluation of cosmetics (Third revision).

### 2.2.2. Microbial Inhibition Testing

The *in vitro* microbial inhibition of pure natural oils (macadamia oil, apricot oil, olive oil, argan oil, and jojoba oil) and beard oil formulation was evaluated using the Kirby-Bauer disk diffusion method. Agar plates were inoculated with standardized bacterial cultures of *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), and *Propionibacterium acnes* (ATCC 6919) by uniformly swabbing the suspension to cover the entire agar surface. Discs, pre-soaked in the respective oil samples, were gently placed at the center of the agar without applying pressure or disturbing the discs. The plates were incubated at 36.0±0.1 °C for 24 h to promote bacterial proliferation, with *S. aureus*, *E. coli*, and *P.*

*aeruginosa* subjected to aerobic conditions, while *P. acnes* was incubated anaerobically. After the incubation period, the diameters of the inhibition zones surrounding each disc were measured and documented for further analysis.

### 2.2.3. Efficacy Test

#### 2.2.3.1. Selection of Clinical Study Participants

The study was conducted on 39 men (panelists) aged between 20–29 years for 4 weeks. The panelists were selected based on the inclusion and exclusion criteria. Inclusion criteria include age, regular beard appearance, and normal testosterone level (blood test). Exclusion criteria include history of disease and medication. Before screening, each participant signed a study-specific Informed Consent Form. The panelists were given enough time to read and understand the information provided, ensuring that they were aware of the implications of participating in the study. The informed consent was obtained in accordance with the ICH-GCP Code of Ethics. Clinical coordinators assisted the individual dermatologists in the trial during the informed consent process.

#### 2.2.3.2. Washout Period

The selected panelist's beard was completely shaved and underwent a washout phase for 14 days. Panelists were instructed not to use any beard growth products. The washout phase should ensure that the panelists had a normal 14-day beard at baseline appointment. After 14 days, the panelists came to the study center and underwent baseline assessment (D0) as a normal 14-day beard growth with methods as explained in Section 2.2.3.4.



**Figure 1.** Evaluation of the product (a) hair clipping, (b) shaving test area, (c) phototrichogram, and (d) digital imaging.

**Table 2.** Scoring criteria of visual grading assessment; The worst condition (0), mild (score 1–3), moderate (score 4–6), best possible condition (7–9).

Criteria	Score 0	Score 9
Appearance	Patchy look, visibility of skin under the beard area	Improved appearance. Shinier, healthier, and fuller looking beard
Growth of Beard	Thin and poor growth	Thick and healthy beard
Example		

### 2.2.3.3. Application of the Product

Following the baseline assessment, the panellists were instructed to shave their beards and apply the test product once a day. The test product was initially applied at the study center, under the observation of a clinical coordinator to ensure the product was used in the right amount and site. This was followed by a dermatologist evaluation for skin tolerance (erythema, dryness, oedema, urticaria, or any allergic reactions) and a subject self-assessment. The panelists were requested to report to the study center after 2 and 4 weeks for the following evaluations using the same methodology (Section 2.2.3.4.).

### 2.2.3.4. Evaluation of the Product Efficacy

The panelists visited the study center twice during each evaluation cycle, with a two-day interval between visits. The schedule of assessment is as stated at [Table 1](#).

Before assessment, the panelists were acclimatized for 10–15 min to a temperature of  $22 \pm 5$  °C and relative humidity of  $30 \pm 10\%$ . This acclimatization procedure was done to standardize conditions and minimize variability caused by external factors, ensuring that the study results are reliable and scientifically valid. The evaluation method was using standard Trichoscan assessment protocol ([Figure 1](#)). On day 0, 14, and 28, the fully grown beard was photographed, then 5 hair fibers were clipped from the test areas and their lengths were recorded. The beard was shaved off to a very short uniform length ( $\pm 0.5$ mm) and the evaluation

site of the beard was marked by a stencil marker. The evaluation includes: beard length (as initial length to determine beard growth rate) and beard density by Phototrichogram, beard thickness by Caselite Imaging, dermatologist evaluation, trichologist evaluation, and subject self-assessment. Panelists were asked to keep their beard unwashed (to keep the mark) for 2 days then come again to the study center for trichoscan assessment and complete the evaluation cycle. On day 2, 16, and 30, the evaluation site was photographed again to check beard length and compare with previous data. The increased hair length was calculated for beard growth rate.

### 2.2.3.5. Visual Grading Assessment

The assessment of customer perception and satisfaction was conducted by visual grading by an trichologist and subject self-assessment by a questionnaire. Each panelist was assessed on a scale of 0 to 9 (from worst to best possible condition), and the mean score was recorded. The visual grading assessment score was stated in [Table 2](#).

### 2.2.4. Statistics

The Shapiro-Wilk test was conducted on the baseline values for each parameter using R software to assess the normality of the data distribution. A p-value less than 0.05 indicated a deviation from normality, Wilcoxon signed-rank Pratt Lehmann test was used to compare each follow-up visit and baseline measurements. Conversely, a p-value greater than 0.05 indicated the data followed a

normal distribution, allowing for the application of the paired t-test for these comparisons. The significance level for all statistical analyses was set at 0.05 ( $p < 0.05$ ), and all tests were performed using two-tailed testing.

### 3. RESULTS AND DISCUSSIONS

#### 3.1. Stability and Safety Tests

The formulation combined 7 natural Mediterranean oils: such as apricot kernel oil, olive oil, macadamia seed oil, argan kernel oil, soybean oil, jojoba seed oil, and avocado oil. The results of the single patch test showed that this formulation is non-irritating, while the HRIPT confirmed it is hypoallergenic with no observed negative reactions. In terms of stability of physical appearance, the beard oil remains stable at all stability conditions for 3 months. The yellowish color originally comes from the natural oil, no significant changes observed in terms of color, odor, and sensory feel.

#### 3.2. Microbial Inhibition Test

The formulation, along with each individual oil, was systematically assessed for antibacterial activity against gram-positive bacteria (*S. aureus* and *P. acnes*) and gram-negative bacteria (*E. coli* and *P. aeruginosa*). Table 3 shows the majority of the oils exhibit inhibitory effects on *P. acnes*, the bacterium implicated in acne pathogenesis. Notably, macadamia oil and argan oil demonstrated antibacterial efficacy against *S. aureus*, while olive oil effectively inhibited *E. coli*, and apricot oil showed activity against *P. aeruginosa*. The

combination of these oils in the beard oil formulation effectively prevented the growth of *P. acnes*, addressing the therapeutic needs of facial hair products designed to mitigate acne formation. However, the beard oil did not show any inhibitory effects on other microorganisms, likely due to the lower active concentration of each oil in the final beard oil formulation.

#### 3.3. Beard Growth Rate

This parameter was assessed by phototrichogram, a noninvasive method for measuring hair growth activity. Phototrichogram can capture high-resolution images at intervals and analyze these hair growth parameters: hair density (number of hair per cm area), hair length, and hair growth rate (increased length per day). The study protocol involves panelists visiting the center two days apart during each evaluation cycle to assess growth rate changes after product usage. The test product significantly improved the beard growth rate from baseline (118.89%) to 134.23% ( $p < 0.0001$ ) at week 2 and 147.78% ( $p < 0.0001$ ) at week 4. Overall, the improvement was up to 24.30% compared to baseline as shown in Figure 2. Improvement of beard growth rate indicates that the beard can grow faster compared to non-treatment growth (baseline).

#### 3.4. Beard Density

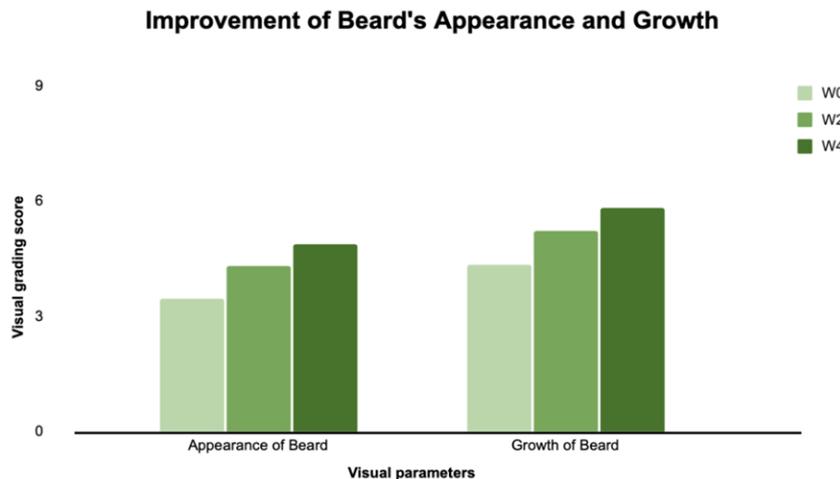
This parameter was also assessed by phototrichogram, quantifying the number of hairs per square centimeter. The beard density significantly improved from baseline (41.73%) to

**Table 3.** Bacterial growth inhibition test result on natural oils and beard oil formulation.

Natural Oil	Bacteria (zone of inhibition)			
	<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>P. acnes</i>
Beard oil formulation	-	-	-	10 mm
Apricot oil	-	-	12 mm	-
Olive oil	-	16 mm	-	20 mm
Macadamia oil	14 mm	-	-	14 mm
Argan oil	16 mm	-	-	18 mm
Jojoba oil	-	-	-	18 mm



**Figure 2.** The improvement of beard growth rate, beard density, and beard thickness after 2 and 4 weeks of beard oil application.



**Figure 3.** The improvement of beard growth rate, beard density, and beard thickness after 2 and 4 weeks of beard oil application.

50.08% ( $p < 0.0001$ ) at week 2 and 61.94% ( $p < 0.0001$ ) at week 4. Overall, the improvement was up to 48.43% compared to baseline as shown in [Figure 2](#). More beard grows from the skin, making the beard area look fuller than a non-treatment beard.

### 3.5. Beard Thickness

This parameter was assessed by Caselite Imaging. This method uses 3D polarized light technology to scan the area and measure the beard thickness from the beard cross section. The beard thickness significantly improved from baseline of 31.19% to 34.05% ( $p < 0.0001$ ) at week 2 and 36.91% ( $p < 0.0001$ ) at week 4. Overall, the improvement was up to 18.33% compared to baseline as shown in [Figure 2](#).

### 3.6. Appearance and Growth of Beard

A well-grown beard has a dense, thick, voluminous appearance, and also feels smooth to the touch. This parameter was assessed by trichologist evaluation and subject self-assessment to determine the visual changes in the beard's appearance and texture before and after the product application. Trichologist grading revealed significant enhancement in beard appearance, with greater fullness and a healthier look after 2 and 4 weeks of product use, as shown at [Figure 3](#). By self-assessment questionnaire, all panelists agreed that the beard growth improved after using the beard oil and were satisfied with the result. The panelists noticed that the beard grew denser, darker and fuller after 4 weeks of application. The beard was also reported to be smoother because the beard oil

provides nourishment to their beard. Based on dermatological assessment, no erythema, dryness, oedema, urticaria, or any allergic reactions happened after 4 weeks application.

The beard growth improvement of two panelists was shown in Table 4, comparing the beard before and after using the product for 2 and 4 weeks. The panelists already had a normal 14-day beard at baseline (W0) then the beard was shaved off. This result showed the beard appearance significantly improved after 2 and 4 weeks applications.

### 3.7. Role of Natural Oils

As mentioned earlier, oxidative stress in the dermal papilla cell or hair follicle leads to hair loss and depressed hair growth [10]. Free radical scavenging enzymes in skin tissues and hair follicle cells may offer a protective mechanism for ROS, but continued exposure can reduce these antioxidant enzymes. Increase of endogenous antioxidant capacity may be needed to improve cell protection from oxidative damage [9][10][13]. Research showed that elevated intracellular ROS leads to oxidative stress and induces hair follicles to enter catagen phase [16][17]. Improved antioxidant capacity of hair follicles can slow the acceleration of catagen phase, therefore maintain proper hair growth [16]. The use of antioxidants may improve hair growth by scavenging ROS and supporting cell growth, as mentioned in research using antioxidants as adjuvant therapy of hair loss [18][19].

The formulation combined seven natural Mediterranean oils: such as apricot kernel oil, olive

oil, macadamia seed oil, argan kernel oil, soybean oil, jojoba seed oil, and avocado oil, which historically have good efficacy for hair. All of these oils provide a rich source of unsaturated fatty acids, including oleic and linolenic acids, which enhance the appearance of the beard, as indicated by subject self-assessments. The emollient properties of these oils offer hydration which maintains moisture levels in the scalp and hair [20]-[27]. The unsaturated fatty acids also form a protective barrier around the hair shaft to prevent hair damage and enhance the hair softness. Argan oil, apricot oil, olive oil, jojoba oil, avocado oil, macadamia oil, and soybean oil are rich in the antioxidant components, such as vitamin E, phenolic compounds, and carotenoids, protect the hair from oxidative stress, including UV-induced damage, thereby mitigating hair loss and promoting hair growth [21][25]-[30]. This efficacy is evidenced by significant improvements in the growth rate, thickness, and density of the beard observed after 2 and 4 weeks of application. Moreover, avocado oil and soybean oil also impart anti-inflammatory effects to enhance scalp health and foster the healing of damaged tissues caused by the UV radiation [25][26]. The antioxidant, anti-inflammatory, and emollient effect of natural oils are suitable to prevent and repair scalp and hair damage. It nourishes, reduces hair loss (due to oxidative stress), and improves hair growth. The exact mechanism of beard oil formulation in enhancing beard growth still needs further study. *In vitro* testing should be done to understand mechanisms of each oil in terms of hair growth and

**Table 4.** Beard appearance of two panelists at baseline, Week 2, and Week 4 assessment.

Panelists	Beard Appearance		
	Baseline	Week 2	Week 4
Panelist 24			
Panelist 25			

nourishment. These findings suggest that the application of hair oil containing apricot kernel oil, olive oil, macadamia seed oil, argan kernel oil, soybean oil, jojoba seed oil, and avocado oil contributes to overall enhancement of the panelists' beards. This formulation also can be used to improve scalp hair growth as alternatives for hair loss therapy.

#### 4. CONCLUSIONS

Formulation contains of seven Mediterranean oils, namely apricot kernel oil, olive oil, macadamia seed oil, argan kernel oil, soybean oil, jojoba seed oil, and avocado oil, was significantly effective in improving beard growth, density, and thickness. It was also effective in imparting antibacterial effects against *S. aureus*, *E. coli* and *P. areuginosa*. It was non-irritating, hypoallergenic and safe for topical use.

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Conceptualization H. Z., D. P. and R. S.; Methodology and Data Curation, H. Z. and D. P.; Resources, Validation and Writing – Original Draft Preparation, D. P.; Formal Analysis, Visualization, Project Administration, and Writing – Review & Editing, H. Z.; Supervision and Funding Acquisition, R. S. All authors have read and agreed to the published version of the manuscript.

#### Conflicts of Interest

H. Z., D. P., and R. S., are employees of Paragon Technology and Innovation, which fully paid for this study.

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