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Vacuum Therapy in Association with Plant Active Compounds, Prebiotics and Probiotics for Treatment of Androgenetic Alopecia, Female Pattern Hair Loss and Telogen Effluvium

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Abstract

The aim of this work was to develop and test an alternative hair loss treatment using vacuum therapy in association with cosmetic emulsions containing plant-based active compounds, prebiotics, probiotics and growth factors, inducing remission of androgenetic alopecia, female pattern hair loss and telogen effluvium alongside their associated symptoms.

Keywords: Hair vacuum therapy • Androgenetic alopecia • Microbiome • Telogen effluvium • Female pattern hair loss

Introduction

For human beings, hair plays an important social and psychological role, and its loss can directly influence self-stem in a negative manner. The intense routine of dermatological clinics is proof to this statement. Hair is one of the most "active" annexes of the skin, connected in a dynamic manner with immunological, endocrine, circulatory and nervous systems. Hair grows in a cyclical manner, alternating phases of rest with phases of intense mitotic activity. Through the follicle, hair crosses the dermis and reaches the epidermis [1]. Hair growth comprises three different phases: (a) anagen phase – intense mitotic activity of the hair bulb; (b) catagen phase – mitosis is interrupted and apoptosis of matrix and shaft cells begins; (c) telogen phase – resting phase [2,3].

Androgenetic alopecia (AGA) is a clinical condition characterized by hair loss; it has genetic causes and is aggravated by circulating androgens. It is more common in females than in males, on which it is known as female pattern hair loss (FPHL) [2,3]. In these conditions, testosterone is transformed into dihydrotestosterone (DHT) by the actions of the 5-α-reductase enzyme [2,3]. On the other hand, telogen effluvium (TE) is characterized by the loss of not only scalp hair, but of body hair as well. This happens due to the sudden and increased amount of hair which enters the telogen phase, resulting in diminished hair density. This condition can also develop due to physiological variables, such as after birth due to the increased amount of circulating estrogens released by the placenta, and also due to stressful events, such as surgeries, infections, hemorrhages, among others [3].

Nowadays there are several clinical therapies and aesthetic approaches aimed at treating the aforementioned conditions. Still, it is vital first for the cutaneous barrier to be healthy and the skin microbiome balanced, as these prevent proliferation of pathogen microorganisms and maintain skin pH stable, preventing physiological alterations of the scalp. The integrity of the cutaneous barrier can be preserved mainly by properly hydrating; there are several bioactive compounds of topical use which possess antioxidant, anti-inflammatory and depigmenting properties, among others, and also promote cell renewal and regulate local microbiomes [4]. Cosmeceutical trends aim to explore new cosmetic compounds, including prebiotics and probiotics, improving the symbiosis of local skin microbiota. Nutritious substances or modulating substrates which benefit the skin microbiome are known as prebiotics. Probiotics and their products (either generated by action of enzymes or of fragments of cell wall) regulate skin homeostasis via a rich ecosystem of tegument microorganisms, and are known as post-biotics [4].

Epigenetics involves the molecular mechanisms which connect environmental factors and gene expression; epigenetic regulation thus modulates gene expression [5]. Rigorous analysis of hair shaft color, strand caliber and shape, among others, evidences hair loss

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and which are the deficiency sources responsible for such. These associations are often linked to epigenetic regulation [5]. Bioidentical growth factors such as endothelial vascular peptides can induce cell reprogramming at the basal layer of the epidermis, modulating epigenetic elements and improving skin properties.

Vacuum therapy is an aesthetical procedure which can be used in several manners, involving either electronic, mechanic or electrical suction pumps. This procedure can promote hyper-vascularization, hyper-oxygenation, draining of interstitial and lymphatic fluids and muscle relaxing [6,7]. Mechanical skin stimulation is capable of stimulating collagen and elastin synthesis and also increases cell nutrition and oxygenation, and is nowadays widely used for treatment of fibroedema gelloid, improving blood and lymphoid local circulation [6,7].

Methods

Human subjects

The study included 25 volunteers of both genders aged between 18 and 65. The subjects were assessed by a dermatologist and by a trichologist. The participants assessed for hair loss suffered from unspecific hair loss, baldness or telogen effluvium. Only subjects that met the study criteria were included in it; the subjects were healthy and had non-damaged skin at the area of the test, did not change their routine hygiene habits, showed up to the tests at the specified time and signed a form attesting their participation was of free will. The study excluded subjects that: (a) were pregnant women, (b) were breastfeeding women, (c) made use of immunosuppressive, anti-histaminic, non-steroidal anti-inflammatory and corticoid medication up to 30 days before the selection for the study (or up to 3 months in the case of subjects that used corticoid medication), (d) suffered from adrenogenital syndrome or had either ovarium, adrenal or pituitary gland cancer, (e) had polycystic ovaries, (f) suffered from active skin pathologies (either local or disseminated), (g) had a relevant clinical history or evidence of alcohol abuse or of other drugs, (h) had intense exposure to sunlight at the area of evaluation, (i) underwent aesthetical or dermatological treatment at the evaluation area up to 4 weeks before selection, (j), suffered from either local or general skin diseases. A biologist, a chemist and technical recruiters rounded up the professionals involved with the study. The study was unicentric and blind, and aimed to investigate three products from the routine cosmetic market in either under normal usage conditions or in association with vacuum therapy for a period of 57 days (D57). The proposed treatments were applied to subjects in order to reduce hair loss, promote hair strengthening and growth of new strands. This study was carried out in accordance with the norms by National Basis of Research Registry for Unified Research involving human subjects (Plataforma Brasil). The study was approved by the Committee for Research Ethics (CEP) under CAE number 07857119.9.0000.822 in February 24th, 2019. The experimental design was developed by the Ecolyzer clinical research laboratory in São Paulo, Brazil.

The participants were subjected to two hair washes using the Antiqueda shampoo. After the second wash, hair was dried with a towel and after a 3 minute pause the Epigem lotion was applied to the scalp covering the whole baldness area. The lotion remained on the scalp for a 15 minutes period. Then, vacuum therapy (Multiplataform equipment Stim Hair, 150023 – Brazil) was used on the scalp in a circular motion using specific suction cups which caused tissue movements and local hyperemia; the equipment generated negative pressure of 520 mmHg and was used upon the scalp for a 15 minutes period. After the end of the vacuum procedure, the participants were subjected to epicranial massage which stretched skin tissue; the massage was performed mostly using the opponent muscles of the thumbs, consisting of drifting movements towards parietal and occipital lymph nodes. The hair was then again dried with a towel and 10 drops of the Nanofactor solution were applied to the scalp. This procedure was repeated at D0, D7, D14, D21, D28, D35, D42 and D49. Participants were instructed to wash their hair at home 2 times per week using the Antiqueda shampoo and then apply the Epigem lotion before going to sleep and again on the next day.

Evaluation of the efficacy of the cosmetic procedure/vacuum therapy involved clinical analysis by a dermatologist/trichologist using a digital phototrichogram, sensorial analysis carried out by a trained technician and assessment of questionnaires answered by the participants (evaluation of subjective value).

The visual analysis and the phototrichogram assessed the subjects for type of hair loss, hair strand color, amount/caliber, density, oiliness and presence of erythema or pruritus. Each aspect was assessed using the following scale: 0=intense, 1=moderate, 2=light, 3=normal. At the final evaluation at day 57, the dermatologist/trichologist assessed scalps for growth of new hair strands, their caliber/thickness, and also assessed the repigmentation of new hair strands should the participant had grayed hair. The sensorial evaluation had the technician assessing hair parameters at D0 and at D57; the parameters assessed were volume, frizz, softness, hydration, caliber, density, oiliness and general hair appearance, and were rated from 0 to 10, 0 being the worst score and 10 being the best possible score. The evaluation of subjective value was carried out at D57; the subjects answered a questionnaire rating hair growth, speed of growth, caliber of hair strands, hair loss, strengthening of hair strands, hair volume, baldness area, oiliness, redness, itching and repigmentation of grayed hair. The evaluation used either the Rikert scale or another specific scale containing 5 levels of agree/disagree: 1=agree completely, 2=agree, 3=neither agree nor disagree, 4=disagree, 5=disagree completely.

The digital phototrichogram is a non-invasive method which captures images in a standardized manner for hair evaluation in shaved areas of scalp. The images obtained using this method were assessed using a digital microscope or a dermatoscope with 10x-200x lens (Dermall, China). Images were captured with a high-resolution camera (Canon, T3i, Japan) at D0, D1, D28, D56 and D57. Images were standardized by coupling a spacing tube to the equipment which kept the distance between camera and scalp constant. LED lighting was also standardized. Images obtained at D0 and D1 were taken on pairs immediately and 24h after scalp had been shaved (Figure 1); the images were analyzed and compared using the software Image Pro Plus (IPP) v.6, assessing hair strand growth, caliber/thickness, phase (either telogen or anagen), density (number of strands per square centimeter) and pigmentation of grayed hairs.

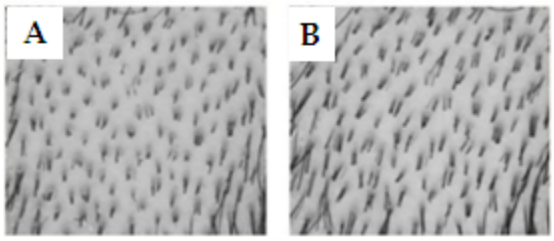


Figure 1: Pair of phototrichogram images from a participant (A) Immediate shaving (D0) and (B) Shaving 24h later (D1).

The images obtained from the scalp of the participants were standardized using a molded cap containing a perforation atop of it, which allowed the images to always be taken from the same area, from D0 up to D57. This procedure ensured the data obtained were precise and representative of the same area of the scalp, as shown in Figure 2.

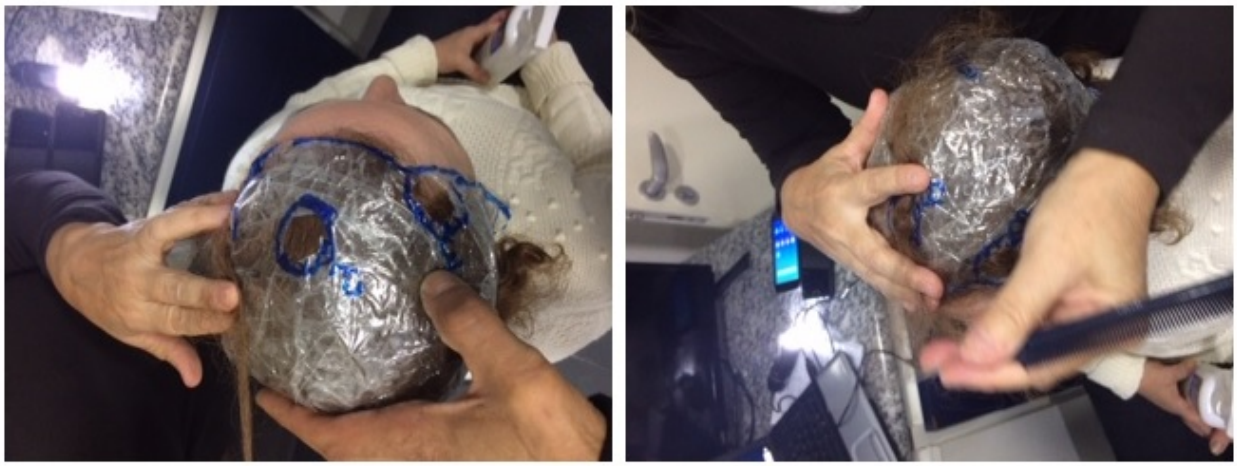


Figure 2: Photoimages showing the perforated molded cap on the head of a participant showing how the same area was analyzed throughout the study from D0 to D57.

Results

All 25 participants were present in all visits at the specified times of study and concluded the study with no complaints or showing any signs of dermatological reactions due to the use of the products tested. Table 1 compiles participant information regarding age, gender, and type of hair loss.

Table 1: Sampling data from the participants of the study.

		Causes for hair loss					
Participants N=25	**Participant age (years)**	**AGA/FPHL**	**TE**	**Stress**	**Hormone deficiency**	**Emotional factors**	**Severe diet**
Male N=6, 24%	28 to 49	N=6 100% (AGA)	N=5 83.33%	N=0	N=6 100%	N=0	N=0
Female N=19, 76%	19 to 65	N=4 21,05% (FPHL)	N=18* 94.74%	N=12; 63,16%	N=4 21.05%	N=2 10.53%	N=1 5.25%

Note: AGA: Androgenetic Alopecia; FPHL: Female Pattern Hair Loss. Sampling data of the participants of the study; TE: Telogen Effluvium; *only 3 female participants suffered from female pattern hair loss alongside telogen effluvium (15.79%).

Initially, the results of the cosmetic treatment in association with vacuum therapy for volume (amount and caliber of strands), density, oiliness, erythema and pruritus were assessed by the dermatologist and the trichologist at the first (D0) and final (D57) days of treatment.

Volume (amount and caliber) of hair strands: the index of hair loss intensity of the participants was assessed (N=25) and rated as 0=intense (8% at D=0 vs. 0% at D=57), 1=moderate (44% at D=0 vs. 8% at D=57), 2=light (40% at D=0 vs. 48% at D57), 3=normal or absent (8% at D=0 vs. 44% at D=57). There was overall improvement of hair loss indexes of 80% (n=20) at D57 in comparison to D0.

Density of hair strands (scalp cover): the index of scalp cover reduction of the participants was assessed (N=25) and rated as 0=intense (16% at D=0 vs. 0% at D=57), 1=moderate (48% at D=0 vs. 8% at D=57), 2=light (36% at D=0 vs. 80% at D57), 3=normal or absent (0% at D=0 vs. 12% at D=57). Participants overall had only a slight reduction of scalp cover reduction at the end of the study.

Oiliness of scalp: the index of scalp oiliness of the participants was assessed (N=15) and rated as 0=intense (0% at D=0), 1=moderate (60% at D=0), 2=light (40% at D=0 vs. 93% at D=57), 3=normal or absent (0% at D=0 vs. 7% at D=57). There

was an improvement of scalp oiliness of 67% (n=10) in comparison to D0.

Scalp erythema: the index of erythema on the scalp of participants was assessed (N=25) and rated as 0=intense (4% at D=0), 1=moderate (0% at D=0), 2=light (80% at D=0), 3=normal or absent (16% at D=0 vs. 100% at D=57). There was an improvement on erythema presence of 84% (n=21) in comparison to D0.

Scalp pruritus: the index of pruritus on the scalp of participants was assessed (N=25) and rated as 0=intense (4% at D=0), 1=moderate (0% at D=0), 2=light (8% at D=0), 3=normal or absent (88% at D=0 vs. 100% at D=57). There was an improvement on pruritus presence of 12% (n=3) in comparison to D0.

Growth of new hair strands: it was observed that at D=57 68% (n=17) of the participants showed growth of new hair, and 32% (n=8) of the participants had those hair strands of good quality.

Caliber of new hair strands on the scalp: it was observed at D=57 that 52% (n=13) of the participants showed growth of new hair strands of thicker caliber in comparison to the hair strands assessed at D=0, 36% (n=9) of the participants had even thicker hair strands growing, and 12% (n=3) of the participants had hair strands growing at the same thickness as seen at D=0.

Color distribution of hair (N=25)/repigmentation of new hair strands in participants with grayed hair (N=7): at the first visit 28% (n=7) of the participants had grayed hair; among these, at the final visit (D=57) grayed hair became lightly repigmentated for 86% (n=6) of the participants and moderately repigmented for 14% (n=1) of the participants.

In addition to the visual assessment, analysis of the phototrichogram was carried out as well, as shown in Table 2. Hair volume (from 70 at D=0 to 80 at D=57, p=0.045) and strand density (from 9.95 at D=0 to 12.34 at D=57, p=0.029) significantly increased due to application of cosmetic products in association with vacuum therapy.

Even though there was no significant statistical difference for hair thickness (from 5.49 at D=0 to 5.56 at D=57, p>0.05), the clinical evaluation evidenced the caliber of the strands increased, which is probably due to the observation of the hair distribution on the area lacking hair which is larger in comparison to the area assessed by the phototrichogram.

Table 2: Statistically significant parameters as assessed by the phototrichogram.

Parameter	Value D=1	Value D=57	p
Number of strands per area unit	70	80	=0.045
Strand thickness	5.49	5.56	>0.05
Strand density	9.95	12.34	=0.029
Strand growth rate	30.7 %	31.0%	>0.05
Number of strands at anagen phase per area unit	68.5	78.9	=0.038
Median of strands at telogen phase	4	2	>0.05
Strand pigmentation (density of white strands)			N=4*

As for the growth of new strands, clinical evaluation evidenced that 68% of the participants had a few new strands growing in comparison to the results from the phototrichogram (30.7% at D=0 to 31.0 at D=57, p>0.05), which resulted in no significant difference; however, the amount of strands at anagen phase (68.5 at D=0 to 78.9 at D=57) increased significantly. These effects can probably be improved should the time of treatment be extended. This hypothesis also applies to strand repigmentation, as the dermatologist observed an increase of 86% of strand pigmentation.

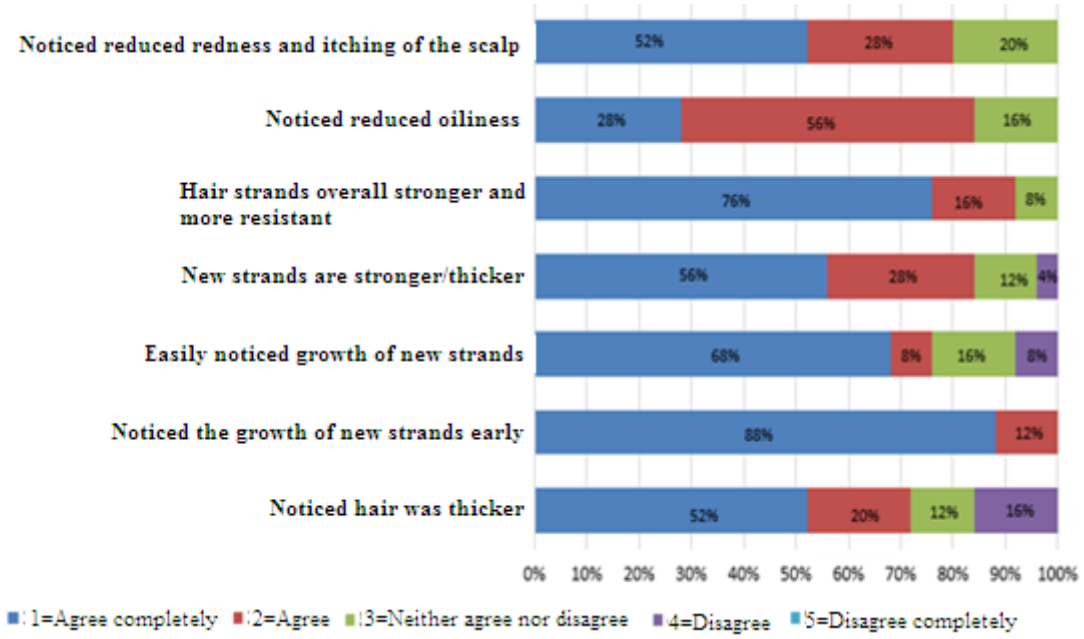
Clinical analysis also evidenced the products used were efficient in reducing scalp oiliness, erythema and pruritus, which interfere with hair strength and growth of new strands. Subjective analysis indicated the participants noticed a quicker growth of new and thicker hair strands, corroborating the hypothesis that hair loss becomes reduced due to increase of hair volume and density as quantitatively assessed.

The results for the sensorial analysis, as carried out by a professional technician, regarding hair volume, brightness, frizz, softness, hydration, caliber, density, oiliness and overall appearance at days 0 and 57 are as follows:

I – After analysis by the technician, it was evidenced scores for most parameters were increased from scores 3 to 8 after 57 days of study.

II – The improvement indexes of the assessed parameters at the initial visit (D=0) in comparison to the results at the final visit (D=57) evidenced ratings ranging from 36 to 92%. Improvement indexes were 80% for hair volume, 84% for hair softness, 84% for hair hydration, 68% for hair brightness, 36% for frizz, 48% for strand caliber, 56% for hair density and 60% for oiliness. These data reinforce the final results for improvement indexes which reached 92% for overall hair parameters after 57 days of study.

As for the subjective analysis, the participants answered a questionnaire regarding certain parameters of the study after 57 days, such as presence of thicker hair, quicker hair growth, hair loss, hair strengthening and strand resistance, hair volume, oiliness, redness, scalp itching and strand repigmentation (in the case of participants that had greyed hair). Some of these results are shown in Graph 1.



Graph 1: Subjective analysis carried out at D=57 regarding scalp redness and itching, oiliness reduction, overall hair aspect, faster strand growth, strand strengthening and new strand caliber (n=25).

According to the participants (N=25): 52% fully agreed there was reduction of redness and scalp itching; 28% fully agreed there was oiliness reduction while 56% moderately agreed; 76% fully agreed hair grew stronger and more resistant; 56% fully agreed hair grew thicker while 28% just agreed; 68% fully agreed hair grew more easily; 88% fully agreed noticing new hair strands growing; 52% fully agreed noticing new hair strands as thicker.

Other parameters were evaluated in the questionnaires but are not shown in Graph 1, such as: (a) hair loss – 60% believed there was a moderate reduction of hair loss; (b) volume – 56% believed there was a moderate volume increase; (c) area lacking hair – 92% believed there was no reduction in the area lacking hair; (d) growth of new hair strands – 68% believed noticing growth of new hair strands after 14 days of treatment; (e) strengthening of strands – 64% believed after 14 days of treatment hair strands were stronger; (f) strand pigmentation – 57% neither believed nor disbelieved the new hair strands had any color; (g) ease of use – 84% of the participants believed the products were easy to apply to the skin; (h) purchase intention – 92% of the participants would certainly purchase the products tested in the study.

Discussion

Scientific literature evidences the efficacy and the mechanism of action of certain individual components found in the studied products. These key ingredients and their respective mechanisms of action are described in Table 3.

Table 3: Active compounds and their respective mechanisms of action as found in the products used in the study.

Active compound	Mechanism of action
Rosmarinus officinalis	Tegument antioxidant and local circulation stimulating agent [8]
Menta piperita leaf extract	Provides freshness during desquamation processes and detoxifies follicles [9]
Arnica montana extract	Balances microbiome, prevents scurf and desquamation easing scalp itching [10]
Daucus carota extract	Antioxidant, beta-carotene rich and minimizes the effects of α-5-reductase enzyme [11]
Serenoa serrulata	Prevents seborrhea, anti-androgenic, anti-eczema and minimizes the effects of α-5-reductase enzyme [12]
Sodium laureth sulfate and disodium laureth sulfossuccinate	Promotes cleaning and facilitates penetration bellow critical micellar concentration [13]
Cocamidopropyl DEA	Promotes soft cleaning [13]
Lactic acid	Promotes cutaneous hydration and regulates pH [13]
Pilocarpus microphyllus leaf extract	Prevents seborrhea and stimulates local circulation [14]
Persea gratissima leaf extract	Hydration agent and antioxidant [15]
Yeast extract	Balances tegument microbiota [16]

Prunus cerasus extract	Keratolytic and antioxidant [17]
Saccharomyces ceriviciae	Probiotic and promotes recovering of cutaneous barrier [18,19]
Malphigia glabra	Extract rich in vitamin C [20,21]
Cooper tripeptide-1	Stimulates collagen synthesis. Antioxidant, cicatrizing and anti-inflammatory [22]
Sh-polypeptide-9	Endothelial growth factor which stimulates the formation of new blood vessels and improves tegument elasticity [23,24]
Sh-oligopeptide-2	Cicatrizing agent and promotes keratinocyte cell proliferation [25]
Xylityl sesquicaprylate	Non-irritant formulation preservative [26]

These products are found in commonly marketed cosmetic products (Antiqueda shampoo, Epigem lotion and Nanofactor solution), which went through specific stability and functionality tests and were dermatologically tested and approved for human use, as defined by the Guide for Safety Evaluation of Cosmetic Products, ANVISA (2012) [27].

Soft surfactants such as lauryl ether sodium sulfate and lauryl ether sodium sulfosuccinate (anionic) when mixed with cocoamidopropyl diethanolamine (amphoteric) contained in Antiqueda shampoo are able to "softly" clean the scalp due to the reduced charge of formed micelles, to the increased size of the micelles formed in these conditions and to the modification of micelle properties when in solution (shampoos have low viscosity); when associated to glycolic extracts, these surfactants reduce disruptions of lipid barriers on the scalp, and when their concentrations are lower than the critical micellar concentrations they can better interact with other extracts increasing skin penetration [13]. Also, the pH of the Antiqueda shampoo was detected as 5.5. All these technological factors in association with antioxidant, anti-inflammatory, anti-microbial and anti-seborrheic effects of extracts, alongside reduction of the action of α-5-reductase [8-11], contributed to reducing erythema, desquamation, discomfort and irritation of the scalp [13]. This evidenced that the integrity of the scalp was better preserved and thus contributed to the permeation of other products in association with vacuum therapy.

The Epigem lotion contains extracts from Serenoa serrulata which prevent edemas, seborrhea and reduces the actions of α-5-reductase [12]. Other extracts found in the formulation are from Pilocarpus microphyllus, Persea gratíssima and Prunus cerasus which prevent seborrhea, stimulate local microcirculation, are keratolytic [17] and relax tissue, also containing high amounts of vitamins A and D [14-16]. The formulation also contains the water-soluble extract fraction from autolyzed yeast, carefully preserved in order to maintain complex B vitamins intact [16]. The formulation also contains Saccharomyces cerevisiae, a protein and aminoacid rich fungi which also contains T and B vitamins; the presence of yeast extracts confers to the formulation status of both pre and probiotic, which helps maintaining integrity and balance of local microorganisms found in scalp and skin [16,18,19]. The extract from Malphigia glabra has antioxidant properties and acts directly upon the scalp and on hair strands via bioflavonoids, pro-vitamin A and mineral salts, which improve sensorial aspects and softness [20,21].

Growth factors, widely studied nowadays, are cytokines secreted by regulating molecules which act on cell regulation and maturation, being responsible for tissue repair and promoting angiogenesis. These compounds are used in trichology as a means to stimulate the growth of new capillaries, which generates new hair follicles due to the formation of deposits of extracellular matrix. Direct actions of growth factors used in trichology include: increase of anchoring proteins, increased vascularization, vasodilating, and inhibition of α-5-reductase, prevention of cell aging, increased melanin fixation and acceleration of hair strand growth [22]. The Nanofactor formulations contain cooper tripeptide-1, Sh-polypeptide-9 and Sh-oligopeptide-2. Cooper tripeptide-1 is naturally found in biological fluids and can increase the number of hair follicles and the growth of hair strands, as it forms compounds bioidentical to the skin, such as glycosaminoglycans, and also possesses actions as antioxidant and anti-inflammatory [22,23]. Sh-polypeptide-9 is a cell signaling protein which stimulates the formation of blood vessels (endothelial growth factor) which aids migration of cells and nutrients promoting collagen production and healing of wounds. This protein is part of a mix of growth factors: EGF, FGF1, IGF1, FGF2 (hydration and cell revitalization synergy) [24]. The Sh-oligopeptide-2 is also a wound healing and antioxidant agent; it is composed of 70 aminoacids and is also known as insulin growth factor (similar to EGF), and promotes activation of keratinocytes [25].

There is also xilityl sesquicaprylate, which acts as a preserver in cosmetic formulations and causes less irritation than other compounds [26].

The association of all of these properties, alongside maintaining the integrity of the lipid barrier of the scalp ensures local hair physiology is preserved [28]. Such fluid cosmetic formulations containing (a) micelle systems with amphoteric charge (Antiqueda shampoo), (b) polymeric system/anionic emulsion (Nanofactor solution) and (c) maltodextrin based encapsulated system (Epigem lotion) interacted temporarily with the corneum layer of the scalp, reducing its impedance and allowing the permeation of actives to deeper epidermis layers [28]. These formulations allow such not causing local irritation and inflammation, not interfering negatively with the efficacy of other cosmetics associated with vacuum therapy [28-30].

Scientific literature and current medical opinion state the importance of preserving skin microbiome, calling it the "first skin". Changes in its functioning lead to dysbiosis, which impairs permeation of active substances through the skin barrier [30,31].

Thus, in order to aid permeation of actives used in cosmetic formulations vacuum therapy was used, which is commonly used in the treatment of fibroedema gelloid (FEG), as it promotes vasodilation, vasoconstriction and negative pressure oxygenation

[28,32]. This technique was here employed alongside other treatments to promote remission of androgenetic alopecia, female pattern hair loss and telogen effluvium [32].

Vacuum therapy used in the treatment of FEG, via negative pressure, exerts upon subcutaneous tissue increase of muscular and tissue blood flow, removing subcutaneous infiltrate from interstitial fluids and stimulating metabolic exchanges. Mechanical stimulation of subcutaneous tissues evidences that vacuum therapy restarts natural production of collagen and elastin, effects also seen due to therapy with platelet-rich plasma [33], which stimulate cell rejuvenation due to negative pressure [2,3,6,32].

The area subjected to vacuum becomes flushed red (caused by either erythema or hyperemia) due to dilation of arteries stimulated by sympathetic neurogenic mechanisms which release vasoactive substances. Negative pressure promotes arteriolar contraction, increasing blood flow and activating dormant blood vessels, which receive a great amount of red blood cells, quickly promoting redness and increase of temperature [2,3,6,32].

Symptoms of androgenetic alopecia, female pattern hair loss and telogen effluvium caused strictly by genetic factors can be aggravated or anticipated due to epigenetic factors. These factors can be either internal or external and do not modify DNA, but can lead to diseases and physical and mental issues [5,34]. The Italian Society of Trichology considers hair as a health marker associating somatic-physical trichology and expression of specific proteins with stress physiology. This explains genome alterations and allows for understanding of epigenetics [35].

In this context, the present work delineated an experimental study using a clinical protocol associating: (a) vacuum therapy as an alternative therapeutic technique for hair areas (there are no studies which associate this therapy with symptoms of AGA, FPHL and TE such as inflammation, desquamation, tissue fibrosis, seborrhea and greasiness); (b) epicranial massage stretching scalp tissue using hands; (c) cosmetics which use amphoteric micelles for soft cleaning, encapsulated pre and probiotics, polymeric thickeners and emulsifiers associated with growth factors; (d) cosmetic products of physiological pH, low viscosity and containing bioactive compounds which do not interfere with local microenvironment and maintain scalp lipid barrier integrity.

Vacuum therapy comprised tissue displacement, hyperemia and local blood congestion. The physiological response involves increased leukocyte flow, increased calcium apport and better oxygenation [2,32]. Other reflex effects include follicle opening due to suction, reversal of scalp rigidity and fibrosis, skin pH rebalance and reduction of oiliness and inflammation caused by alopecia. These benefits associated with the bioactive compounds used in the formulations increased hair strands in bald areas and improved the caliber of new hair strands (these strands are visible to the naked eye, as shown in Figure 3; which is a videodermathoscope image of the frontal section of the skin after 8 sessions at day 45 of the study).

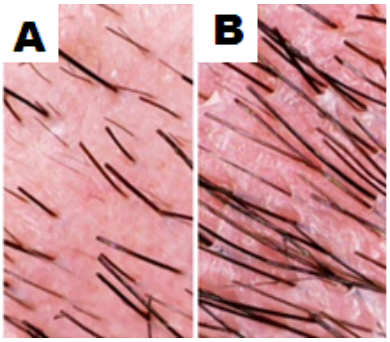


Figure 3: Image of the frontal skin section of a male participant. Vacuum therapy upon androgenetic alopecia and telogen effluvium. Image pairs from the same participant at day 0 and after 8 therapy sessions at day 45.

Table 4 describes the factors leading to hair loss as grouped by similarity.

Table 4: Reasons or factors for hair loss as grouped by similarity: Hormones, stress, and emotional state or severe diet.

Factors leading to hair loss				
Participant group	**Hormones**	**Stress**	**Emotional state**	**Severe diet**
01	6 Males	—	—	—
02	4 Females	—	—	—
03	—	12 Females	—	—
04	—	—	2 Females	—
05	—	—	—	1 Female

From the participants grouped in Table 4, 3 females from Group 2 suffering from FPHL and TE and 3 males from Group 1 suffering from AGA and TE were selected. Both these groups of participants suffered from hormonal abnormalities, which lead to α-5-reductase activation and subsequent rarefying of hair culminating in hair loss. The phototrichogram from both groups were compared, as shown in Figure 4.

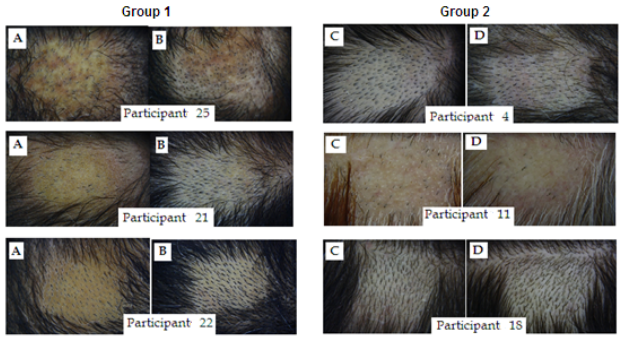


Figure 4: Phototrichogram images from Group 1 (3 males suffering from AGA) (A) Shaving after 24 hours (D1) and (B) Shaving after 57 days of study (D57); and from Group 2 (3 females suffering from FPHL) (C) Shaving after 24 hours (D1) and (D) Shaving after 57 days of study (D57).

By comparing the clinical results and phototrichoscopy images from Groups 1 and 2, inflammatory effects at D1 and the effects of the therapy at D57 can be observed. Inflammation as observed can

be characterized by fibrosis and follicular infundibulum colonized by microorganisms. Such exposure to follicular antigens can lead to scalp inflammation; these factors are not often taken into consideration when describing causes for AGA, but were observed in this study in most of the participants.

Studies diverge on some of the factors that influence AGA and FPHL, such as on the use of finasteride, a selective inhibitor of α-5-reductase, very efficient on combating hair loss in males, but not in women which had already underwent menopause [36]. Studies on etiology of AGA and FPHL alongside follicular alterations on both genders evidence miniaturization is a common factor. This event occurs in different areas and in males tends to occur more often in hair shafts than in females [36]; this is also commonly seen in routine clinical practice.

Figure 4 evidences that, for almost all participants, shaved areas at D1 were hyperpigmented; this pigmentation was brownish and surrounding the follicles, indicating local inflammation and external lymphocyte infiltration. Yellow dots represent empty follicular ostia containing plenty of grease and also melanoses due to photo-exposure; these dots also evidence reduction of amount of strands in a same follicular unit. Patients not suffering from AGA have follicles containing up to 4 hair strands.

After shaving at D57, the phototrichogram of the participants from both genders evidenced reductions of the perypillar sign; these had a softer brownish tone and could not be seen in certain areas. Inflammation was reduced as seen in the images obtained from patients 11, 18, 21, 22 and 25 (Figure 4).

As for follicular ostia clogged with sebaceous secretion (yellow dots), at D57 there was emptying due to treatment using emulsions containing active emollient substances in association with negative pressure at 520 mmHg as exerted by the Stim Hair equipment. During epicranial massage, it was evidenced at the end of the study that scalp tissue mobility was increased, and that scalp appearance was much improved and free of desquamation processes alongside reduced hyperemia and erythema.

Comparing the phototrichogram images as seen in Figures 3 and 4 with the results from Table 2, it is possible to determine that different methodologies indeed increased amount and density of hair strands per area unit alongside induction of hair strands to anagen phase. This must be attributed to vacuum therapy in association with the use of pre and probiotic plant substances alongside growth factors.

The clinical assessment of the dermatologist (Table 5) and the information gathered by the questionnaires answered by the participants (Graph 1) evidence that the therapy employed in this study was efficient in causing remission of the symptoms caused by AGA, FPHL and TE. Graph 1 (subjective analysis) shows 52% of the participants reported reduction of redness of itching, 28% reported reduction of oiliness, 76% reported overall improvement of hair parameters and 56% reported noticing growth of new and stronger hair strands. In comparison to the results described by the dermatologist, oiliness and erythema improved by 67% (n=10) and 84% (n=21), respectively; as for pruritus, 12% (n=3) of the participants suffered from the condition at D0, but none of them still suffered from it at the end of the study at D57.

Table 5: Summary of data obtained from direct clinical assessment as carried out by a dermatologist (symptoms associated with hair loss).

Symptoms	Improvement score
Oiliness	67% (N=10)
Erythema	84% (N=21)
Pruritus	12% (N=3)
Overall aspect	92% (N=23)

Note: N: Number of participants; Total of 25 participants.

The analysis of the results obtained for vacuum therapy in association with cosmetic treatments as described by the dermatologist and by the trichologist regarding qualitative and quantitative growth of hair strands showed significant improvement even in a short time frame: 80% (n=17) for hair strand volume (amount and caliber), 80% (n=20) for density (coverage of scalp), 52% (n=13) for hair caliber and 32% (n=8) for growth of new hair strands. The therapy also induced repigmentation of hair strands for all 25 participants; 7 participants had greyed hair, and from these, 6 had slight repigmentation and 1 had moderate repigmentation. This evidences vacuum therapy in association with cosmetic treatments activates melanocytes, and this effect should be stronger given a greater study time.

Conclusion

Alternative treatment using vacuum therapy in association with active plant substances, prebiotic and probiotics and growth factors was successful in causing remission of AGA, FPHL and TE as well as preventing associated symptoms at significant levels. There was decrease of hyperemia and erythema with absence of desquamation events; these are therapeutic effects of the vacuum therapy in association with bioactive substances which promoted oxygenation, vasoconstriction, vasodilation and rebalancing of local microbiota.

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