



A revolutionary and at the same time natural formulation that provides lashes real growth

Nowadays, makeup consumers are looking for formulations that go beyond aesthetics. Eyelash mascaras aims to improve the esthetic appearance temporarily, providing to darken, lengthen, and thicken eyelashes by using a combination of waxes, pigments, and resins [1]. Prominent eyes and eyelashes are often considered a sign of beauty and can be associated with increased levels of attractiveness, confidence, and well-being [2]. With that said, a new natural-based ingredients formulation was developed to a multifunctional eyelash mascara that, besides the temporary better aesthetics, also improves the growth phase of follicles providing lashes' growth and volume in a long term.

Results and discussion

The innovative mechanism of action of this biofunctional combination of natural ingredients prolongs the anagen phase of the eyelashes, inducing the growth of the lashes length, in addition to strengthening their anchorage in the follicle, preventing hair loss through the reduction of the telogen phase, protecting its integrity.



Longer anagen phase
Strengths lashes anchorage in the follicle
Prevents lashes loss through the reduction of the telogen phase

Intracellular metabolism improvement

Rich in flavonoids, antioxidants and aminoacids. Increases the density of eyelashes

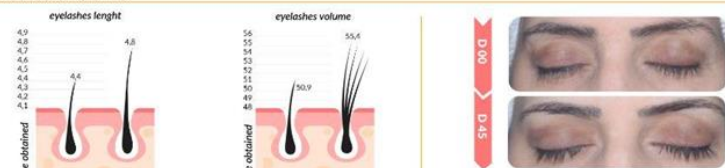
Naturally accelerates the hair growth cycle

Gene expression in vitro assay:

There was a significant increase in the expression of the β -Catenin in HEK293T with a fold change of 1.34. β -Catenin part of the canonical pathway of WNT signaling, it is expressed during the anagen phase, increasing the duration of this phase of the capillary cycle [3]. For both conditions, HEK293T and HHDP, there was an increase in BCL-2 (4.16 and 1.27 resG respectively), a gene that encodes a protein involved in the control of apoptosis, having an anti-apoptotic function [4]. Its expression is increased in the anagen phase of the cell cycle, decreased in the catagen phase and abolished in the telogen phase. Together, these genes are key biomarkers related to the maintenance and prolongation of the anagen phase. It was also detected a significant increase in VEGF α , a growth factor that induces follicular vascularization helping to pre-regulate apoptosis and plays an important role in the anagen-catagen transition being secreted by the dermal papilla [5]. In addition, treatment with the active induced an increase in CK15, a biomarker of stem cell potential, in neopapillae, demonstrating the potential of the active in aging prevention and hair growth [6]. Cytokeratin 15 is also continuously expressed in the outer sheath of the hair follicle, acting in the formation of the fiber.

	HEK293T	HHDP
β -Catenin	1.34***	0.98
BCL-2	4.16***	1.27***
VEGF α	1.87***	2.26***
CK15	1.42	6.70***

In vivo test results after 45 days of use:



The product promoted an increase of 8% in eyelash growth. The product promoted an increase of 9% in eyelash volume. Images obtained from survey participants before and after normal conditions of use of the investigational product in the following conditions D0 (baseline) and D45 (final)

The product perception evaluation is also a strong indication of the fulfillment of consumer acceptability, presenting favorable purchase results in 87% and product of the eyelash growth action in 80%.

Cosmetic Evaluation Questionnaire



Survey volunteers' responses to the questionnaires applied after 45 days of using the product

Materials & Methods

In Vitro:

Baicalin was elected as a model for in vitro validation. In vitro tests were performed to evaluate the effect of baicalin on primary human keratinocytes and dermal papilla cell spheroids. Cells in monolayer or three-dimensional model were treated with 0.032% active in culture medium and gene expression for β -catenin, BCL-2, VEGF and CK15 was quantified by qPCR.

Cell Culture:

HEK cells (Human Epidermal Keratinocytes), obtained from Cascade Biologicals were used. Cells were maintained in culture with Epilife medium (M-EPI-500-CA - Gibco) containing HKGS (Human Keratinocyte Growth Supplement, S-001-5 - Gibco) at 37°C and 6% CO₂. HHDP cells (Human Dermal Papilla Cells) were seeded in six-well low attachment plates (Corning, model 3471) at 2.37x10⁵ cells per well in DMEM containing 10% FBS without antibiotic. The cells were kept at 37°C and 6% CO₂ for a period of 24h. After this period, the spheroids were transferred to 96-well plates for further testing.

HEK293T cultures and HHDP spheroids were used, with 105 HEK293T cells plated in 12-well plates and 3x10⁵ HHDP cells in 6-well Low Binding plates (Corning).

The active was prepared at 0.32 mg/mL (0.032%) in DMEM with 10% FBS. After 24 hours of incubation with the actives, RNA extraction was performed with the RNeasy Mini Kit (74106, Qiagen), and the samples were quantified in Nanodrop and preserved at -80°C. Reverse transcription was performed with the High-Capacity cDNA Reagent RT kit (4368814 - Life Technologies) according to the manufacturer's recommendations. For each sample, 200ng of total RNA were considered as a template for cDNA synthesis. Gene expression was detected by the Real Time PCR (qPCR) methodology through the TaqMan system (4444963 - Life Technologies) with InverGen toriad probes (BCL-2, CK15, β -Catenin, VEGF α). All reactions were performed on 384 wells plate in triplicate using the reaction solution described in table 1.

In Vivo:

The following natural plant-based active ingredients were evaluated in this study:



Conclusions

Eyelashes have an important role in determining beauty, and as such, prominent eyelashes are a highly sought-after attribute. The combination of in vitro and in vivo tests demonstrates the action of the natural plant-based actives present in the color cosmetics formulation, to promote growth and improve the volume of eyelashes through the prolongation of anagen phase and maintenance of the stem cell potential of follicular cells. It is believed that this study opens up a new frontier for makeup with valuable knowledge for the development of new color cosmetics with benefits consumer center.

Acknowledgments

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