International Journal of Herbal Medicine
Available online at www.florajournal.com

E-ISSN: 2321-2187
P-ISSN: 2394-0514
www.florajournal.com
IJHM 2023; 11(4): 01-06
Received: 03-05-2023
Accepted: 07-06-2023

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Clinical trial to assess the safety and efficacy of hair grow serum product in volunteers with hair fall and healthier hair

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DOI: https://doi.org/10.22271/flora.2023.v11.i4a.870

Abstract
All genders, ages, and ethnicities experience hair loss, which has both physical and psychological implications. The currently available synthetic drug-based conventional therapy for hair loss is still not perfect and has a number of drawbacks. Both their efficacy and the safety of their use are frequently questioned. It has spurred interest in less harmful alternative treatments, such as homeopathic formulations and/or their active ingredients. In healthy male and female volunteers with hair loss, this clinical trial was created to assess the effectiveness and safety of a hair serum formulation comprising Arnica, Jaborandi, *Cantharis*, *Calendula*, Embelica Off., and Bacopa mon. The study included 30 subjects in total, who used the test product every day for 90 days. The test product's effectiveness at boosting hair growth rate, hair density, anagen hair, telogen hair, and the density of vellus and terminal hair was assessed using TrichoScan®. Both dermatologists and subject self-assessment questionnaires compared hair thinning and hair fall decrease to their baseline conditions. In comparison to the baseline measurement, there was a considerable improvement in the rate of hair growth, hair density, vellus hair density, terminal hair density, hair thinning, and hair density without bulb. During the trial, adverse events were not documented. There were no reports of skin intolerance during the study, and the test product was considered dermatologically safe to use.

Keywords: Anti-hair fall serum, Vellus hair density, hair growth, TrichoScan®, hair follicles

1. Introduction
People of all ages are susceptible to the prevalent condition of hair loss [1]. Androgenetic alopecia, the most prevalent kind of hair loss, is characterized by the development of very fine vellus hairs as a result of the shrinkage of the hair follicles, giving the impression of baldness [2]. Autologous hair follicle transplantation and therapeutic intervention with medications that stimulate hair growth are currently accessible alopecia treatment options. There are only two hair growth-promoting medications on the market that have received FDA approval, notably minoxidil and finasteride [3]. Due to adverse pharmacological reactions as skin irritation, sexual dysfunction, and circulatory issues, these medications have a limited number of applications [4]. Additionally, if used for an extended period of time, some steroidal medications often used to treat recurrent dandruff and scalp inflammation, the conditions that contribute to less patterned hair, may have negative side effects [5]. There has been an increase in interest in researching and developing novel therapeutics to treat alopecia, taking into account the psychosocial repercussions of hair loss and the unfavorable side effects of existing treatments. Many societies have employed a variety of plant extracts and homeopathic formulations as traditional hair tonics over the years. Therefore, a logical strategy for creating novel therapies for hair loss is thorough scientific research to assess the potential of alternative treatments, such as Homeopathic supplements, to promote hair growth. Anagen, catagen, and telogen phases make up the hair growth cycle [6]. Dermal papillae cells (DPCs), a subset of specialized fibroblasts found in the hair follicle bulb, are crucial for controlling the hair growth cycle when these phases switch [7]. In order to design a therapeutic for the treatment and/or prevention of hair loss, factors impacting the actions of DPCs are crucial [8]. The anagen initiation of multipotent epithelial stem cells is influenced by a number of signaling molecules, such as Wnts, Sonic hedgehog (Shh), and transforming growth factor-beta (TGF-), among others [9]. A rational strategy for creating innovative medicines for the treatment of alopecia would be to target these complex biochemical signaling networks that control hair development [10].
More than 30% of adults, according to a recent study by the National Center for

Complementary and Integrative Health, use unconventional methods to address their hair loss. Nearly 30.2 billion dollars are spent on hair growth products in the USA alone [11], making this a multibillion dollar industry. Natural product-based topical and oral supplements have long been used to treat hair loss. Minerals, vitamins, and amino acids are frequently recommended alternatives [12]. Unfortunately, there are no formal investigations linking the effectiveness of biotin supplementation with hair growth, despite the fact that biotin has been associated to hair loss [13]. Additionally, doctors urge against using biotin supplements to treat hair loss since they may interfere with testing for thyroid hormone and thyrotropin and interact with tests for troponin and N-terminal pro-brain natriuretic peptide thyroid levels and cardiac syndromes [14]. There are several homoepathic hair loss treatments available, but few of them have solid clinical support. A safe and efficient hair loss management product is thus urgently needed.

Numerous new aetiologies of hair loss are being discovered thanks to improved research methods [15–17]. As a result, the proper topical cosmetics mix and multi-targeted strategy may reduce hair loss and produce quicker results. Additionally, consumers frequently use Homeopathic drugs since they have less adverse effects and higher compliance [12]. Arnica, jaborandi, *Cantharis*, *Calendula*, Embelica Off., and *Bacopa monare* all ingredients in the investigational product known as hair serum that promotes hair growth. A common herb contains elements including vitamin C, phosphate, calcium, and iron that promote the growth of dermal papilla and thicken hair. Additionally, it is a strong inhibitor of the enzyme 5-alpha reductase, which changes testosterone into dihydrotestosterone, which causes androgenic alopecia [18–21]. False albinism is brought on by a selenium shortage, which affects the production of more than 25 mammalian enzymes and results in body hair loss. By encouraging one of the angiogenetic growth factors, the isomeric selenopeptide PeptiSeLect® promotes hair growth [22]. Since a long time ago, coconut and its compounds have been used in hair cosmetics like shampoo and conditioners due to their numerous documented health benefits, which include effects that reduce dandruff and hair fall [23]. With its antioxidant properties, peanut shell extract preserves the hair strand by lowering lipid peroxidation, protein degradation, and tryptophan degradation [24-25].

In the current investigation, we examined the effectiveness and safety of a hair growth serum for reducing hair loss and enhancing follicular density in healthy male and female individuals.

2. Methods Ethics and Informed Consent

Following independent ethics committee permission, the trial was carried out at MS clinical Research in Bangalore (Clinicom Ethics Committee). The Good Clinical Practice Guideline and the principles outlined in the Declaration of Helsinki and its later amendments were followed during the study's execution. The clinical trial registry of India (CTRI) received a prospective registration for the study with the registration number CTRI/2019/08/020742. Before being enrolled in the study, each subject provided their signed and date informed permission.

2.1 Study Design

This open-label clinical trial examined healthy adult male and female volunteers with self-perceived hair loss to determine the effectiveness and safety of hair grow serum. 30 patients between the ages of 25 and 50 who had hair loss that was graded on a 10-point photo numeric scale as being between grades 3-6 were involved in the study. In addition, subjects who agreed to refrain from using hair dye, hair treatments with oils or spa treatments, perming, or straightening were included. Subjects with a hair density of more than 100 and less than 200 hair follicles per square centimeter as determined by TrichoScan® measurement, complaining of hair loss and hair damage, were also included. Participants in the study were not smokers, did not engage in crash dieting or excessive drinking, and had not taken part in a comparable study within the previous three months.

Subjects who underwent hair growth therapy within three months of screening, who have scalp conditions that could interfere with the study, who have recently undergone cancer chemotherapy, and who have a history of alcoholism or psychiatric disorders, including trichotillomania, are all excluded from the study.

There were ten visits in all during the study. The individuals were assessed during the first visit (day 15), which served as a screening visit, using the research inclusion-exclusion criteria. The test region was also shaved during this visit. Two days following shaving (day 13), a density examination was used to validate the final eligibility. On the first (day 15) and last visits, the participants had to complete a "Quality of Life" questionnaire (day 90).

The individuals were given neutral shampoo to use at home following visit 1 and were told not to use it more than three times per week for the two weeks of washout. Following a two-week washout period, baseline evaluations were conducted (day 0), and individuals were administered the test substance. And for three (3) months, participants were told to leave the test product in their hair as a leave-in serum using 2 mL (for men) to 3.5 mL (for women) once daily at night after dividing their hair into sections. Additionally, they were told to keep using neutral shampoo. During the therapy phase, a follow-up assessment was done at the end of each month for three months.

TrichoScan® was used to assess the effectiveness of the test product for promoting hair growth, hair density, anagen hair, telogen hair, the density of vellus hair, and terminal hair (Trichoscan Professional V3.7.27.124, Tricholog GmbH & Datinf GmbH). The subjects were contacted two days prior to each assessment visit so that the assessment area could be shaved.

The measurement site was chosen such that hair in the immediate area is combed for shaving two fingers from the parting, on the frontotemporal regions' receding hairline, or on the vertex. Hair was pulled through the mask after it was put on, and a tidy 1 cm^2 of space was left behind after shaving. After masking the surface area, dye was applied, and after 15 minutes, the area was cleaned using an alcohol-based solution. Images were acquired using a camera while pressing the lens into the moist evaluation region to ensure that no trapped bubbles existed. The software Trichoscan was used to load the captured images and immediately begin the analysis. The same region was located and evaluated throughout the subsequent trips.

The 10-point MSCR photo-numerical linear scale, which is a more sensitive version of the Norwood and Ludwig scales for Indian males and females, respectively, was used to rate the degree of hair loss. In order to correlate the MSCR scale, we also used the Norwood scale for men and the Ludwig scale for women during the baseline and final visits.

A dermatologist used the hair comb test and the hair pull test

to examine variables including hair thinning and reduced hair fall. With the use of the subject's self-assessment questionnaire, the subject's hair and scalp quality were assessed. At the screening and final visits, patients' quality of life improvement was assessed using quality-of-life questionnaires. Based on the occurrence of adverse events, both by the participant and the dermatologist, the study product's safety was assessed.

2.2 Analytical Statistics
30 participants made up the sample size for the statistical analysis. While frequency and percentage are shown for categorical data, mean and standard deviation are used for continuous data. From the beginning of treatment through its conclusion, the efficacy parameters were contrasted. Using the non-parametric Wilcoxon signed rank test, a p value was calculated over the SAQ domain between screening and the completion of the treatment. The comparison of quantitative variables was performed using the two-tailed Student's t-test (age, hair fall reduction, improved hair growth and hair thickness). The effectiveness of the product was evaluated using a paired t-test.

3. Results
3.1 Baseline Characteristics and Demographics
The hair growth serum, 2 to 3.5 mL once day at night, was the subject of this monocentric clinical experiment. Out of 46 screened subjects, 42 healthy adult male and female volunteers with hair loss were enrolled and finished the trial; one was ruled a screen failure and the other three were lost to follow-up (Table 1).

Table 1: Patient Characteristics and Baseline Demographics

Parameters	Hair Serum (n=30) Male Subjects: 2mL, Female Subjects: 3.5mL
Age, years Mean (SD) Range	38.4 (5.16) 25-50
Gender category, n (%) Male Female	15 (50) 15 (50)
Race category, n(%) Asian–Indian heritage	30 (100)
Hair density >100and<200 as per30 (100)	Tricho Scan® measurement

Abbreviations
n, number of subjects; SD, standard deviation.

Efficacy
Dermatological Assessment
Hair Comb Test: The results of Dermatological assessment for Comb Test for hair fall with bulb and without bulb were noted to be significantly less at Day 60 and Day 90 compared to base line. The percentage reduction was 59.38 % and 82.56 % respectively for hair fall with bulb and hair fall without bulb at the end of the study (Fig. 1).

Hair Thinning
Hair thinning was improved significantly at both Day 60 and Day 90 over base line. Test product showed an improvement of 8.01 % and 16.45 % in hair thinning at Day 60 and Day 90, respectively, compared to baseline (Fig. 2).

Hair Fall Test
There was no significant difference noted in hair fall based on linear photo numeric scale at all-time points compared to baseline (Figure 3A). The grading in 26 subjects remained constant while the grading for three subjects increased from 3 to 4 and for one subject, it improved from 6 to 4.

Hair Pull Test
Hair pull test is used to determine how tightly hair is anchored to the hair papilla. The mean number of hairs removed per pull significantly reduced from 2.56 to 1.96 (45.4%) at the end of the study compared to baseline. Statistically significant change was noticed as early as day 30 (Fig. 3B).

Instrument Assessment
Hair Density by TrichoScan®
Statistical analysis of the mean hair density rate measured by TrichoScan®in the test product showed a significant improvement as early as 30 days and at all-time points compared to baseline (Figure 4A). The density increased from 169.35 ± 20.7 per cm2 to 178.01 ± 19.57 per cm2 resulting in a percentage change of 8.17%.

Hair Growth Rate by TrichoScan®
With the continuous application of test product, significant improvement was noted in hair growth rate at day 30, day 60 and 90 compared to baseline. The mean growth rate improved from 305.39 to 359.56 µm/2days resulting in 18.36% change (Fig. 4B).

Anagen and Telogen % by TrichoScan®
The percentage of anagen and telogen was maintained similar throughout the study period; however, there was no decline in the mean percentage (Figure 4C).

Vellus and Terminal Density by TrichoScan®
The assessment for vellus and terminal density is performed on the TrichoScan® Software. There was a statistically significant improvement noted in terminal density at all-time points compared to baseline (Figure 4D). There was a percentage change of 14.51 and 5.13 in vellus and terminal hair density respectively at visit 10 compared to baseline.

Subject Self-Assessment Questionnaire
At all study time periods compared to baseline, subject self-assessments indicated statistically significant improvements in the subjects' total hair fall rate, hair volume, hair texture, and itchy scalp. After visit 9 (Day 60), there was no longer any itch, and by applying the test product consistently, hair loss ceased at Day 90. (Table 2).

Quality of Life Questionnaire
At the screening and follow-up visits, subjects were required to complete the quality of life questionnaire. 92–100% of the

respondents' quality of life improved, according to six surveys about hair loss and how it affected their daily lives (Table 3).

Figure 1 Dermatological assessment for Comb Test. Number of hairs collected after the hair comb test were counted and separated as hair fall with bulb and hair fall without bulb at visit 1, visit 6, visit 8 and visit 10; Values are expressed in Mean ± SE; *p <0.04by*t*-test.

Figure 2 Hair Thinning evaluations by Image comparison. Hair fall was measured using MSCR 10-point photo-numeric hair thinning scale which was compared with the image taken from VISIA CR imaging, lower the grade better the result, values are expressed as Mean ± SE, *p <0.04by*t*-test.

Figure 3 (A) Hair fall assessment by linear photo numerical scale. Hair fall was assessed using MSCR 10-point photo-numeric hair thinning scale compared with the scalp of the subjects directly during their study visits; Lower the value better the result; values are expressed as mean grade ± SE. (B) Hair pull test assessment results. Hair was firmly tugged away from the scalp as fingers slide along the hair shaft and the number of extracted hairs was counted. Values are expressed as mean number of hairs per pull ± SE, *p <0.04by*t*-test.

Figure 4 TrichoScan® Assessment in 1 cm^2 shaved area of the scalp. (A) Assessments of hair density as number of hairs per cm^2. (B) Assessments of Hair Growth rate as length of hair grown per 2 days. (C) Assessment of anagen and telogen %. (D) Density of terminal and vellus hair as the number of hairs per cm^2. Values are expressed as mean ± SE; *p <0.04 by *t*-test.

Secondary Outcomes

Safety: During the course of the trial, there were no adverse events or significant adverse events reported. But after using the product, itchiness, dryness, and dandruff that were noted during the baseline visit as part of the "Subjects Self-Assessment and Dermatological Assessments" disappeared. During the duration of the trial, none of the participants had erythema, allergic reactions, folliculitis, oiliness, burning, or boils on their scalps.

Table 2: Changes in Self-Assessment Questionnaire, Mean (SD)

Product (N=30)	Baseline Day 0	Visit 6 (M1) Day 30	Visit 8 (M2) Day 60	Visit 10 (M3) Day 90
Hair fall rate	2.64 (0.64)	3.39 (0.78)	4.39 (0.78)	4.70 (0.48)
Hair texture	2.59 (0.61)	2.89 (0.45)	3.27 (0.40)	3.65 (0.46)
Hair volume	2.73 (0.53)	2.73 (0.43)	2.85 (0.49)	3.0 (0.51)
Grading hair fall	1.55 (0.52)	0.45(0.6)	0.03 (0.17)	0(0)
Scalpitching	0.83 (0.60)	0.04 (0.33)	0(0)	0(0)

Notes: N, number; SD, standard deviation; M, month; hair fall rate: 1 = more than 100 (worst possible condition), 2= 50–100, 3= 30–50, 4= 10–30, 5= less than 10 (best possible condition); hair texture: 1 = rough and frizzy (worst possible condition), 2= rough, 3= normal, 4= soft, 5= very soft (best possible condition); hair volume score: 1=very less volume (worst possible condition), 2 = less volume, 3 = average, 4 = good volume, 5 = excellent (best possible condition); grading hair fall: 0 = no itching (best possible condition), 1 = negligible itching-no concern, 2 = mild itching, 3 = moderate itching, 4 = severe itching, 5 = very severe itching-compromises the daily routine.

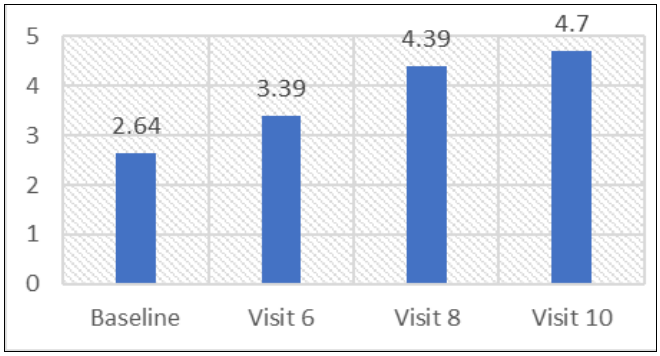

Fig 1: Hair fall rate

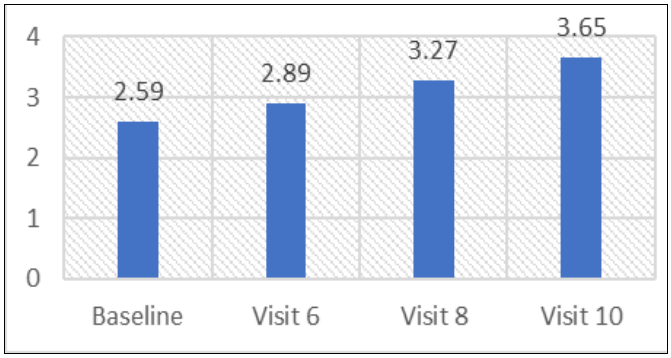

Fig 2: Hair texture

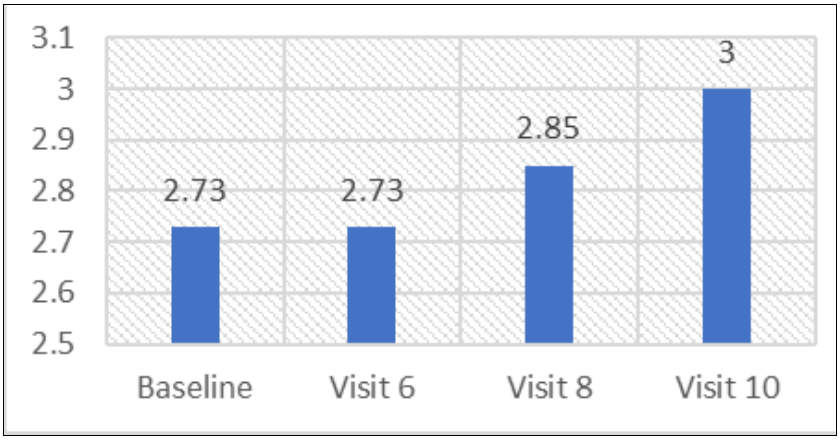

Fig 3: Hair Volume

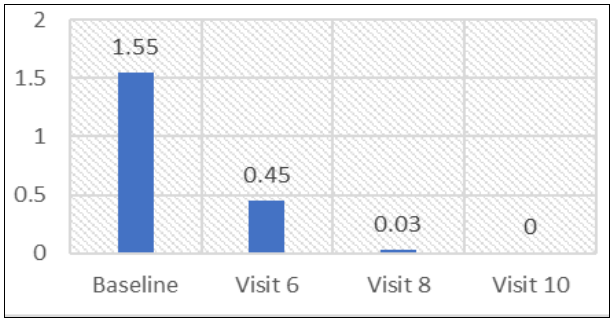

Fig 4: Grading hair fall

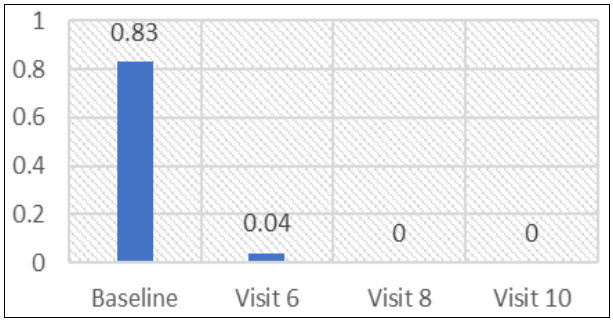

Fig 5: Scalp itching

Table 3: Quality of Life Questionnaire (Change from Base line to Final Visit)

Questions	Visit 1 (Base line)					Visit 10 (Final Visit)				
	Often	Sometimes	Rarely	Always	Never	Often	Sometimes	Rarely	Always	Never
Q1	6(14.29%)	15 (57.14%)	6 (16.67%)	3 (11.9%)	0(0%)	0(0%)	0(0%)	2 (9.52%)	0(0%)	28 (93.33%)
Q2	6 (23.81%)	20(57.14%)	1 m (7.14%)	3 (11.9%)	0(0%)	0(0%)	0(0%)	4(9.52%)	0(0%)	28(93.33%)
Q3	7 (30.95%)	17 (47.62%)	2(4.76%)	4 (16.67%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	30 (100%)
Q4	8(23.81%)	15(52.38%)	6 (21.43%)	1(2.38%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	30(100%)
Q5	4 (11.9%)	20 (69.05%)	1 (4.76%)	5 (14.29%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	30(100%)
Q6	11 (38.1%)	15 (40.48%)	5 (9.52%)	6 (11.9%)	0(0%)	0(0%)	0(0%)	2(4.76%)	0(0%)	29(96.66%)

Notes: Values are expressed as the number of subjects responded for each category and percentage of subjects in parenthesis. Q1: Do you feel uncomfortable, frustrated, or stressed because of your hair fall? Q2: Do you feel that your physical appearance has deteriorated due to your hair fall, and do you look in the mirror too often or avoid looking in the mirror entirely? Q3: Do you feel uncomfortable when people in the community ask you about your hair fall? Q4: Do you avoid your friends or refrain from attending social situations because of your hair fall? Q5: Do you get desperate thinking that your hair fall will never improve? Q6: When treating your hair fall, have you ever thought that you are just wasting time and money?

4. Discussion

In both male and female volunteers who are experiencing hair loss, the current study demonstrates that a hair serum formulation with the active components jaborandi, *Cantharis*, *Calendula*, Embelica Off. and Bacopa mon. enhances hair density and stimulates hair growth. Thus, the therapeutic effects of the hair grow serum comprising these substances were seen in the prevention of hair loss and the promotion of hair growth in human volunteers. The formulation is moderate, safe, and soft on the scalp because it does not include parabens, formaldehyde, or synthetic colors.

A common and bothersome symptom that lowers a person's quality of life is hair loss. A sizable portion of the population, both male and female, is impacted. Treatment for hair loss is required since it can lead to psychosocial consequences including sadness and anxiety.

Since ancient times, homeopathic remedies have been employed in traditional medical systems to treat alopecia. For use in hair treatment, more than a thousand plant species have been researched. Due to the improved blood flow, *Rosemary* oil, grape seed extract, *Hibiscus rosa-sinensis*, sage, and nettles all help to prevent hair loss. Alternative therapies for alopecia include Ginkgo biloba, emu oil, and green tea extracts, which are known to inhibit 5-alpha reductase and lower dihydrotestosterone. The present formulation of the clinically tested innovative hair grow serum contains components that have been researched for the treatment of alopecia with a variety of methods to lessen hair loss and encourage hair growth.

Vascular endothelial growth factor (VEGF) promotes hair growth by increasing the proliferation of cells around hair follicles, according to a number of preclinical and *in vitro* studies. It maintains the equilibrium of the hair cycle by promoting VEGF activity and inhibiting 5-alpha reductase activity, which reduces hair loss and increases hair growth. The fruit of the plant that feeds the hair has various phytoconstituents, including alkaloids, terpenoids, flavonoids, pectin, saponins, tannins, ascorbic acids, carbohydrates, and many other substances. It also has antioxidant and antibacterial properties, which further guards against infections-related hair loss. At all-time points, there was less hair breaking, suggesting that the test product increases the tensile strength of the hair.

Nutritional considerations have an impact on the multifactorial disease of hair loss. Micronutrient supplements may aid in reducing hair shedding, according to recent studies. An increase in hair density, hair shaft diameter, percentage of anagen hair, and mass hair index were observed after three months of therapy with a lotion containing sandalwood odorant, which also reduced hair fall. The anagen-telogen ratio did not alter significantly in the current investigation, but TrichoScan® measurements of hair density, hair growth rate, vellus, and terminal density showed substantial improvements over baseline at all study time points. The scale grade changed in direct proportion to the change in hair density; the higher the hair density, the lower the grade. In the self-assessment questionnaire, the subjects acknowledged an improvement in their hair's progressive growth rate as determined by TrichoScan®. Additionally, subjects significantly improved their hair volume and texture over baseline at all research visits, as measured by a self-assessment questionnaire.

The limited sample size and open-label, single-arm, non-comparative study design are two of the study's drawbacks. Additionally, a comparative study design with a greater sample size, longer length, and a wider population range can increase the scientific value of our clinical investigation.

5. Conclusion

In order to regulate hair thinning and stimulate hair growth, this revolutionary hair grows serum may be a secure solution. It is light, safe, and soothing on the scalp because it doesn't include parabens, formaldehyde, or synthetic color. With a 7.77% increase in follicular hair density, the test product was

generally successful in reducing hair loss in more than 97% of the research group. At the conclusion of the trial, the majority of the subjects reported an improvement in their quality of life as it related to hair loss. The product's color, smell, texture, and effects on hair fall received overwhelmingly good subject evaluation. Because there were no adverse events associated to the study product documented throughout, it may be assumed that the test product is safe to apply topically. The findings of this study demonstrated that the three-month use of the Hair Grow Serum formulation to healthy male and female volunteers experiencing hair loss was both safe and effective.

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