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KEY POINTS

- Solutions to address hair loss have met varying levels of success. As such, novel approaches are needed.
- The present article proposes a *Cucurbita pepo* (pumpkin) *var. styriaca* extract to supply nutrients and activate follicular autophagy, in turning energizing hair growth.

Activating Autophagy to Energize

Scalp Hair and Eyelash Growth

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Consumer interest in hair growth products continues to rise. The global market was estimated at US $581.5 million in 2022 and is expected to reach $810.1 million by 2028. Products in demand extend beyond those that stimulate regrowth to include products that lessen or forestall loss and thinning. Such demand is driven in part by lifestyles and increased incidences of illnesses that lead to balding over time.[1]

The quest to develop effective hair loss treatments has been a long one and solutions have met varying levels of success along the way. As such, novel approaches to address this widespread consumer concern are needed. The present article proposes a *Cucurbita pepo* (pumpkin) *var. styriaca* extract to supply nutrients and activate follicular autophagy, in turn energizing the growth phase of the hair cycle.

Hair Growth Cycle

For the present discussion, an explanation of the biology would first be helpful. On average, about 90% of hairs on the human scalp are in the growing (anagen) phase, while 5% are in the transition (catagen) phase and 10-15% are in the resting (telogen) phase.[2] Dermal papilla cells are responsible for the synchronization and orchestration of cyclical hair growth process. New follicular keratinocytes are supplied from the bulge – a stem cell niche in the region of the arrector pili muscle. The follicular keratinocytes travel via the outer root sheath to the hair bulb where they proliferate, expand and cornify (see **Figure 1**).

Any malfunction caused by malnutrition, aging or an extrinsic or intrinsic factor can impair healthy hair shaft production and bring it to a standstill, resulting in hair loss.

After leaving the basal bulb region, a nascent hair traverses approximately 4 mm to emerge from the scalp surface in a process that takes about two weeks.[3] This keratinization/cornification process combines different biological, biochemical and biophysical mechanisms, and it starts above the Auber's Line; i.e., the "line" across the widest portion of the bulb below which cells are undifferentiated.[4, 5]

The terminal differentiation of follicular keratinocytes consumes significant amounts of energy and requires extensive synchronization and organization. The zone in the hair bulb where this happens is called the *ring of fire* (see **Figure 1**). This is the area where the greatest transformation of cells occurs. Eventually all cellular components are broken down, recycled and converted into new building blocks that are used to form hair shafts. Apart from the enormous amount of energy required, the cells also need a reliable recycling system.[6-8]

It is not surprising that the highest level of mitochondrial activity and the greatest autophagy (self-digestion) rate are observed in this zone. Here, cell membranes provided by the Golgi apparatus are used to enclose material to be recycled. The fusion with lysosomes allows the complete degradation of the captured valuable material to form amino acids, sugars, nucleotides and fatty acids – the basic building blocks for all new macromolecules to be formed in cells. This allows the remaining intact mitochondria to be fed again for the production of energy, i.e., adenosine triphosphate (ATP), so that stalled cellular processes can be restarted.[9-11]

Autophagy maintains the constant division of cells, but it is also particularly relevant to the terminal differentiation process in hair follicles and the epidermis – the so-called keratinization process. Hair shafts especially require a high level of deposition of keratins and their tight cross-linking; for this highly oxidative environment, these are essential. (For more on cross-links in hair, see **Page 38**).

Such a highly organized process needs constant monitoring and a reliably functioning supply chain. This means that any malfunction caused by malnutrition, aging or an extrinsic or intrinsic factor can impair healthy hair shaft production and bring it to a standstill, resulting in hair loss.

To optimally supply cells with nutrients and ensure autophagy is activated, a *C. pepo var. styriaca* extract upcycled from press cake was developed. In vivo and ex vivo studies were carried out as described here and compared with in vitro results described elsewhere.

Test Materials

Test extract: To develop the test extract, press cake remaining after oil production of organic *C. pepo var. styriaca* was subjected to extraction in water. This aqueous extract[a] is a rich source of bioactive compounds with interesting nutraceutical properties, such as free amino acids including carboxypyrrolidine cucurbitine, polyamines (spermidine and spermine), polyphenols, proteins and carbohydrates.[12]

Test formulations: Scalp hair tonics were developed containing 1% and 3% active ingredient with water, alcohol denat. and propanediol. Eyelash/eyebrow gel formulations were created with and without 5% active ingredient and water, citric acid, xanthan gum, gellan gum, sodium benzoate and potassium sorbate.

Test Methods

Two double-blind in vivo studies were carried out, one for the scalp hair and the other for eyelashes. Eyebrows also were assessed visually (not shown) and through subjective perception.

The global alopecia market is expected to reach US $14.2 billion by 2028, expanding at a CAGR of 8.1% from 2021 to 2028.

Source: Grand View Research, Inc.

[a] *Hairvivine Pro (INCI: Propanediol (and) Water* (Aqua) *(and)* Cucurbita Pepo *(Pumpkin) Seed Extract (and) Citric Acid) is a product of Rahn AG.*

Scalp hair method: The scalp hair study assessed the effects of the test tonics on hair density and loss in 39 Caucasian volunteers presenting with hair loss (ages 18-70 years). A test tonic was applied daily by gently massaging it into the scalp either immediately following washing or on dry scalp. Measurements were performed on Day 0 and Day 120.

To achieve the same experimental conditions, all volunteers received a neutral, commercially purchased shampoo and were instructed to wash their hair at least three times per week. In addition, volunteers were instructed to not wash their hair 48 hr prior to a measurement visit, and to not comb their hair 24 hr prior to the visit to maintain hairs near the end of the telogen phase and thus avoid an artificial reduction in the percentage of hairs in the telogen phase. The study was performed without placebo but with two different active ingredient concentrations, as noted.

Density measurements: Hair density was measured using a system[b] capable of counting the number of hairs in a defined area (generally per cm^2).

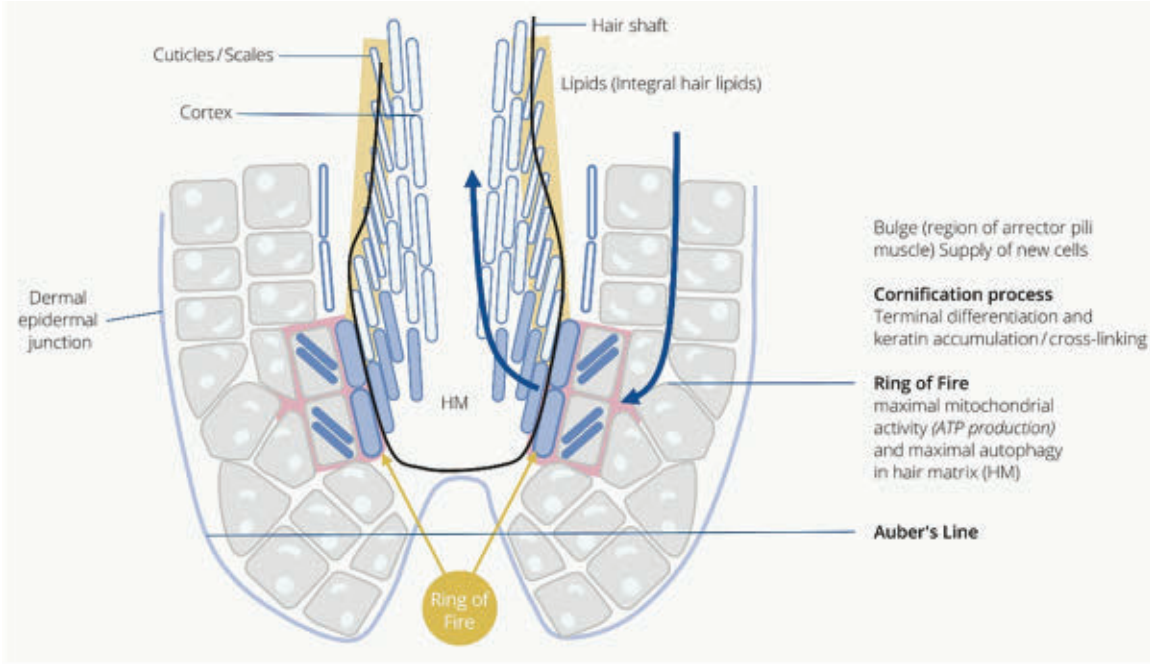
Hair loss measurements: Hair loss was evaluated by washing and combing tests. A technical assistant washed and combed each volunteers' hair. The hairs that fell out during each process under standardized conditions were collected and counted.

Hair aspects: The general aspects of volunteers' hair were also evaluated on the basis of subjective perception. A five-point scale was employed (5 = completely agree, 1 = strongly disagree). Volunteers who responded with scores of 5 or 4 were considered to be satisfied with the results of the treatment.

Eyelash method: To assess the effects of the test gels on eyelashes, 40 Caucasian female volunteers were enrolled in a placebo-controlled study. Volunteers used the 5% formula twice daily for 56 consecutive days. Eyelash length/diameter/volume were quantitatively measured using a 3D digital three-dimensional microscope system[c] at Day 28 and Day 56.

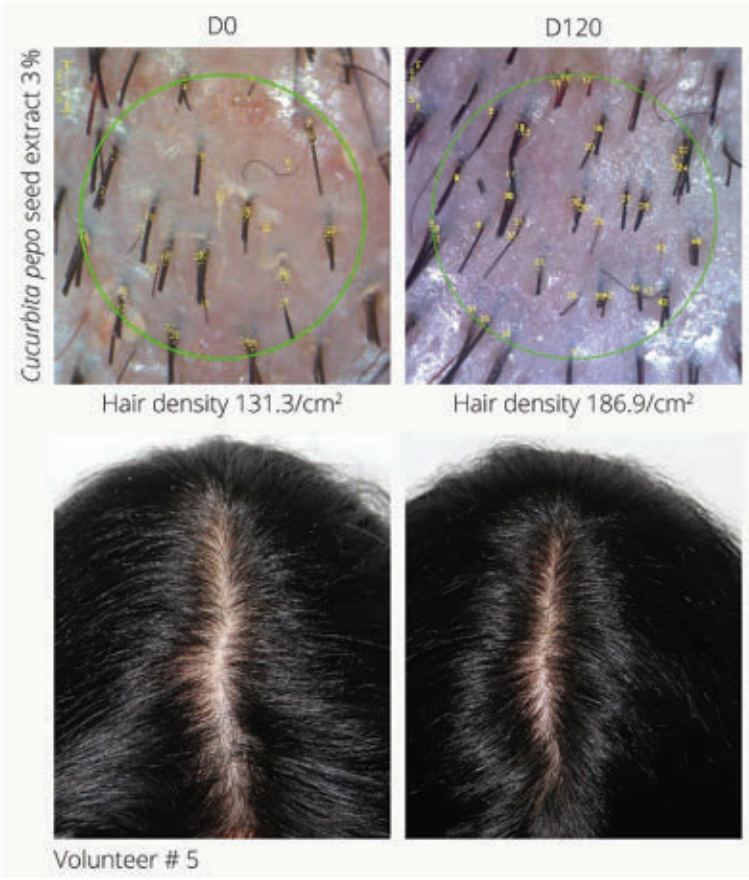
[b] *TrichoScan, Tricholog GmbH & Datinf GmbH*

[c] *HIROX-RX 2000*



The constant supply of new keratinocytes from the bulge region pushes the cells inward. On their way to the hair bulb, they begin to differentiate in the so-called ring of fire. Here, mitochondrial activity as well as autophagy are maximized in order to pave the way for their terminal differentiation into hair shafts.

Figure 1. The cornification of follicular keratinocytes requires considerable energy.



*The area of the green circle is 0.25 cm²

Figure 2. Visible effects on hair density

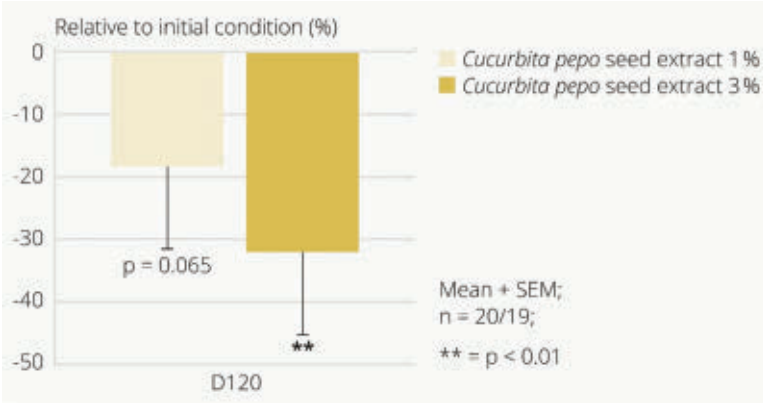


Figure 3. Hair density data

Eyelash/eyebrow aspects: The volunteers also evaluated the general aspects of their eyelashes and eyebrows. For the survey, an eleven-point scale was employed (10 = completely agree, 0 = strongly disagree). Volunteers who responded with scores equal to or greater than 6 were considered to be satisfied with the results of treatment.

Ex vivo method: An ex vivo study was carried out using a human hair follicle culture as described by Philpott et al.[13] Briefly, complete hair follicles (anagen phase) obtained from Caucasian female donors ages 30-55, from nape and peri-auricular skin, were prepared and seeded in 12-well plates (1 follicle/well) in a culture medium with (0.01%) and without (0.00%) the test compound. The development of each hair shaft was monitored for seven days by measuring the length of each follicle.

In vitro method: An in vitro study of the effects of the extract on autophagy and mitochondrial fitness was performed using normal human epidermal keratinocytes (NHEK). The molecular effects have been described elsewhere.[14]

Statistics: Statistical analyses to determine significant changes were performed using the paired (in vivo studies) or unpaired (ex vivo study) student's *t*-test. The statistical values shown in black in the figures are results in comparison with the initial condition; the values in blue are the results compared with placebo treatment.

In vivo Results: Scalp Hair

Scalp hair density: Hair density measurements revealed the *C. pepo* seed extract was capable of dose-dependent stimulation of hair growth. The images captured[b] for volunteer No. 5 (treatment with 3% active ingredient) on day 0 and day 120 are shown in **Figure 2** (upper panel). General images of the frontal and mid-scalp

The aqueous *C. pepo var. styriaca* extract is a rich source of bioactives and nutraceutical constituents, such as free amino acids.

region of the head are shown in the lower panel.

The width of the hair part spacing down the center of the scalp visibly decreased, compared with D0; extrapolation of the measured area to the whole scalp revealed approximately 39,000 new hairs. In general, hair density increased on average by up to 14.5% following treatment with 3% test active (n = 19), and by up to 6.8% with 1% active (n = 20; see **Figure 3**).

Scalp hair loss: A healthy scalp normally loses 50 to 100 hair shafts per day, and different stress triggers may cause the transition anagen hair to telogen hair (by up to 70%). This can cause hair thinning and hair loss.[15] The results of this study showed that on average, hair loss decreased by 32% and 18% after 120 days of treatment with 3% and 1% active ingredient, respectively (see **Figure 4**).

Subjective perception: The test volunteers perceived a decrease in hair loss after 45 days of test product application. This was the case

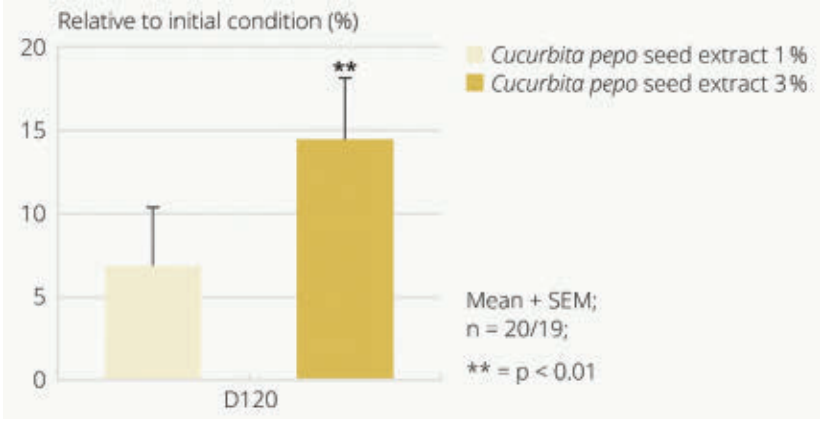


Figure 4. Effects on hair loss

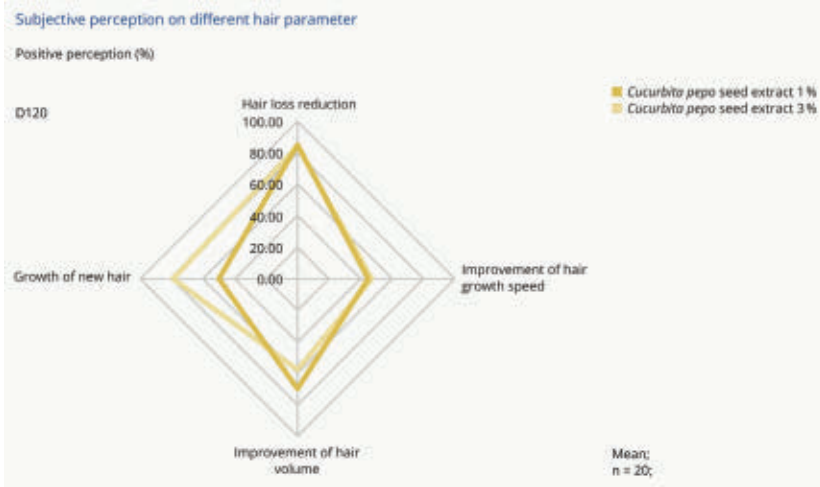


Figure 5. Subjective perception of hair parameters

for 84.2% of volunteers in the group using 1% *C. pepo* seed extract, and for 90% of volunteers in the group using 3% *C. pepo* seed extract (see **Figure 5**).

In addition, the rate of hair growth was perceived to occur sooner, by 42.1% in the 1% test ingredient group (n = 20), and by 50% in the 3% test ingredient group (n = 19) after 45 days of treatment. An improvement in hair volume also was registered by 36.8% of volunteers in the 1% test ingredient group, and by 45% of volunteers in the 3% test ingredient group. Finally, the growth of new hair was reported by 42.1% and 40.5% of volunteers after 45 days in the 1% and 3% groups, respectively.

In vivo Results: Eyelashes

Eyelash length/diameter/volume: Application of 5% *C. pepo* seed extract resulted in time-dependent improvements in eyelash growth, diameter and volume (see **Figure 6b-c**). At both measurement time points (D28 and D56), the improvement was significant in comparison with the initial condition and the placebo.

The test treatment increased eyelash length on average by up to 2.1% and 4.7% after 28 and 56 days, respectively; the difference between the placebo and test formula was greater by a factor of 4 at Day 28 and by a factor of 6.6 by Day 56. Furthermore, there was an average 1.1% and 3% increase in eyelash diameter after 28 and 56 days, respectively; the results for the two treatments differed by a factor of 16 by day 28 and a factor of 11 by day 56.

Finally, eyelash volume improved on average by 5.3% and 11.3% after 28 and 56 days, respectively; results for the placebo and test treatment differed by almost a factor of 6 by day 28 and by a factor of 7.8 by day 56. Visible effects at D28 and D56 for volunteer No. 13 are shown in **Figure 6a**. Improvements to eyelash length and the thickening of eyebrows are already apparent after just 28 days of treatment.

Subjective perception: Volunteers also reported an overall improvement of various eyelash and eyebrow conditions; note that only the eyelash data is provided above (see **Figure 7**). The active ingredient treatment outperformed placebo in all parameters.

Ex vivo study: In the ex vivo study, follicles exposed to 0.01% *C. pepo* seed extract improved hair shaft progression by an average rate of 388%.

In vitro study: As noted, in vitro results are report elsewhere[14] but briefly, 1% of the test ingredient for 24 hr under normal conditions significantly increased the induction of autophagy. Histochemical analysis of the Golgi apparatus revealed an increase in autophagosome formation by 80%, compared with non-treated cells. Gene expression of MAP1LC3A confirmed the induction of autophagy, as the corresponding protein is incorporated into the membrane of autophagosomes. The activation of autophagy also resulted in an increase in

levels of healthy and active mitochondria, providing enough energy for the effective terminal differentiation of follicular keratinocytes.[14]

Discussion and Conclusions

Dysfunctional mitochondria can form due to general aging, but this process is also promoted by disturbances in the metabolism of keratinocytes on their way to becoming corneocytes or hair shafts. Of course, extrinsic ROS and stress are factors that can contribute to dysfunctional mitochondria. ROS can directly disrupt the respiratory chain, halting the production of ATP. This means the cells' energy balance collapses and the results are lack of energy and diminished capability for terminal differentiation.

Enhanced autophagy can break this cycle. Autophagy is important to the terminal differentiation process in the epidermis and hair follicles. The keratinization process needs vast amounts of energy for the synchronized transformation of entire cell material into hair keratin and membrane complexes. Autophagy cleans up the cellular waste as well as dysfunctional mitochondria and delivers a high level of energy for the keratinization process in hair follicles.[6-8]

The results of the ex vivo hair shaft experiment impressively demonstrate a significantly increased growth rate. This is due to the important nutrients present in *C. pepo* seed extract. In addition, the autophagy experiments showed that, contrary to expectation, autophagy was activated, and mitochondrial fitness was increased.[14, 16] Since these two processes are essential for keratinization and hair growth, *C. pepo* seed extract offers a solution to boost hair growth.

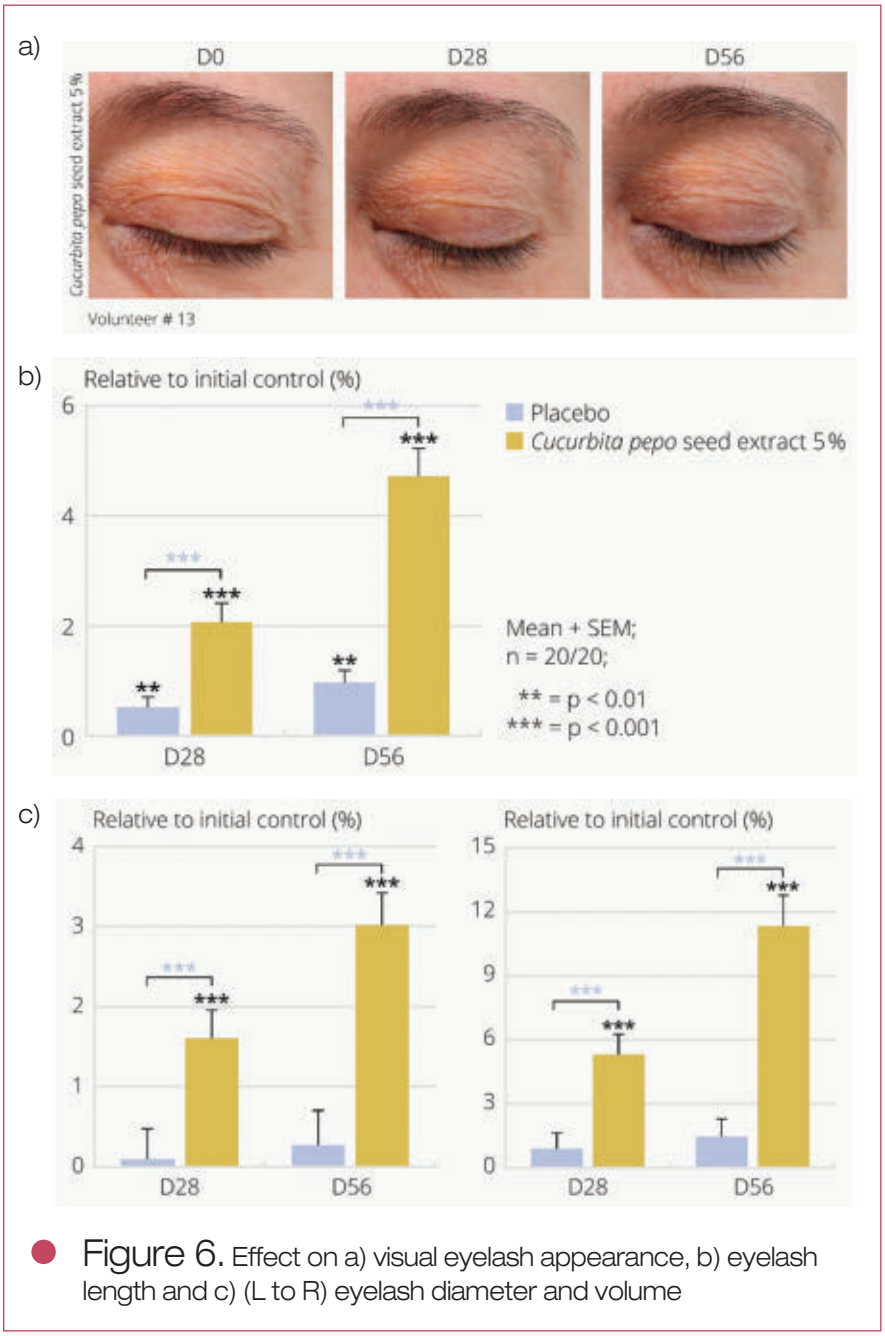


Figure 6. Effect on a) visual eyelash appearance, b) eyelash length and c) (L to R) eyelash diameter and volume

Results of the ex vivo hair shaft experiment demonstrate a significantly increased growth rate.

This was made apparent by the results of the in vivo studies: hair density was improved on average by 14.5% (n = 19) following treatment with 3% extract for 120 days. At the same time, hair loss was reduced by 32%. Eyelash parameters such as length, diameter and volume were also significantly improved over the placebo treatment.

Overall results indicate the test ingredient supplies cells with targeted nutrients, thus activating autophagy and delivering what the cells need to produce vital and strong hair.

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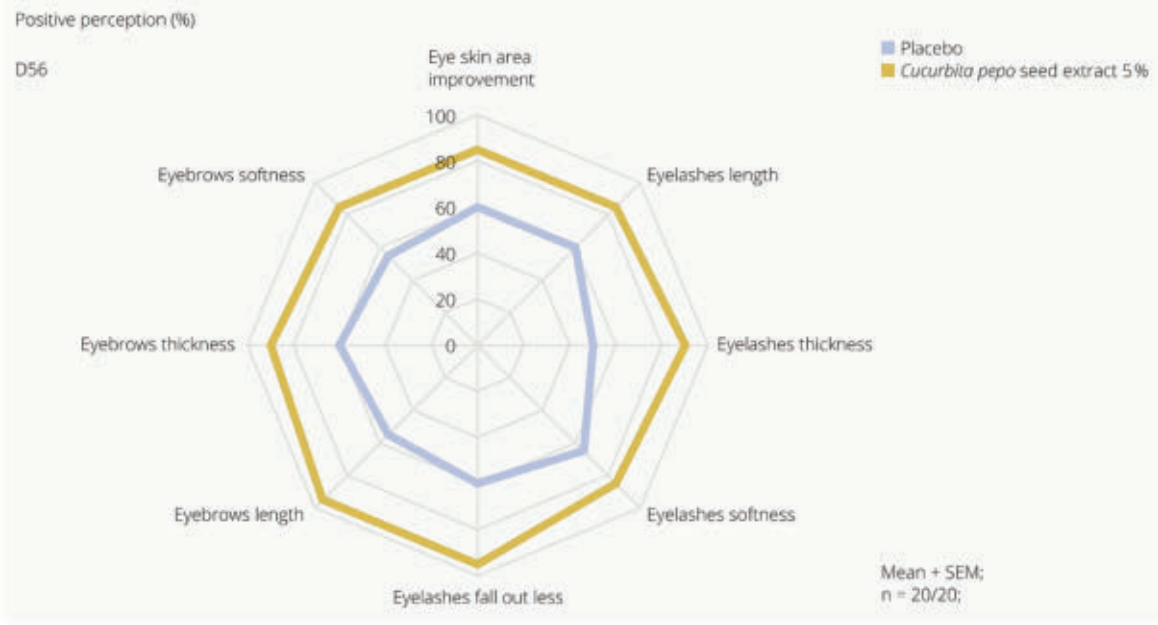

Figure 7. Subjective assessment of eyelash and eyebrow parameters

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