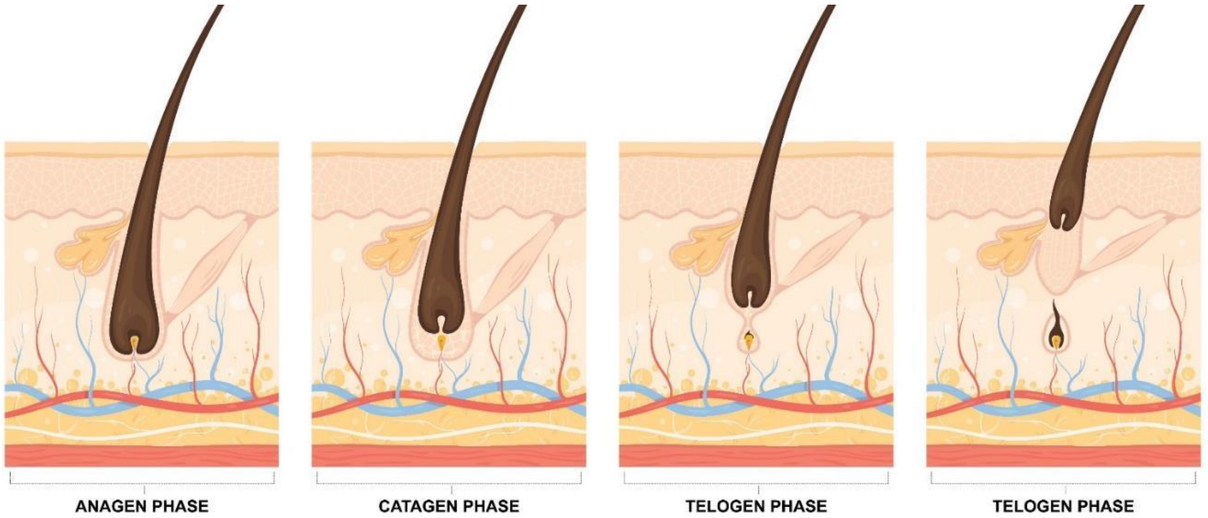
Sinerga
SKIN EVOLUTION

TRICHO-SOLVE®

complete solution against hair loss

HAIR GROWTH CYCLE

→ In physiological conditions



1. Growing phase:

3,5 years –The hair is actively growing and extending. About **90%** of the hair are in this phase.

2. Transition phase:

3,4 weeks - The **follicles atrophy** and **vascularization ceases**.

3. Resting and shedding phase:

2,5 months – Begin when the bulb reaches the erector pili muscle. Consequently, the formation of **new hair displaces the old ones.**

HAIR LOSS TRIGGERS

Hair loss refers to an **abnormal condition**, acute or chronic, **of hair replacement**, in which the amount of hair falling is visibly greater than the amount of hair being grown.

Trichological pathologies could be of

Inflammatory origin

Characterized by an inflammation of the scalp or piliferous areas, induced by both **internal stressing agents** (genetic predisposition, stress) and **external stressing agents** (season changes, lack of sleep, hair dryer pollution, UV rays).

Hormonal origin

Induced by the action of androgens on the hair bulb (the most common forms is **androgenetic alopecia**).

Androgens shorten the anagen phase and promote follicular miniaturization, leading to gradual hair thinning.

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HORMONAL ORIGIN

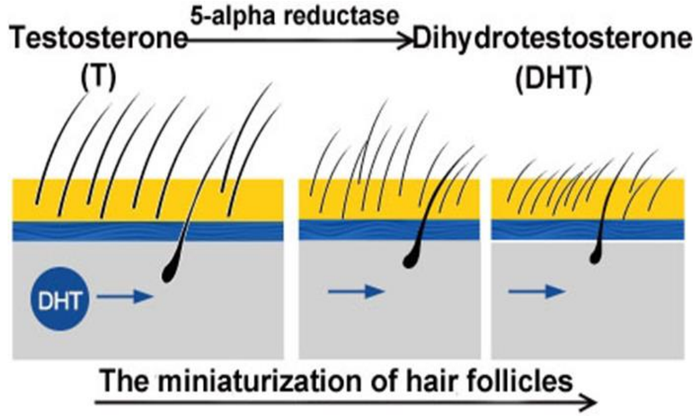
Hair loss disorders of **hormonal origin** involve dysfunctions in the number of androgenic hormones (**DHT** - **dihydrotestosterone**) that deeply affect hair follicles. This unbalance is often caused by over-productive enzymes that regulate their metabolism.

What is the connection between DHT and hair loss?

DHT (dihydrotestosterone) is the result of the metabolism of testosterone; this conversion is **regulated by the enzyme 5α-reductase.**

Excess of **DHT**, caused by the overexpression of 5α-reductase**,** leads to:

acceleration of the anagen phase → miniaturization of hair follicles → regression to vellus hair



Androgenic alopecia is a form of hormonal hair loss caused by a higher production of dihydrotestosterone and higher levels of 5 alpha-reductase. This symptom can happen in both male and female, although the severity and frequency are greater in men than women and in some specific ethnic groups.

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INFLAMMATORY ORIGIN

Another trigger for hair loss could be inflammation, in which **IL-1 α** is involved.

What is the connection between IL-1α and hair loss?

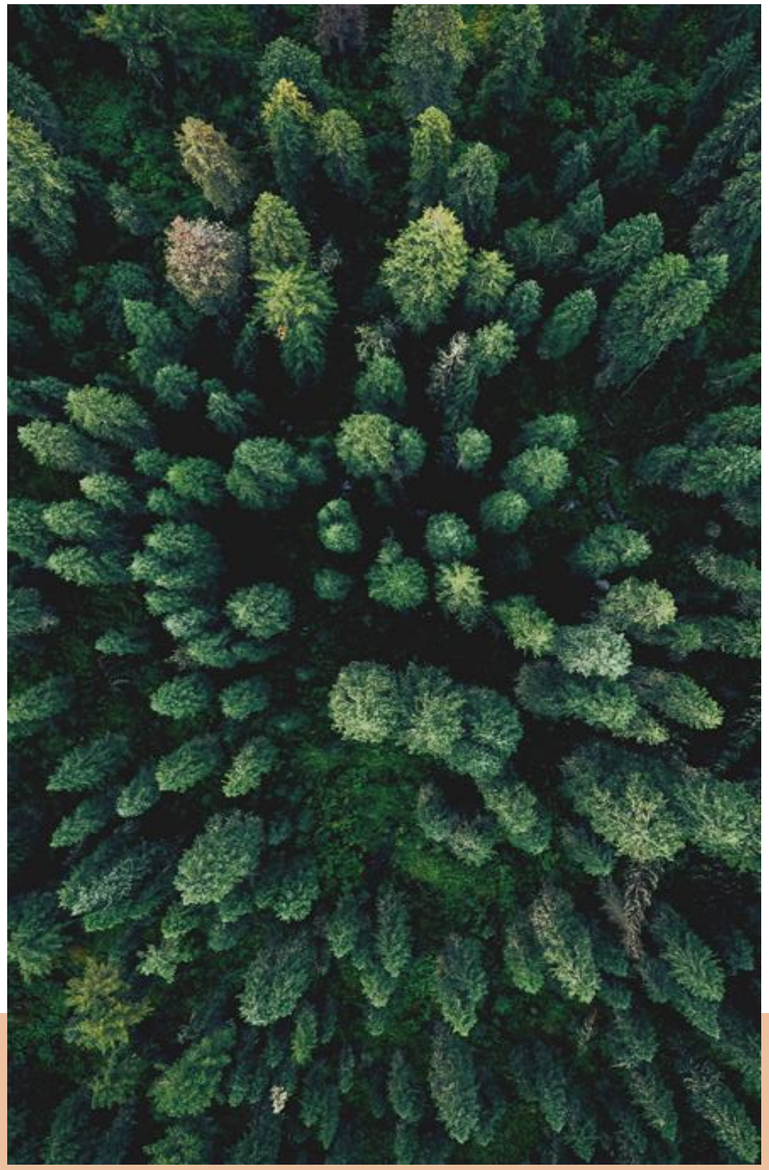
IL-1 α is a highly **pro-inflammatory cytokine** that promotes the recruitment of T-lymphocytes to inflamed tissues, **activating the immune reponse**.

The production of IL-1 α, in a inflammatory conditions, lead to:
inhibiting hair follicle growth → inhibiting hair fiber production

Thus, **IL-1 α may play a role in inflammatory hair loss conditions**, such as alopecia areata, through a direct growth-inhibitory effect on hair follicles[1].

Alopecia areata is an inflammatory autoimmune disease characterized by hair loss; a major role played by **focal inflammatory lesions**, reflecting the role of local cytokine production in the development of patchy hair loss[2].

1. Harmon CS, Nevins TD. IL-1 alpha inhibits human hair follicle growth and hair fiber production in whole-organ cultures. Lymphokine Cytokine Res. 1993 Aug;12(4):197-203.
2. Tazi-Ahnini, R., McDonagh, A., Cox, A. et al. Association analysis of IL1A and IL1B variants in alopecia areata. Heredity 87, 215–219 (2001).



TRICHO-SOLVE®

INCI Name: Picea Abies Extract, Cocoyl Proline, Glycerin

Natural active ingredient containing **Picea Abies**, derived from conifer bark, a purified source of Lignans, rich in polyphenols and vehiculated trough a **patented technology based on lipoaminoacids, diols and triols**.

✓ **PATENTED TECHNOLOGY:**

Green solubilization of Picea Abies Extract for a better bioavailability

TECHNICAL DESCRIPTION

INCI NAME	Picea Abies Extract, Cocoyl Proline, Glycerin
Aspect:	Liquid
Odour:	Characteristic
Colour:	From slightly yellow to amber
pH:	6.0 - 7.0
Preservative system:	Preservative free
Recommended use:	1 - 5 %

COSMOS APPROVED (in progress)

*

MIXED 9-4656-22-100-00

PRESERVATIVE FREE

HALAL In progress

NATURAL ORIGIN INDEX

BIODEGRADABLE

*Available also Chinese inci name, listed in IECIC

High compatibility with all the ingredients typically used in cosmetics

Easy to formulate: add it to the aqueous phase, with a temperature above 40°C

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PROVEN EFFICACY

In silico test

- Molecular modeling: anti-inflammatory effect

In vitro tests

- Stimulation of **protein synthesis**
- Stimulation of **cell proliferation**
- Inhibition of **the inflammatory reaction**
- Inhibition of **5-α reductase**

In vivo test

- Conducted on volunteers as an adjuvant in the treatment of hair loss

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IN SILICO TEST

MOLECULAR MODELLING

Potential targets were analyzed and the **selection of the enzymatic targets** is shown in the table.

Interesting matching score with **Arachidonate 5-lipoxigenase**, enzyme involved in the **inflammatory cascade**, with a key-role in the **hair loss process**[1].

Enzyme involved in the inflammatory cascade

Target Name	MoA Score
Arachidonate 5-lipoxygenase	0,92
Cytochrome P450 3A4	0,86
Cytochrome P450 19A1	0,74
Beta-lactamase	0,67
Histone-lysine N-methyltransferase, H3 lysine-9 specific 3	0,65
Phosphodiesterase 4A	0,65
Lysine-specific demethylase 4A	0,63
Arachidonate 12-lipoxygenase	0,38
Carbonic anhydrase	0,38
Carbonic anhydrase 2	0,37
Uncharacterized protein Rv1284/MT1322	0,37
Ornithine decarboxylase	0,31

→ As inflammation is one of the triggers of hair loss, the matching score of Picea Abies Extract with arachidonate 5-lipoxygenase suggests a potential inhibitory action.

3. Kim, S. N., Akindehin, S., Kwon, H. J., Son, Y. H., Saha, A., Jung, Y. S., Seong, J. K., Lim, K. M., Sung, J. H., Maddipati, K. R., & Lee, Y. H. (2018). Anti-inflammatory role of 15-lipoxygenase contributes to the maintenance of skin integrity in mice. *Scientific Reports*, *8*(1).

IN VITRO TEST

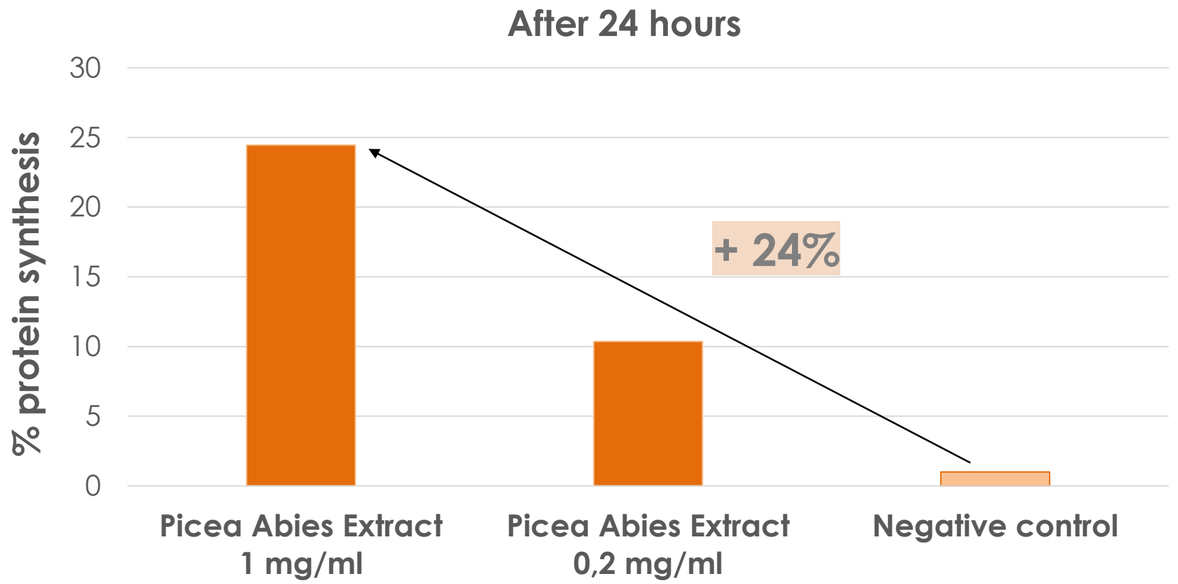
STIMULATION OF PROTEIN SYNTHESIS

The **purpose** of this assay is to asses quantitatively the effects of **Picea Abies Extract** on the total protein synthesis in human hair dermal papilla fibroblasts.

Human **hair dermal papilla fibroblasts**

Cells underwent starvation in serum-free medium for 6 hours before treatment with **0,2mg/ml or 1mg/ml PICEA ABIES EXTRACT**

Bradford assay on total cellular protein content



→ As keratin is the most abundant kind of proteins, the increase in protein synthesis (+24%) implies a significant improvement in hair growth and strength.

IN VITRO TEST

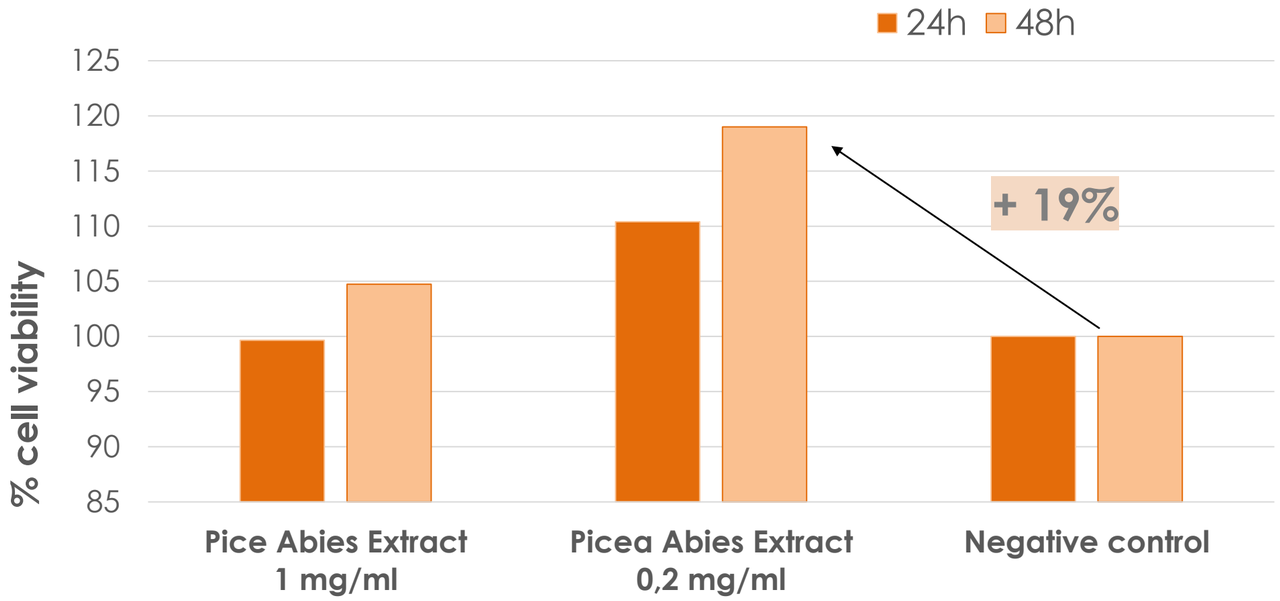
STIMULATION OF CELL PROLIFERATION

The **purpose** of this assay is to asses quantitatively the effects of **Picea Abies Extract** on the stimulation of cell cycle in human hair dermal papilla fibroblasts through the cell viability assay.

Human **hair dermal papilla fibroblasts**

Cells underwent starvation in serum-free medium for 6 hours before treatment with **0,2mg/ml or 1mg/ml PICEA ABIES EXTRACT**

MTT cell viability assay



→ This increase in cell proliferation (+19%) serves as an index of potential stimulation for hair growth and strength.

IN VITRO TEST

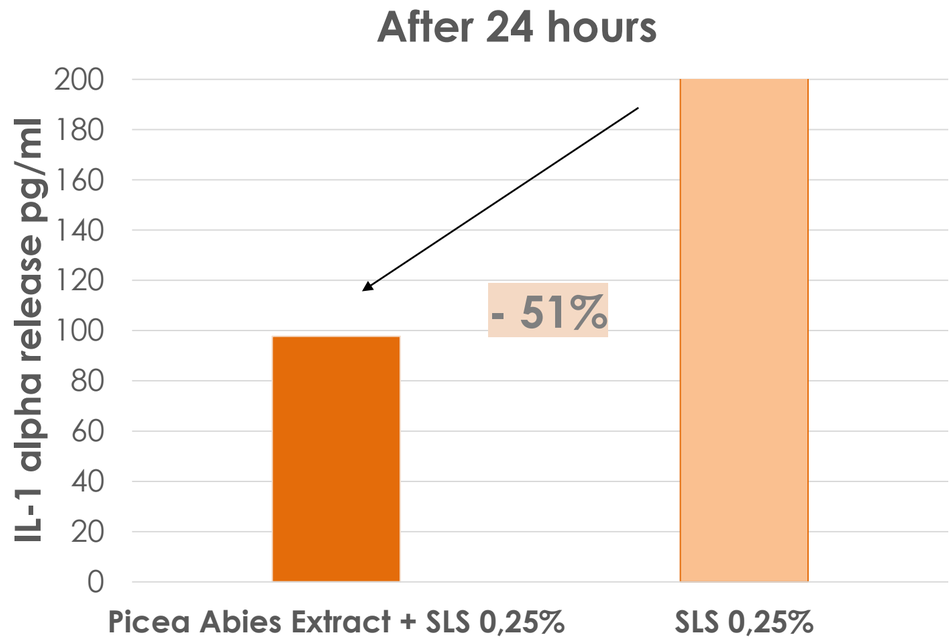
INHIBITION OF THE INFLAMMATORY REACTION

The **objective** of the assay is to quantitatively **assess the effects** of the **Picea Abies Extract** in protecting skin cells and **in** inhibiting the inflammatory reaction.

A **reconstructed** artificial human **skin** model

Pre-treated epidermis for 60 minutes with **0.25% SLS**, then incubated with **PICEA ABIES EXTRACT** for 24 hours

ELISA test on IL1α



→ The decrease in IL-1 alpha release (-51%) demonstrates an in vitro anti-inflammatory activity.

IN VITRO TEST

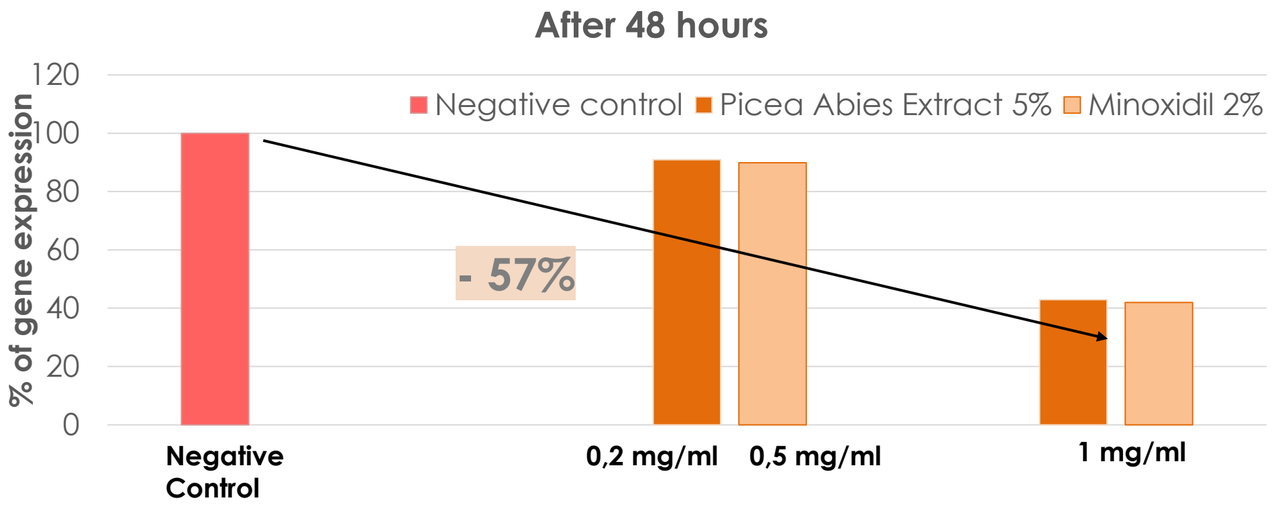
INHIBITION OF THE 5-α REDUCTASE

The **objective** of the *In vitro* assessment is to quantify the modulation of the gene expression of enzyme 5α-reductase on human cells of the dermal papilla.

PICEA ABIES EXTRACT VS MINOXIDIL:

Most common products components of anti-hairloss OTC, reference anti-falling molecule with clinically proven efficacy.

→ The concentrations of the two products were established through cytotoxicity: for Picea Abies Extract, they are 0.2 and 1 mg/ml, while for Minoxidil, they are 0.5 and 1 mg/ml.
At concentrations of 0.2 mg/ml and 0.5 mg/ml, the two products have an equivalent amount of active substance, representing 0.01%.

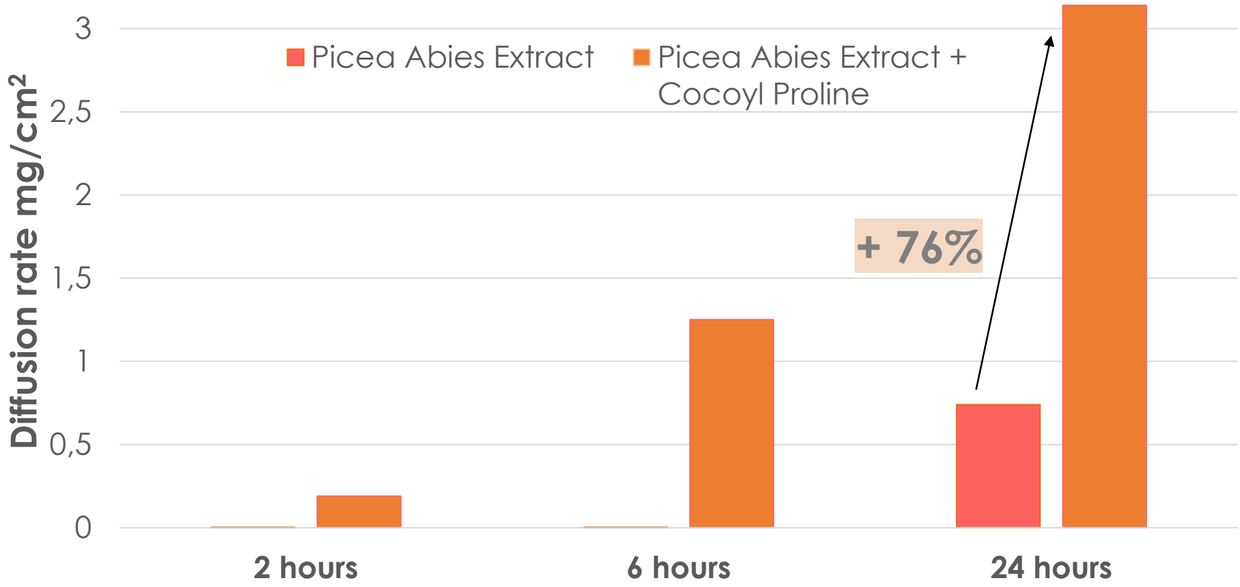


→ Picea Abies Extract 5% is able to reduce (-57%) the expression of enzyme 5-α reductase in human air dermal papilla cells.

SINERGA'S IMPROVEMENT

From the **widespread expertise** in the field of cosmetic ingredients, we have decided to improve **Picea Abies Extract's efficacy** by vehiculating it in a Sinerga's patented green technology: Cocoyl Proline.

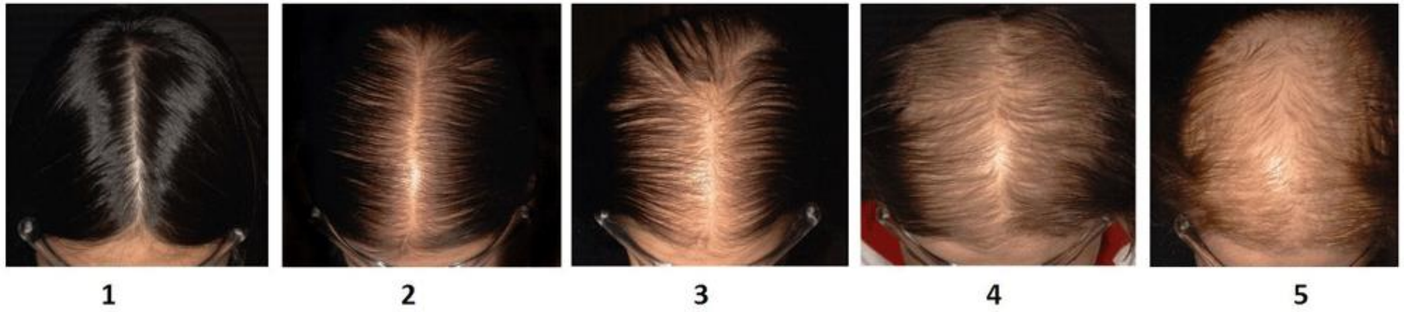
Through **Franz cell testing**, we were able to assess that this vehiculation technology **increases the absorption of Picea Abies Extract by +76%** (compared to the active matter alone), therefore **enhancing its efficacy**.



IN VIVO TEST

EFFECTIVENESS IN HAIR LOSS ADJUVANT THERAPY

Tricho-Solve® was evaluated in vivo through its incorporation into an anti-hair loss lotion.



30 healthy volunteers, 20 -50 years (50% women - 50% men), **with Telogen Effluvium grade 2 and 3,** with thinning or temporary hair loss.

90 days, evaluation timepoints: (T0), (T30) (T90)

Once a day on the scalp with a light massage

3.5ml of hair loss lotion containing thhrico-solve 5%

Density of the hair (hair number/cm2), **growth rate** (mm/day), **% hair in anagen phase**, **number of hair in anagen phase.**

Instrumental analysis*:*

All the previous parameters were calculated with Phototrichogram with Trichoscan

The **phototrichogram** consists in taking macrophotographs of a defined area of the scalp after hair shaving and repeating it after 48 hours.

The period of time is enough to permit the evaluation of the growth of a hair segment; the growth was then evaluated by comparing the two pictures.

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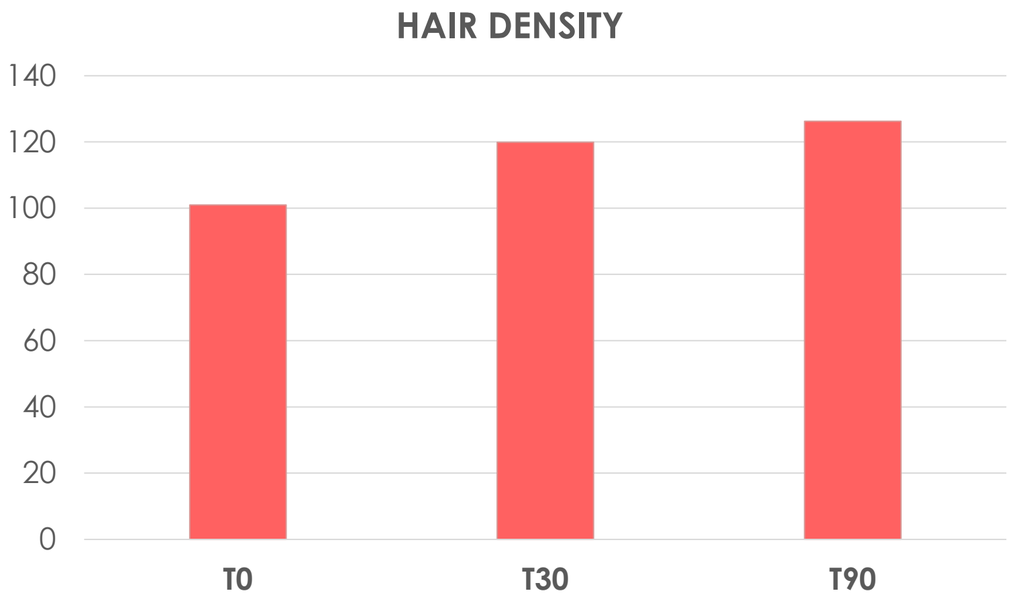
IN VIVO TEST

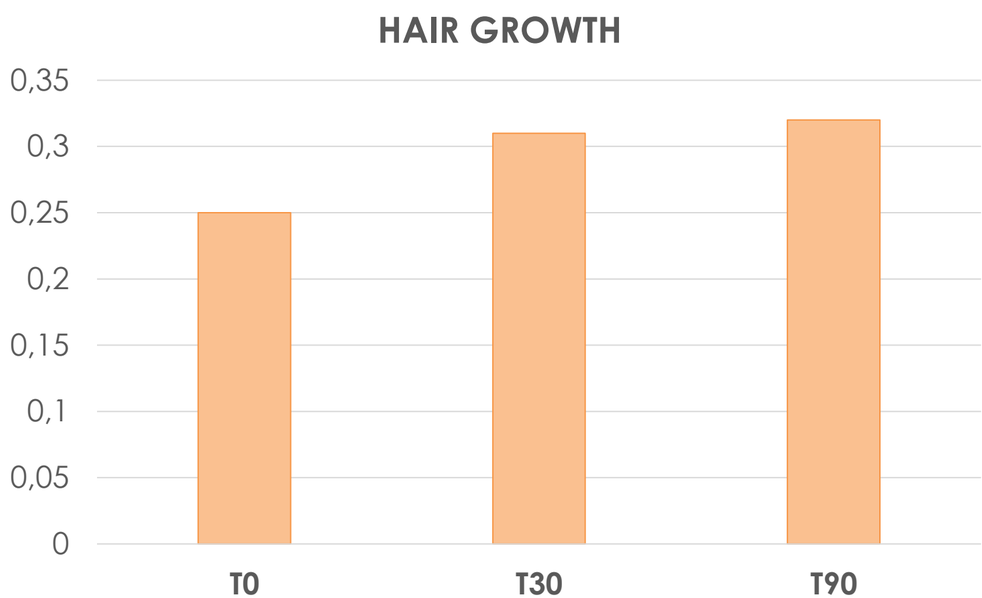
EFFECTIVENESS IN HAIR LOSS ADJUVANT THERAPY



Increases **hair density** (hair/cm2):

+ 20.22% after 30 days

+ 26.71% after 90 days



Increases **hair growth rate** (mm/day):

+ 27.15% after 30 days

+ 30.22% after 90 days

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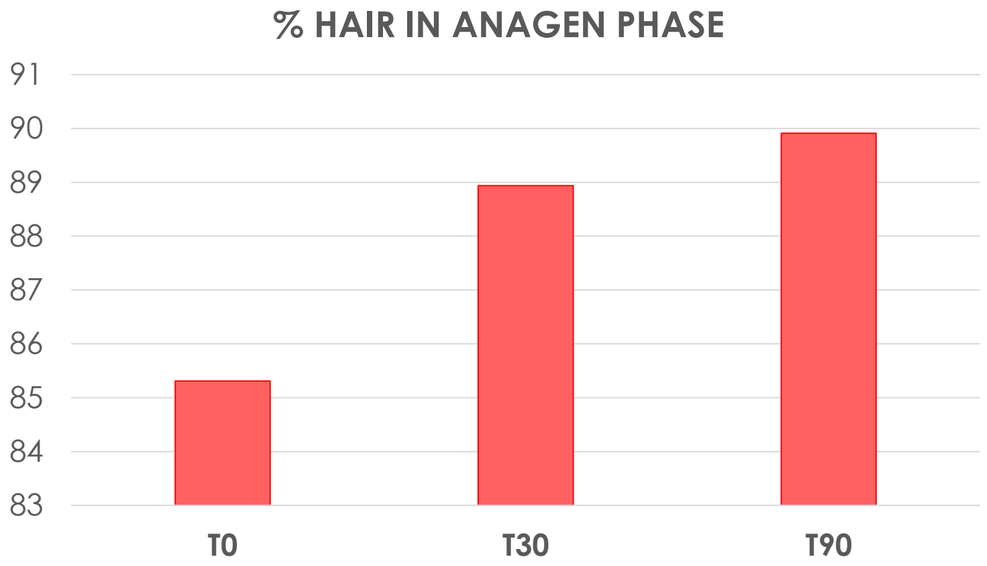
IN VIVO TEST

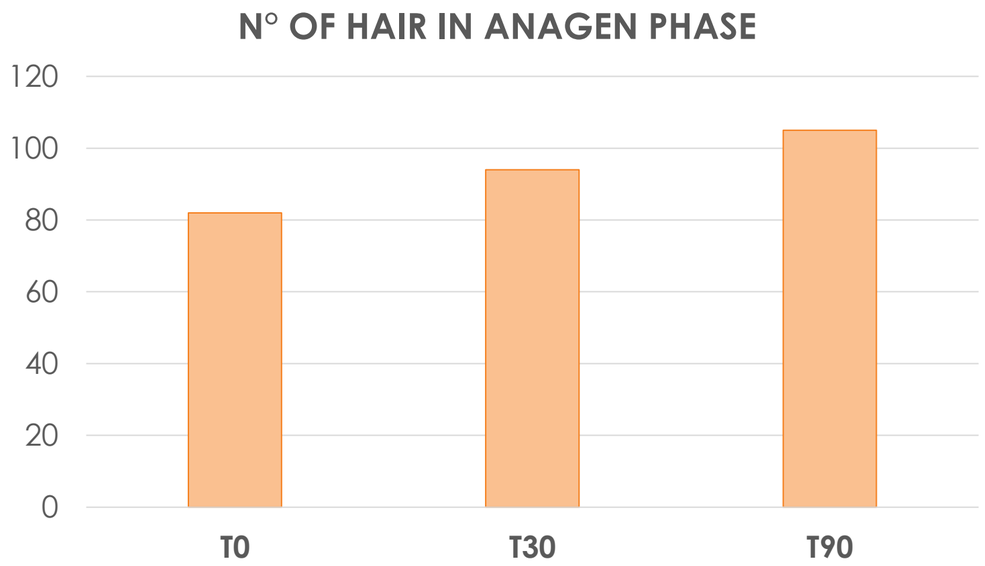
EFFECTIVENESS IN HAIR LOSS ADJUVANT THERAPY



Increases the **% of hair in anagen phase**:

+ 4.39% after 30 days

+ 5.68% after 90 days



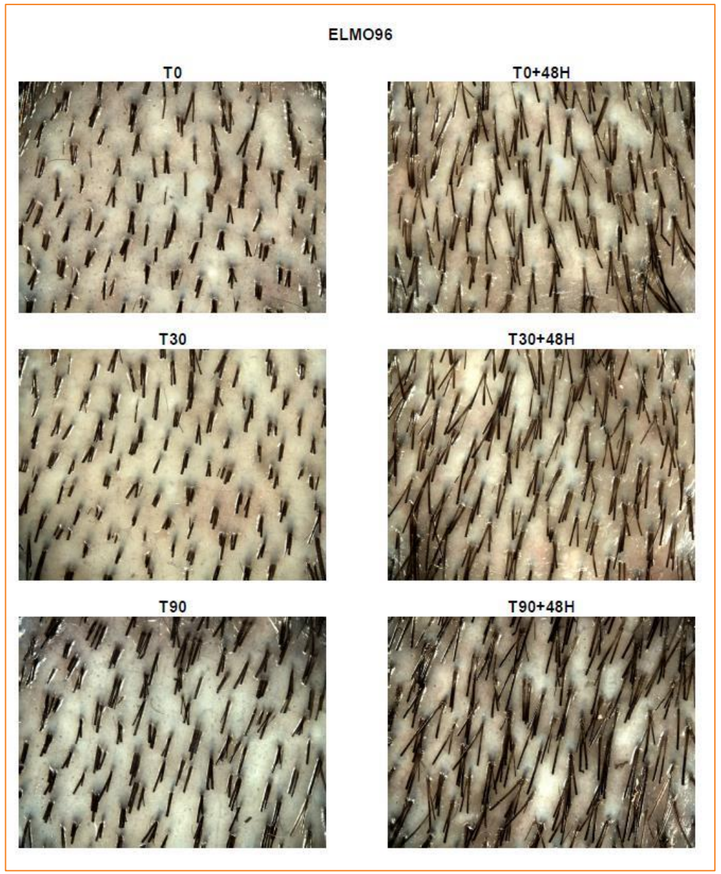
Increases **the number of anagen hairs**:

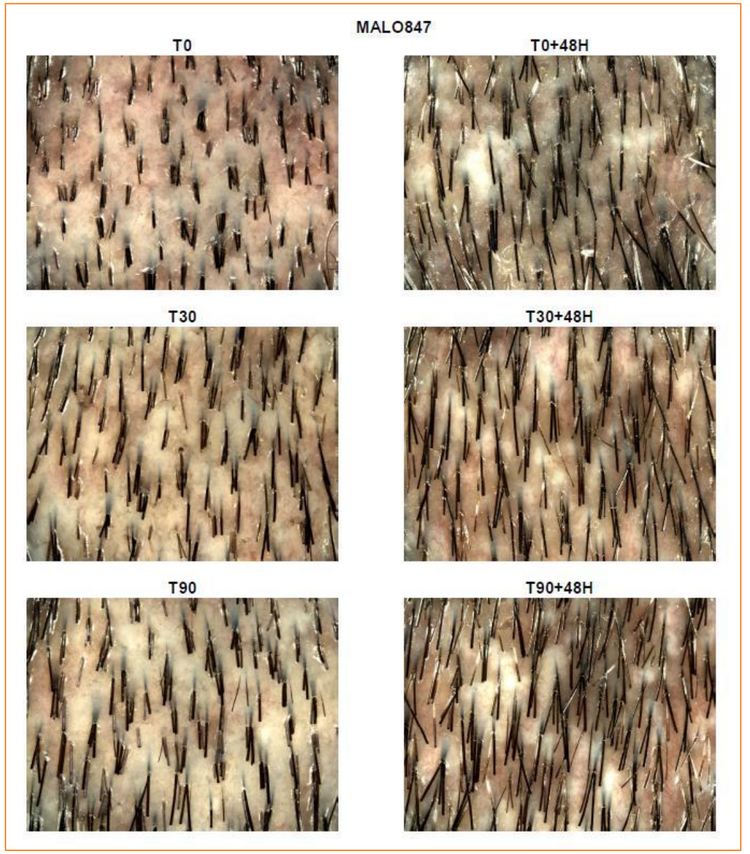
+ 14.27% after 30 days

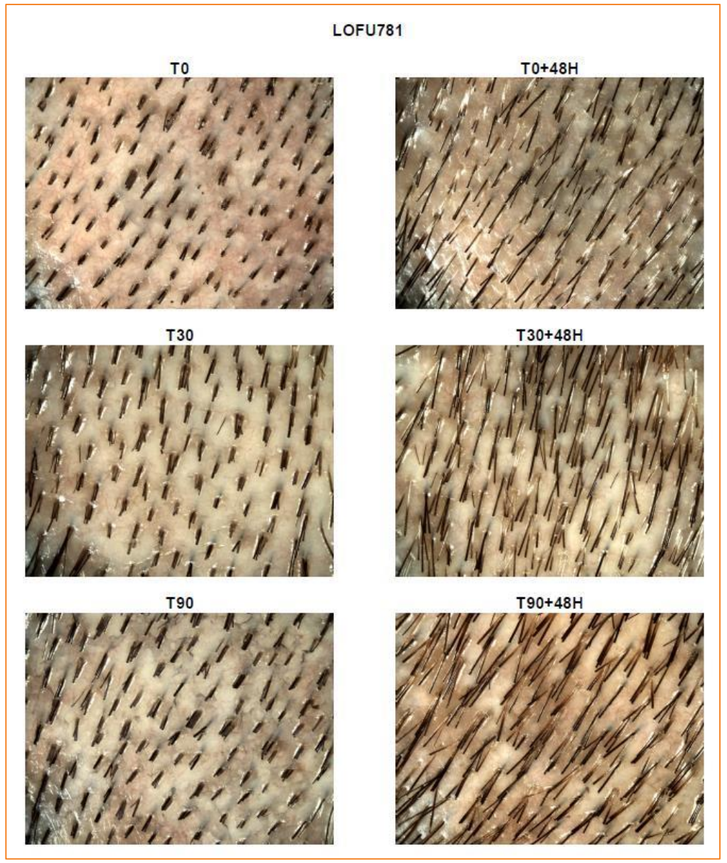
+ 30.49% after 90 days

IN VIVO TEST

RESULTS: REPRESENTATIVE IMAGES OF THE TREATED AREAS





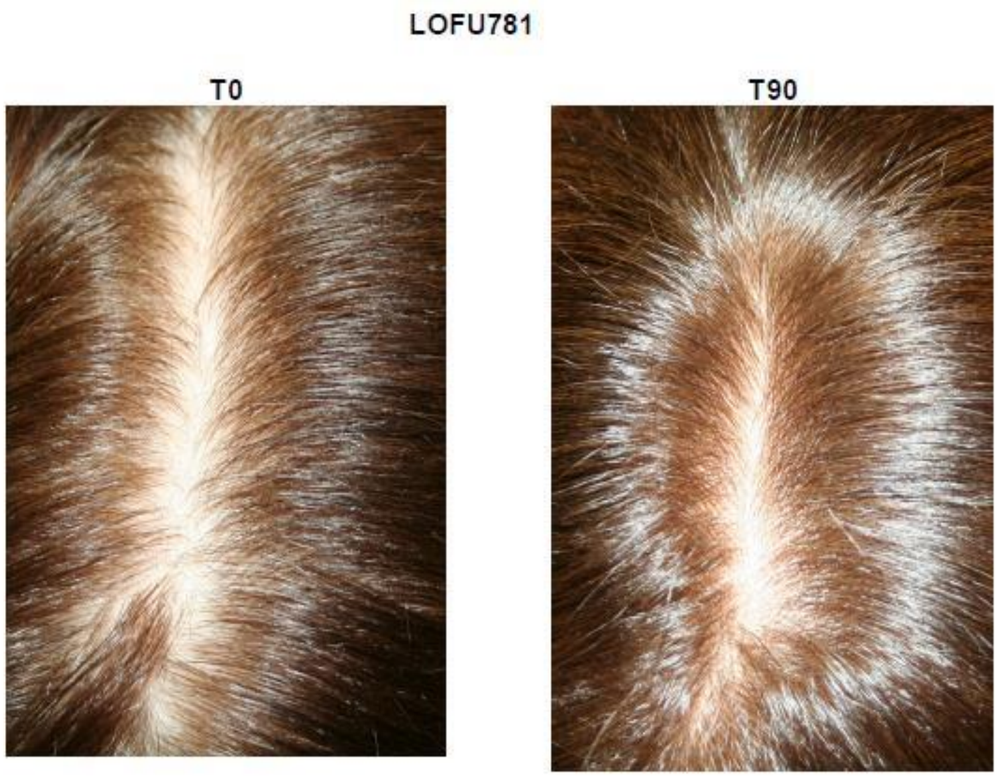


→ Images were taken with phototrichogram 48 hours after shaving at each experimental time point (T0 + 48h, T30 + 48h, T90 + 48h).

Each volunteer applied 3.5 ml of the product to the scalp once a day for 90 days with a light massage.

IN VIVO TEST

RESULTS: REPRESENTATIVE IMAGES OF THE TREATED AREAS



A clinical evaluation of the treated area were also performed, evaluating the state of thinning hair at each experimental time.

STATE OF THINNING HAIR	SCORE
No thinning	0
Slight thinning	1
Pronounced thinning	2
Extended thinning	3
Serious thinning	4

MEAN 2.27 (T0) 1,90 (T30) 1.43 (T90)

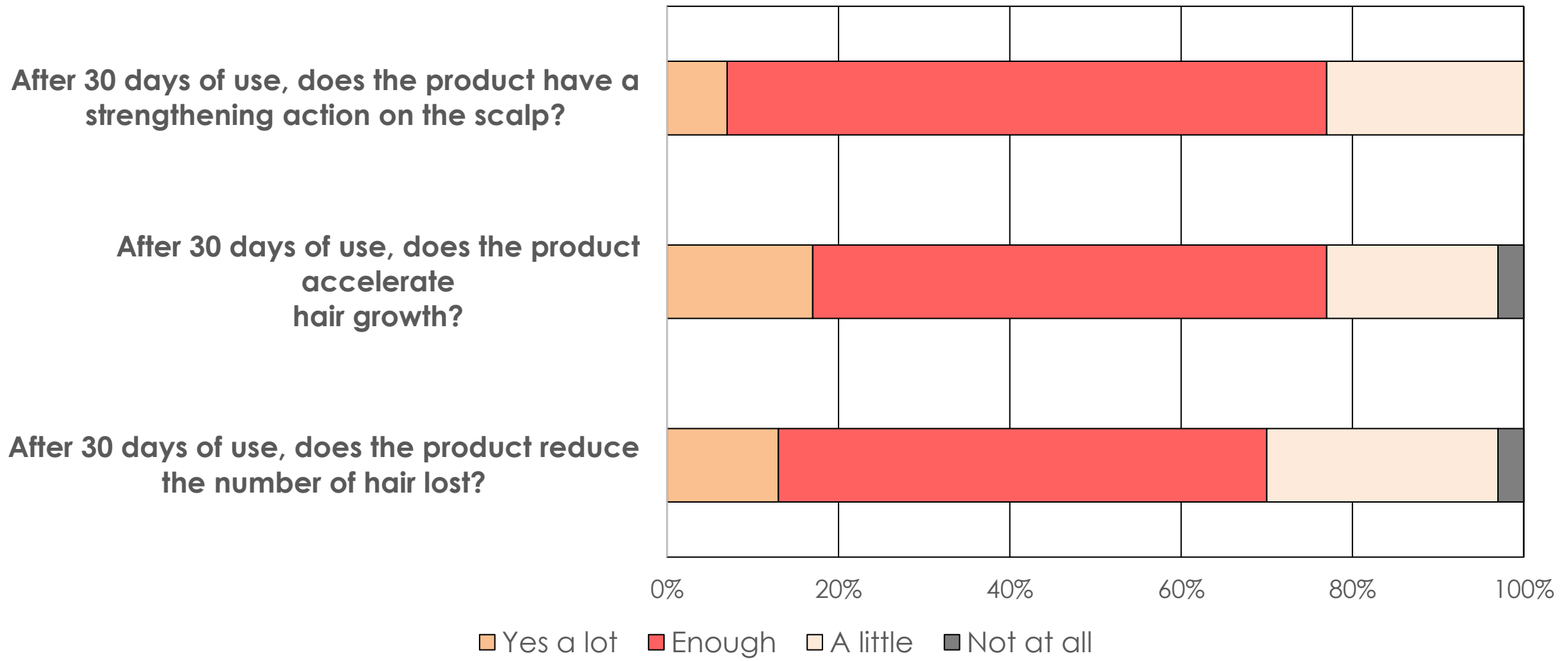
-37%

Even the objective clinical evaluation shows a **general improvement** in the state of thinning hair.
Furthermore, the product was **well tolerated** and **positively judged** by most of the volunteers.

IN VIVO TEST

RESULTS: QUESTIONNAIRE TO OBTAIN JUDGMENT FROM POTENTIAL CUSTOMERS ON PRODUCT PERFORMANCES

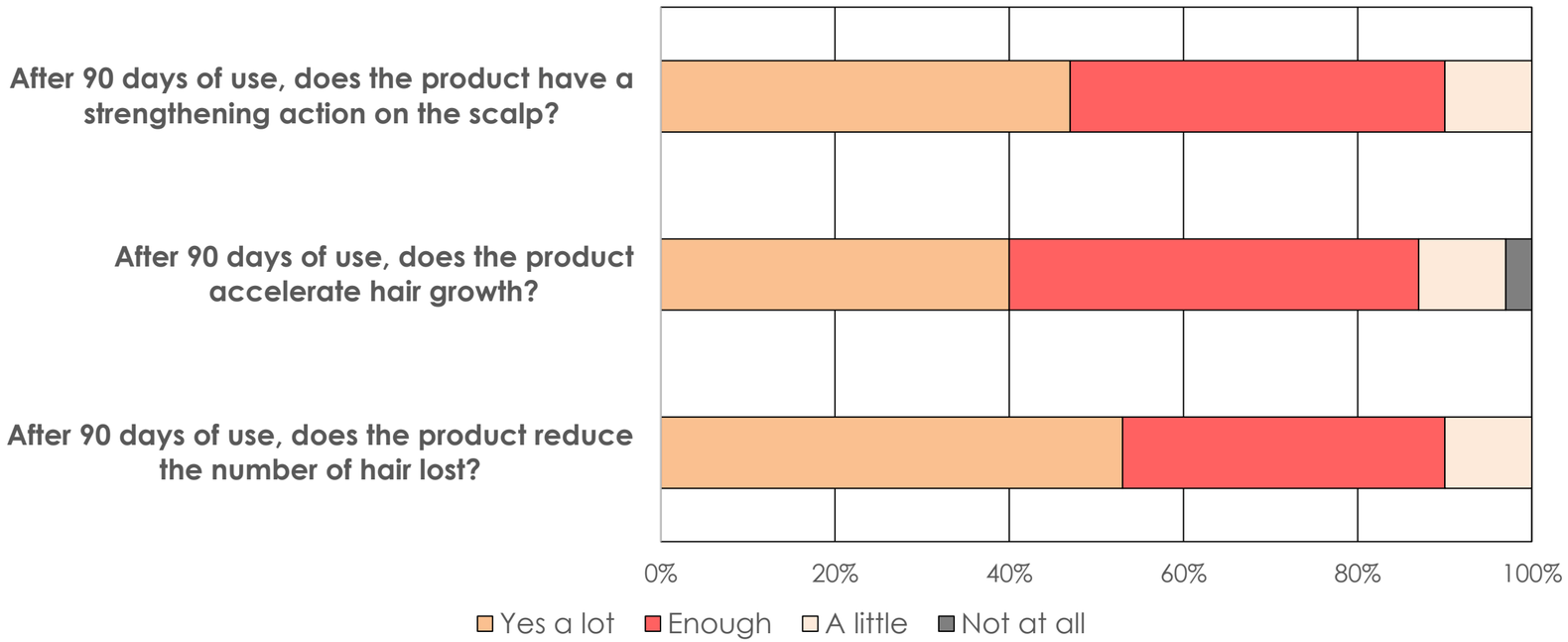
→ After 30 days



IN VIVO TEST

RESULTS: QUESTIONNAIRE TO OBTAIN JUDGMENT FROM POTENTIAL CUSTOMERS ON PRODUCT PERFORMANCES

→ After 90 days



SUGGESTED FORMULATION

HAIR FORTIFYING LOTION FOR HAIR LOSS

- **Tricho-Solve®** (5%) was added to a formulation **simulating a finished anti hair-loss treatment to evaluate its efficacy in vivo**.

- The formulation includes **X-SOLV**, a Sinerga's patented ingredient able to play a **synergistic effect in counteracting hair loss**.

Ingredients (LSIN7294A)	Phase	%
Water	A	To 100
Disodium EDTA		0.10
Sodium Benzoate		0.30
Caffeine		1.00
Niacinamide		0.10
Arginine		1.00
Panthenol		0.30
Biotin		0.01
Potassium Sorbate	A'	0.25
TREALIX® (Trehalose, Hydrolyzed Vegetable Protein)		1.00
Sodium Ascorbyl Phosphate		0.10
Saccharomyces/Zinc Ferment (and) Saccharomyces/Copper Ferment (and) Saccharomyces/Magnesium Ferment (and) Saccharomyces/Iron Ferment (and) Saccharomyces/Silicon Ferment (and) Water		1.00
TRICHO-SOLVE® (Picea Abies Extract, Cocoyl Proline, Glycerin)		5.00
Alcohol	B	30.00
Menthol		0.40
Retinyl Palmitate	B'	0.02
Tocopheryl Acetate	t	0.02
X-SOLVE® (Ethyl Ximenynate, Lecithin)		1.00
Solubilizing agent		qb
Lactic acid	C	qb

SYNERGISTIC ACTION WITH X-SOLVE®

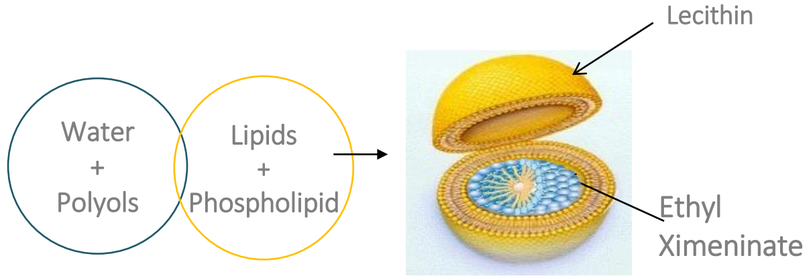
HAIR FORTIFYING LOTION FOR HAIR LOSS

INCI: Ethyl Ximenynate, Lecithin, Caprylic/Capric triglyceride, Glycerin

With a patented encapsulation technology able to better delivery its active compound, it has vasodilatory and anti-inflammatory properties.

Ethyl Ximenynate has a renown **effect** on **preventing** the release of arachidonic acid, one of the main responsible of **inflammatory process.** It has been shown to yield potent inflammatory mediators by two major pathways: through synthesis of leukotrienes and prostaglandins.

Efficacy enhanced by an
Encapsulation Technology By High Pressure Homogenization



Better delivery of the active ingredient

Quick efficacy results

+ 29% Microcirculation improvement compared to non-encapsulated form

Active able to **increase the microcirculation** with vasodilatory properties;

- constant blood flow helps maintain keratin protein in the hair fiber and brings nutrients to the follicle.

increases extracellular **nitric oxide production (+37%)**

Nitric oxide induces **relaxation of vascular smooth muscle** and dilates blood vessels promoting the flow.

increase **actin expression** in fibroblasts by **40%**

It suggests a role in **improving the tone and texture** of the dermis.

CLAIMS AND APPLICATION

- Increases **hair growth**
- Increases **hair density**
- Reduce **hair thinning**
- Prevents **hair loss**
- **Inhibits androgen production**, involved in hair loss disorders
- **Strengthens and fortifies hair**
- **Reduces scalp inflammation**

- **Hair Loss Adjuvant Treatment**: lotions, sprays conditioners and shampoos.
- **Hair-Reinforcing Products**
- **Hair Loss Prevention Products**

Synerga SKIN EVOLUTION