



## Grow Factor #1

### LUMINOUS PERFECTION:

### Skin without spots, Radiant, Resplendent

Is formed by a synergy of ingredients that acts on the three levels of the skin, for an absolute efficiency.

On the one hand, it contains a mixture of amino acids particularly rich in adenosine, which helps in the formation of ATP and improves blood flow, thus obtaining a homogeneous complexion.

**Contains Scelleye, a growth factor similar to Plant-IGF-1**, obtained from *N. Benthamiana*. Scelleye is especially suited for extremely delicate eye skin care. IGF-1 is a polypeptide essential for the formation and development of the skin. Very abundant in the basal layer of young skin.

Contains stabilized vitamin C, which gives the product antioxidant and whitening activity, together with the Glutathione and Sophora Flavescens extract, makes the product to act on three levels:

#### 1. Surface level:

- Antioxidant activity
- Anti-inflammatory effect

#### 2. Level of epidermis:

- Exfoliating activity
- Promotes anti-tyrosinase activity
- Melanogenesis inhibition

#### 3. Level of dermis:

- Inhibition of the enzymatic activity of MMP.
- Increased anti-glycation activity.

It incorporates **biomimetic peptides identical to the growth factors involved in cell renewal, resulting in younger skin.**

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It provides **epigenetic activity**: Epigenetics is the new scientific discipline that allows influencing the behavior of genes to achieve optimal performance.

Today we know that the environment, our lifestyle, emotions ... in definitive, the history of the skin, generates epigenetic factors that influence decisively in our way of aging, more than the genetic inheritance itself.

The key? **COBIOGENOL**, an active ingredient from marine origin that acts like a chemical switch activating the genes "off" by certain epigenetic factors. Normalizing the generation of proteins necessary for the rejuvenation and regeneration of the skin, neutralizing oxidative stress generated by exposure to light and blue light, and re-structuring the stratum corneum, the outermost layer of skin, key in maintaining its Barrier and protective function.

All properties claimed for this product have been clinically demonstrated:

- Antioxidant activity
- Anti-inflammatory effect
- Exfoliating activity
- Anti-tyrosinase activity
- Inhibiting activity of melanogenesis.
- Epigenetic activity
- Protection against Electromagnetic radiation and Blue-Light

## **Introduction:**

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There are several different parameters that influence the aspect and attractiveness of skin in an important way, such as moisturization, firmness or wrinkles. However, one of the concepts that has most influenced the different standards for beauty throughout history is skin pigmentation, or tone.

**A radiant complexion is associated with beauty.** The pigment distribution, melanin, and skin texture vary according to health status and age. An irregular coloration is an indicator of the age of the skin, since the aging is associated with the presence of dark spots.

The difference in skin color is mainly due to the presence of melanin, a pigment that protects from external aggressions, such as UV rays.

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When the body generates too much melanin to protect itself from these aggressions, or simply because of aging, accumulations can be created causing blemishes or changes in skin tone. **This disorder is called hyperpigmentation and can affect all skin types.**

Hyperpigmentation is the third major cosmetic concern, as it is one of the obvious signs of the decline of youth. For this reason, the cosmetic goal is to reduce blemishes and age spots, and to improve the skin tone.

**Grow Factor #1 - LUMINOUS PERFECTION:** Notably illuminates the skin while fully respecting its natural and health characteristics. **In vitro tests showed that the synergistic combination of ingredients contained inhibits 90% melanin synthesis.** Contains a **combination of active ingredients** that bring **luminosity and vitality to the skin, giving it immediate, radiant and healthy appearance** and protects it from external aggressions: **Radiación Electromagnética y Blue-Light.**



- ✓ It allows to recover the luminosity and light lost as a result of the chronological aging, or an undue exposure to the UV rays (photo-aging), as well as numerous aggressions to which our skin is exposed daily (pollution, cold, tobacco, poor diet, lack of sleep, stress, ...).
- ✓ With a continuous use, it manages to homogenize skin tone and reduce unwanted pigmentation.
- ✓ Contributes to reduce wrinkles and lines of expression through the synthesis of collagen and elastin.
- ✓ It increases skin's the face lack of firmness, smooth and elasticity by stimulating the synthesis of collagen which contributes to regenerate the tissue support dermal matrix.

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## BIOACTIVE INGREDIENTS IN **Grow Factor #1**

### **LUMINOUS PERFECTION::**

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#### **SCELLEYE: BIO-MIMÉTICAL PEPTIDE. Plant Growth Factor**

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#### **What are growth factors?**

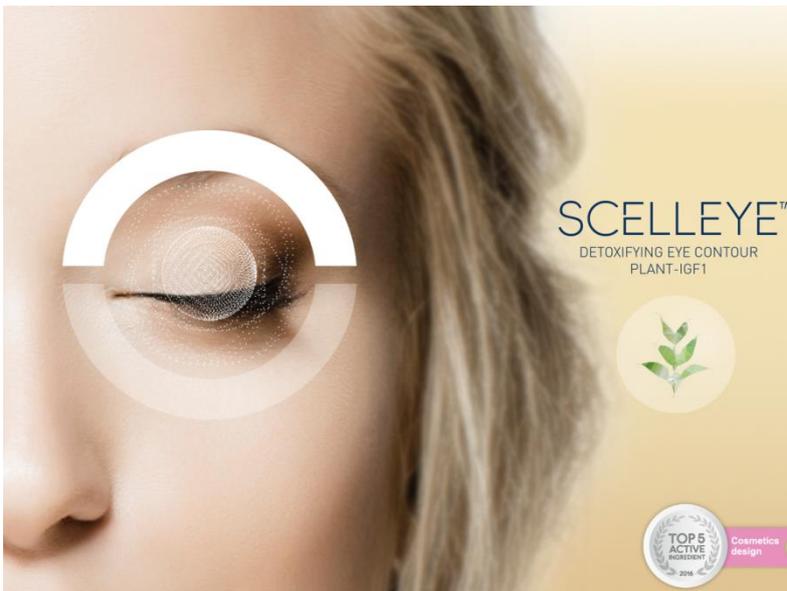
Growth factors are proteins that bind to their own receptors on the cell surface with the primary result of activating cell proliferation and / or differentiation.



Growth factors regulate the expression of the constituent proteins of the extracellular matrix: collagen, elastin, laminin, etc.

#### **What are bio-mimetic peptides?**

**They are synthetic agonists (which have the same function) as natural growth factors and completely mimic their action, providing the same clinical benefits.**



Scelleye is a growth factor similar to Plant-IGF-1, obtained from *N. Benthamiana*. Scelleye is especially suited for extremely sensitive eye care of the eyes, helping to restore the activity of the epidermal basal layers, as

well as activating the proteasome and decreasing carbonylation, for effective purification of damaged proteins.

In addition, it diminishes the crow's feet and the circles under the eyes.

IGF-1 is a polypeptide essential for the formation and development of the skin. Very abundant in the basal layer of young skin.

During UV exposure, it prevents tissue damage. Aging skin has low levels of IGF-1.

Scelleye contains plant-derived IGF-1 from wild plants and protects the delicate skin from the eye's. In addition, it diminishes the crow's feet and the circles under a complete care of the outline of the eyes.

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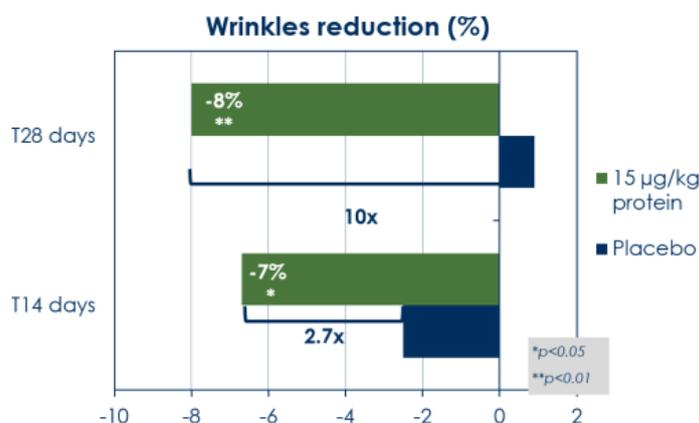
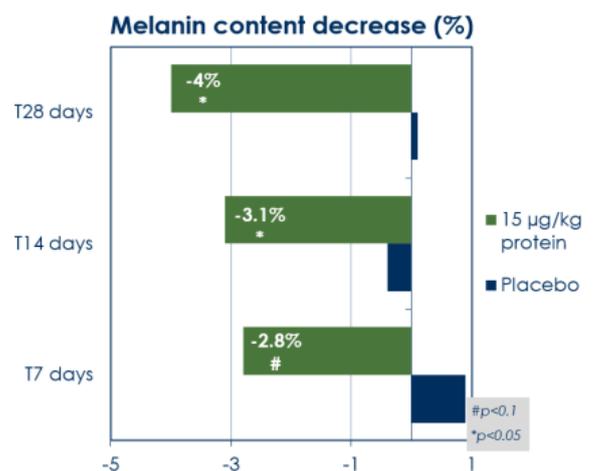
Scelleye Contains plant-IGF-1 from wild plant biofactories to help you design your true beauty covering multiple eye care concerns such as UV, pollutants, wrinkles and dark circles

## In vivo efficacy: double blind assessment of eye care efficacy.

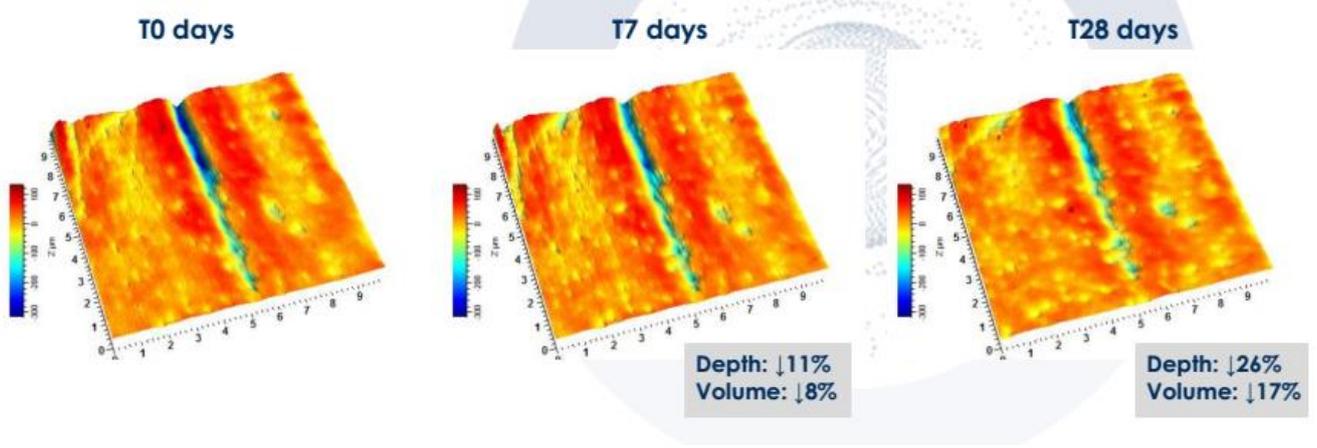
Caucasian women (51 ± 6 years old) showing dark circles, applied a gel cream with 15 µg/kg (0.015 ppm) protein around one eye, twice daily for 28 days. Placebo was applied around the other eye

Evaluation of: Melanin (Siascope), Crow's feet replicas (Visia®), Skin replicas (FOITS)

- 3% significant reduction of melanin content under the eyes after 7 days with Scelleye™
- 4% improvement after 28 days, indicating a dark circles soothing



- 7% and 8% significant reduction of crow's feet after 14 and 28 days with Scelleye™
- 10-fold less wrinkles than placebo after 28 days



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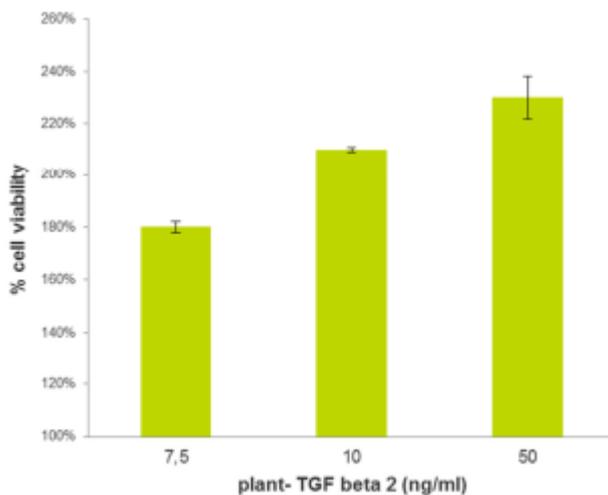
## Plant-TGF $\beta$ 2: BIO-MIMÉTICAL PEPTIDE. Growth factor

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**Plant-TGF  $\beta$ 2** is synthetic bioengineered highly purified Transforming Growth Factor beta 2 produced in plants.

It is a biotech ingredient for the prevention of skin photoaging. Its ability to induce collagen and fibronectin biosynthesis helps improving the appearance of wrinkles.

### Human fibroblast cell proliferation



Dermal fibroblasts are the cells that synthesize collagen and the extracellular matrix proteins to maintain the skin's structure and resilience. As a result of the process of aging turnover of new fibroblast decreases, collagen production slows and the skin becomes thinner and wrinkled.

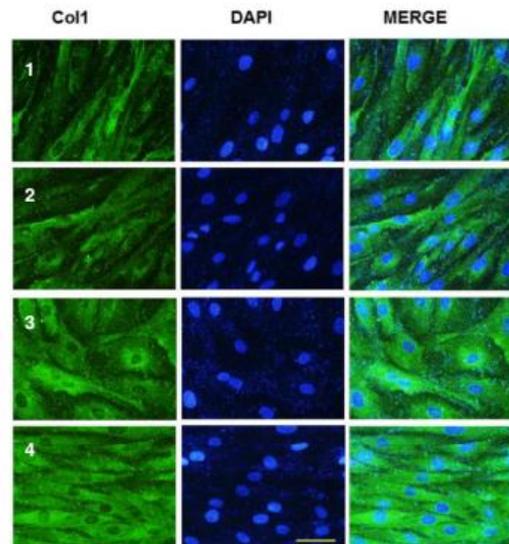
Primary dermal fibroblasts were seeded in 24-well plates and grown up to 40% confluence and treated with different concentrations of plant-TGF  $\beta$ 2 for 48 hours. Cell viability was assessed by MTT assay and showed a dose-dependent stimulation of fibroblasts proliferation.

Proliferation was increased by 210% at 48 hours after 10 ng/ml treatment versus untreated control (base line 100%).

Fibroblast proliferation boosts the skin repairing process stimulating collagen synthesis and the formation of connective tissue.

## Type I Collagen synthesis

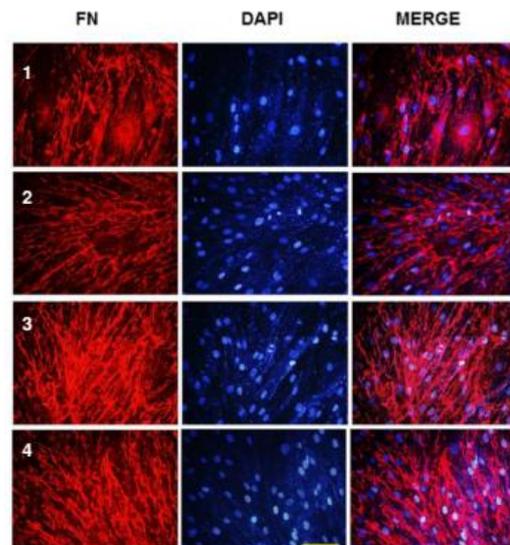
Collagen I expression profile was performed on dermal fibroblasts by indirect immunofluorescence, showing a remarkable intracellular increment of the protein versus the untreated fibroblasts 24 hours after treatment with different concentrations of plant-TGF  $\beta$ 2.



Scale bar 50  $\mu$ m.

## Fibronectin synthesis

Fibronectin profile was performed on dermal fibroblasts by indirect immunofluorescence, showing an extracellular increment of the protein 24 hours after treatment, and a better organization versus the untreated fibroblast after treatment with different concentrations of plant-TGF  $\beta$ 2.



Scale bar 100  $\mu$ m.

1: Untreated Control; 2: 1 ng/ml plant-TGF  $\beta$ 2; 3: 10 ng/ml plant-TGF  $\beta$ 2; 4: 20 ng/ml plant-TGF  $\beta$ 2.

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## COBIOGENOL: Electromagnetic protection, protection against Blue-Light. Epigenetic Science

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**First active ingredient** focused on reducing the biological disorders produced by exposure to **Electromagnetic Radiation.**

Electromagnetic waves are a form of energy consisting in vibrations of electric and magnetic fields. We live in a sea of invisible electronic pollution, which is becoming toxic to our health.

We do not see or perceive them but are increasingly present in our domestic environment. Wherever we might be, non-ionizing radiation is all around us. We are constantly exposed to magnetic fields generated by appliances and domestic electrical installations: mobile phones, WIFIs, microwaves, television, radio, computers, etc.

As technology advances and the use of these devices increase, both at the workplace or private areas, our exposure to non-ionizing radiation is likely to intensify further.

### ▪ **Oxidative Stress**

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The “digitization” of our world means that our cells are exposed to a continuously increased level of non-ionizing radiation, for which they have not adapted. In the last decade, EMR (Electromagnetic Radiation) levels have increased dramatically, and we are starting to realize its negative consequences. EMR is an environmental stress factor for human health, and skin as a physiological barrier is the first

objective of this radiation.

This exogenous stress leads to oxidative cellular stress, the formation of excessive reactive oxygen and nitrogen species and reaction products. It leads to a mitochondrial metabolic dysfunction: ROS causes a disruption of Mitochondrial function and cellular ATP Levels.

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The massive oxidative cell stress leads to chronic inflammation. Several studies [1][1][3] have shown that skin exposure to cell radiation suffers several biological parameter alterations:

- ✓ Massive increase in free radical production (ROS)
- ✓ Massive Increase of pro-inflammatory cytokines
- ✓ Decrease in cell regeneration
- ✓ Reduction in structural proteins: key molecules involved in the stratification of the epidermis
- ✓ Lost in cohesion between keratinocytes
- ✓ TEWL increase: dehydration, the skin becomes more vulnerable and sensitive
- ✓ Stratum corneum weakening
- ✓ Disturbed skin refraction: lost in brightness, becoming dry and uncomfortable

**COBIOGENOL**, is a functional ingredient proved to reduce the skin biological alterations, resulted from the exposure to EMR.

**It helps at the restoration process of the skin**, being a specific anti-stress agent. It is a **concentrated solution of purified marine glycogen**, ready to use at cosmetic skin care preparations.



Glucose is the most important sugar at the cellular level. It plays a very important role in the energetic metabolism of the cells. Its energy is used at the restoration process of the skin.

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### CLINICAL EFFICACY TESTS:

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- **Effects of COBIOGENOL Preventing and protecting from Electromagnetic radiation-induced oxidative stress:**
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Several exogenous stimuli such as ionizing radiation, EMR, UV light, smoke, inflammatory processes and some human diseases trigger off free radicals production, causing severe damage in the mitochondrial membranes provoking the massive release of free radicals (ROS).

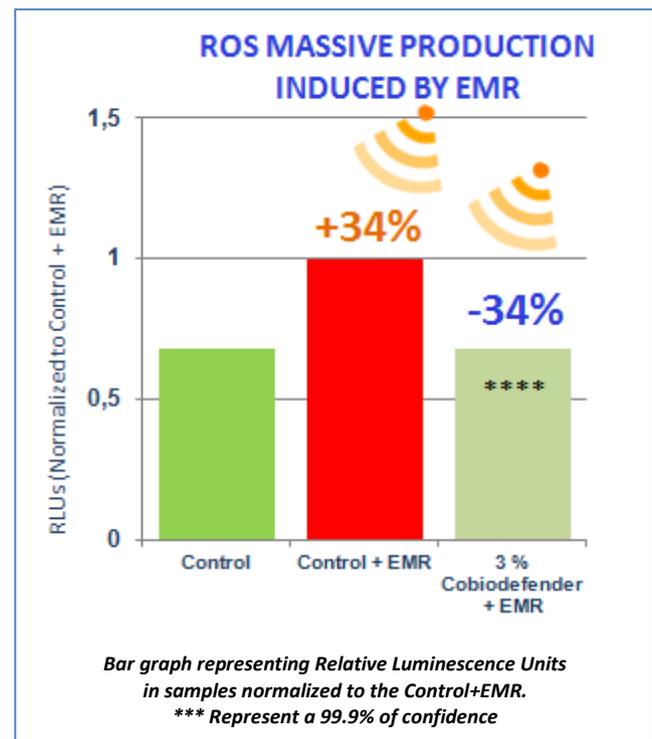


The goal of this study was to evaluate the capacity of COBIOGENOL in protecting cells from the damage caused by induced electromagnetic radiation (EMR), by reducing oxidative stress (ROS).

To this end, Human keratinocyte cells were exposed to 6 hours of EMR generated by a mobile phone to assess the response of skin cells

to the induced oxidative stress and test the potential of COBIOGENOL to prevent EMR-induced ROS.

After 6 hours exposition, EMR induced ROS accumulation  $34,26 \pm 3,13\%$  and treatment with COBIOGENOL **reduced EMR-induced ROS production  $34,82 \pm 3,12\%$**  indicating a protective effect.



- **Cellular rejuvenation**                      **epigenetic**

### **Epigenetics is a new paradigm in the Science of Anti-Aging.**

It is the study of the mechanisms involved in the regulation of gene activity: the biological mechanisms that will switch genes on and off without altering their sequence.

Epigenetic change happens regular and naturally but can also be influenced by several factors including age, the environment (UV radiation, pollution...), lifestyle, emotions and diseases and much more that are yet to be discovered.

These factors discussed above, have the ability to "turn" or "turn off" certain genes, whose effects will be observed physically or physiologically.

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The "on" or "off" effect of genes can be accomplished by three mechanisms:

- 1) **MicroRNAs**
- 2) Histone modification
- 3) DNA mutilation

## **What are miRNAs?**

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MicroRNAs (or "miRNAs") are chemical "switches". They are small fragments of RNA responsible for protein synthesis. These control mechanisms are key elements of epigenetic regulation. Its production is constantly modified by the environment and living conditions, including our emotions. MicroRNAs turn on and off numerous biological processes such as cell survival, skin repair, processes, hair growth...

## **Epigenetics and the future of skin care**

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We can regulate microRNA expression to rejuvenate. miRNAs are involved in regulation processes such as cell cycles, DNA repair systems, reactions to oxidative stress, apoptosis, etc.



During aging and other external aggressions, chromatin ( the genetic material in the nucleus of our cells) becomes senescent and disorganized contributing to premature aging.

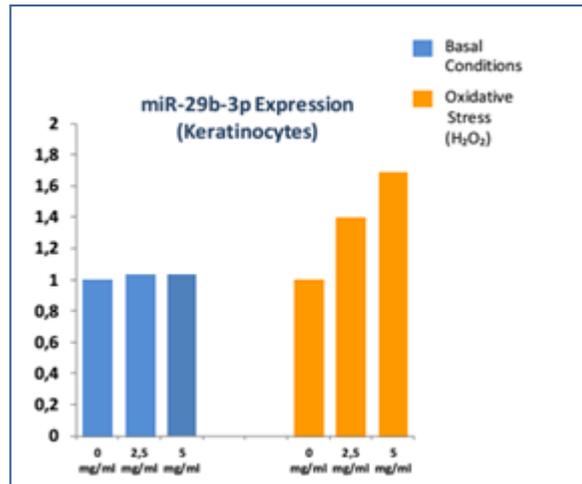
**COBIODEFENDER EMR is a natural epigenetic active ingredient** that can regulate microRNA expression. This means that it has the ability to act on the expression of miRNAs and "**switch on**" or "**switch off**" **certain genes**.

- By **in vitro assays in human keratinocytes** (skin cells), COBIODEFENDER EMR has been shown to have the ability to **rejuvenate cellular chromatin, making aging cells acquire characteristics of young cells**.

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COBIODEFENDER EMR increases the expression of miR-29b-3p, a miRNA that "switch on", or "activates" the synthesis of protein P53: **Protein guardian of genetic material**, which has a **potent antioxidant, anti-stress and anti-aging activity**.

- Under conditions of cellular stress induced by photo-oxidation (photo-aging) and oxidative stress, (by hydrogen peroxide), COBIODEFENDER EMR reduced the levels of **H3K79me3**, a miRNA considered as an **aging clock, inducing a cellular rejuvenation**.



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### Summary of COBIOGENOL activity:

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- ✓ Protection of oxidative stress caused by electromagnetic radiation
- ✓ DNA protection and rejuvenation
- ✓ Anti-aging activity
- ✓ Aged cells acquire characteristics of young cells.

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## GATULINE® SPOT-LIGHT: Depigmentant and correction of senescence spots

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### Age spots: a clinical sign of photo-aging

Age spots, known commonly as **dark spots**:

- Are permanent hyperpigmented marks that appear on UV exposed areas of the skin - also known as solar lentigo
- Their appearance increases over time

**They are skin blemishes** not associated with any medical conditions.



- ✓ Range in color from light to dark brown
- ✓ Located on particular on the hands, face, décolleté, shoulders, arms forehead, and the scalp if bald

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Age spots are one of the main aging concerns named by European and American women after wrinkles and sagging. In France: 20% of women over age 25 suffer from age spots 90% after the age of 50.

The pathophysiology of senescence spots was not well known until now. In addition to the relationship with UV exposure, the rest of the mechanisms involved in the development of hyperpigmented areas have not been specified

It is actually object of great interest in the scientific community plus level of knowledge

- **Causes:**

They are widely admitted: disruption of skin pigmentation, oxidation (ROS) and inflammation. Recent studies have evidenced that solar lentigo could be induced by the dermal environment located beneath the hyperpigmented areas. All active ingredients on the market target the epidermal causes. Few ingredients offer additional anti-oxidation and anti-inflammatory effect, an even smaller amount of products target accumulated melanin. None prevent glycation and dermis degradation, newly identified as key to prevent age spot appearance.

- Degradation of the dermis by MMPs

- Glycation

Gatuline® Spot-Light Is the first ingredient that acts on ALL the causes of senescence spots at all levels of the skin:

- 1) Act in the epidermis**

- Melanogenesis, production and elimination of melanin

- 2) Act in the dermis**

- Protect from photo-aging and MMPs degradation
- Protect the extracellular matrix from glycation

- 3) Act throughout the skin**

- Inhibit inflammation and prevent oxidative degradation

Gatuline® Spot-Light a unique blend for high efficacy.

**Contains:**

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## **Kiwi water: Demonstrated anti-tyrosinase activity.**

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**Demonstrated anti-tyrosinase activity. Hand-picked Italian Kiwi. It is a constitutive vegetal Water, 100% natural that produces a synergistic effect that maximizes the depigmenting potential of Sophora flavescens.**

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## **Sophora flavescens root extract: Medicina tradicional China**

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**Depigmenting potential of skin extensively described in the literature**

**Long History in Traditional Chinese Medicine**

**Described in the Shennong Ben Cao, the oldest Chinese book on medicinal plants.**

**It is rich in active molecules with documented biological efficacy or secondary metabolites,**

**secreted by the plant naturally for communication and its self-defense:**

**✓ Flavanones:**

**• Sophoraflavanone G: Good penetration through the intercellular lipids of the stratum corneum. It remains in the epidermis thanks to its relative hydrophobicity. It remains in the layer of the skin where the melanocytes are located.**

- Inhibition of tyrosinase
- Anti-inflammatory effect
- Anti-oxidation

**✓ Alkaloids:**

**• Matrine: Small size and water soluble. Transport easier to the dermis as it is highly hydrophilic. It reaches the deeper layers of the skin where the extracellular matrix is found, which is very sensitive to degradation.**

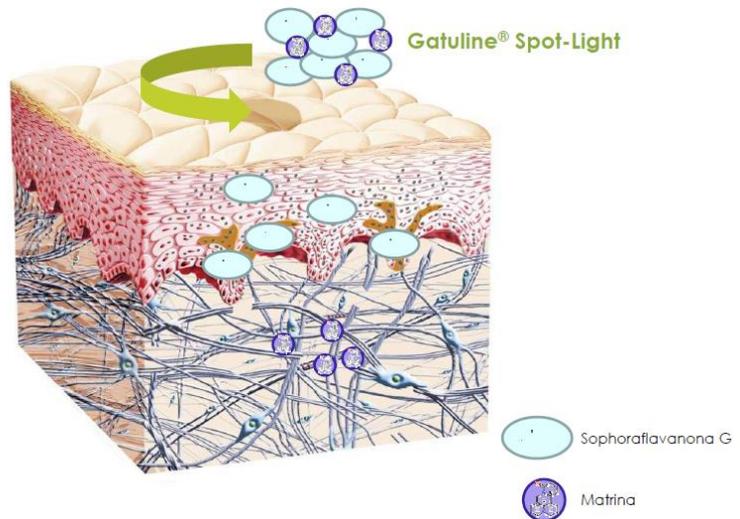
- Inhibition of MMPs
- Anti-inflammatory effect

## MECHANISM OF ACTION: Global: On all the causes of the spots

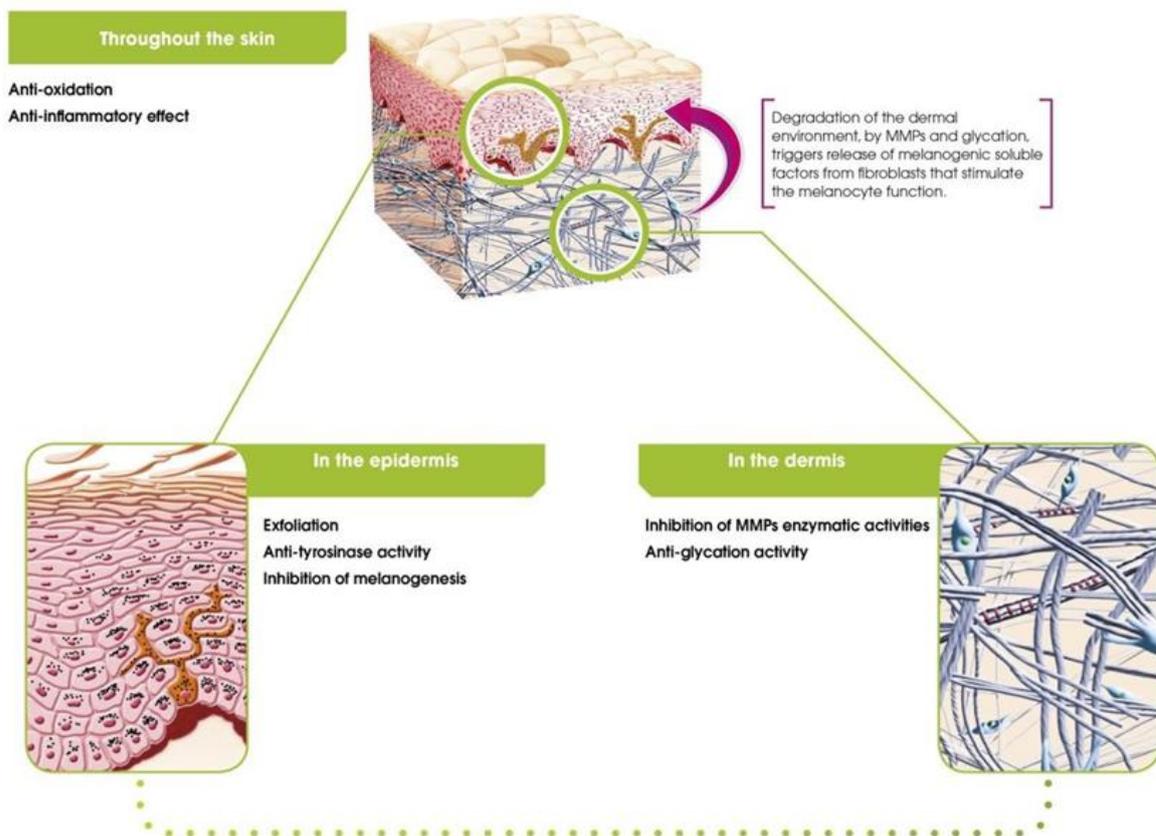
Melanocytes photo-age when exposed to excessive doses of free radicals and inflammation.

When this occurs melanin overproduction results in inadequate distribution of the melanin in the epidermis.

Until recently it was believed that melanogenesis was exclusively regulated in the epidermis by keratinocytes, but new investigations demonstrate the significant role of fibroblasts in hyperpigmentation, particularly when the dermal environment is photo-degraded: The degradation of the dermal environment triggers the Release of soluble factors stimulating melanocyte function by fibroblasts.

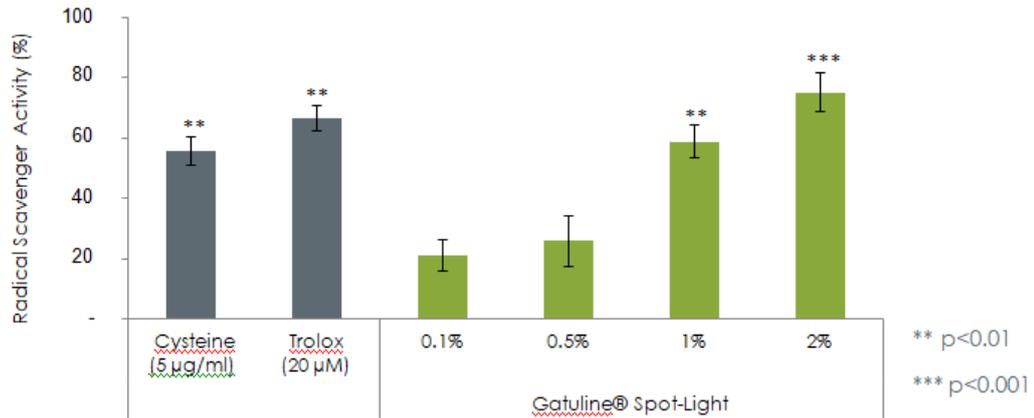


### Triple action for a greater efficiency:



## ■ TROUGHT THE SKIN: Anti-oxidant and anti-inflammatory effect

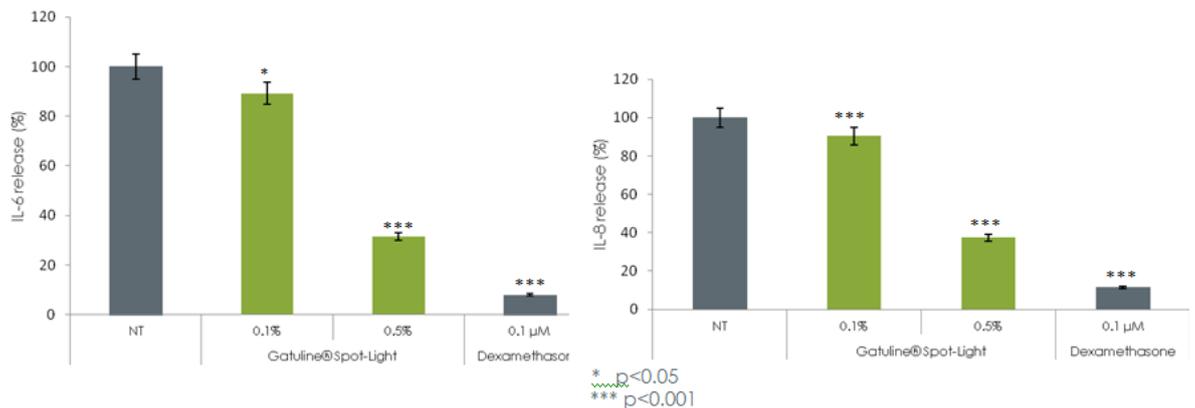
- 1. Anti-oxidation: In-vitro test on DPPH radical:** 30 minutes incubation w/wo Gatuline® Spot-Light. Measure of the absorbance at 518 nm using UV spectrophotometer:



⇒ Up to 75% DPPH radical scavenging activity (dose effect)

- 2. In-vitro test on the Anti-inflammatory effect: Measure of Pro-inflammatory mediators release by keratinocytes**

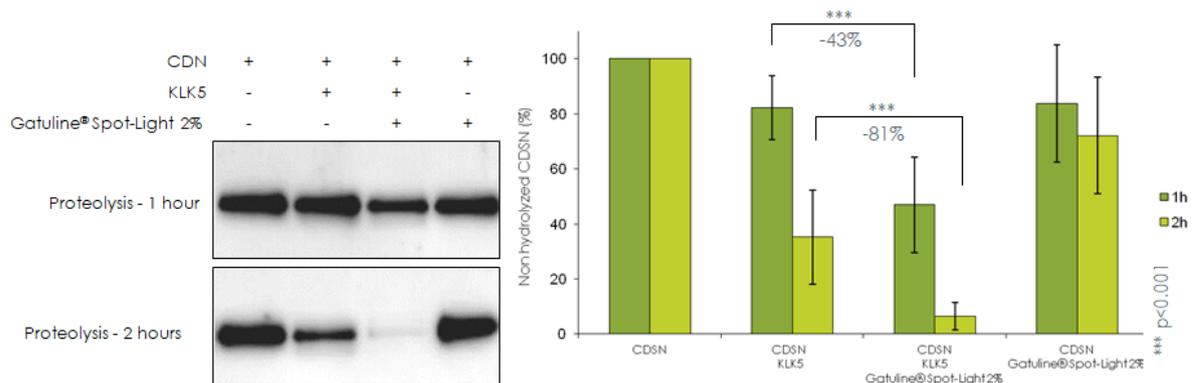
Under PMA stimulation. ELISA dosage



⇒ Strong inhibition of pro-inflammatory mediators release, up to IL-6: 70% and IL-8: 63%

## ■ IN THE EPIDERMIS: Spots's Exfoliation, Anti-tyrosinase activity, Inhibition of melanogenesis.

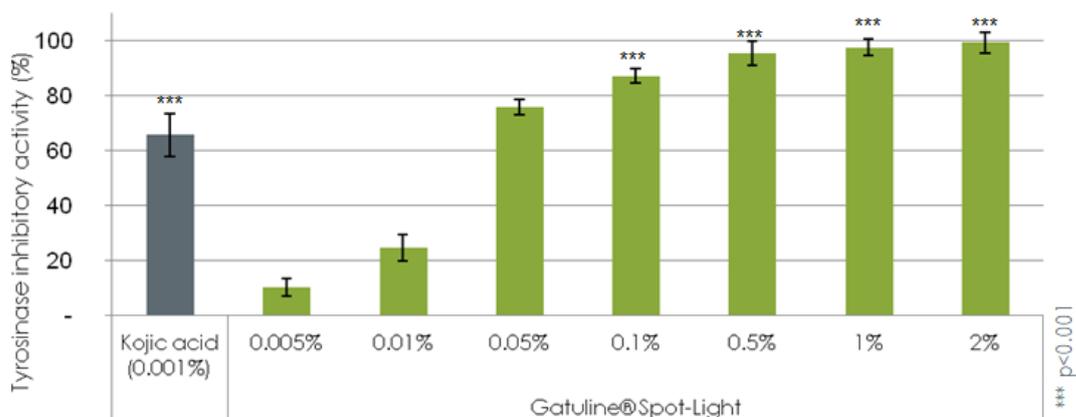
- Increases exfoliation in hyperpigmented areas:** Stimulates the enzymatic degradation of corneodesmosin by kallikrein-related peptidase-KLK5. It modifies the intercellular connections, which facilitates the desquamation:



➡ Up to +81% proteolysis of corneodesmosin due to enhanced enzymatic activity of KLK5

- Inhibition of tyrosinase, the enzyme that directly regulates the amount of melanin produced:**

10 minutes incubation w/wo Gatuline® Spot-Light or kojic acid. Addition of L-dopa, 10 min incubation, and measure of absorbance at 490 nm with a spectrophotometer.



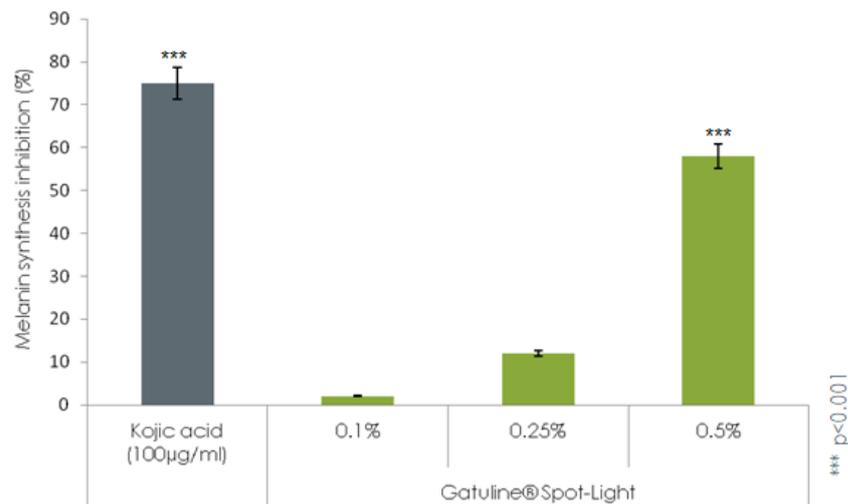
➡ More than 80% tyrosinase activity inhibition from 0.1% Gatuline® Spot-Light

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### 3. Inhibition of melanogenesis

B16 melanocytes. 72 hours incubation with NDP-MSH and w/wo Gatuline® Spot-Light

Quantification of total melanin content by measuring absorbance at 405 nm.



➔ 58% melanin synthesis inhibition with 0.5% Gatuline® Spot-Light (dose effect)

### ■ IN THE DERMIS: Inhibition of the enzymatic activities of MMPs. Antiglican activity.

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#### 1. Inhibition of enzymatic activities of MMPs

**Metalloproteinases (MMP)** are a family of proteolytic enzymes that develop a degradative function, generally directed to the extracellular matrix and its constituents (collagen, elastin ...). MMP-1 (collagenase-1), MMP-2 (gelatinase A), MMP-3 (stromelysin-2), MMP-9 (gelatinase B) and MMP-12 (macrophage metalloateastase) were measured. Incubation for 30 minutes with / without Gatuline® Spot-Light and measurement of absorbance at 412 nm with a spectrophotometer.

**For each metalloproteinase 2 % Gatuline® Spot-Light 2% reduces up to:**

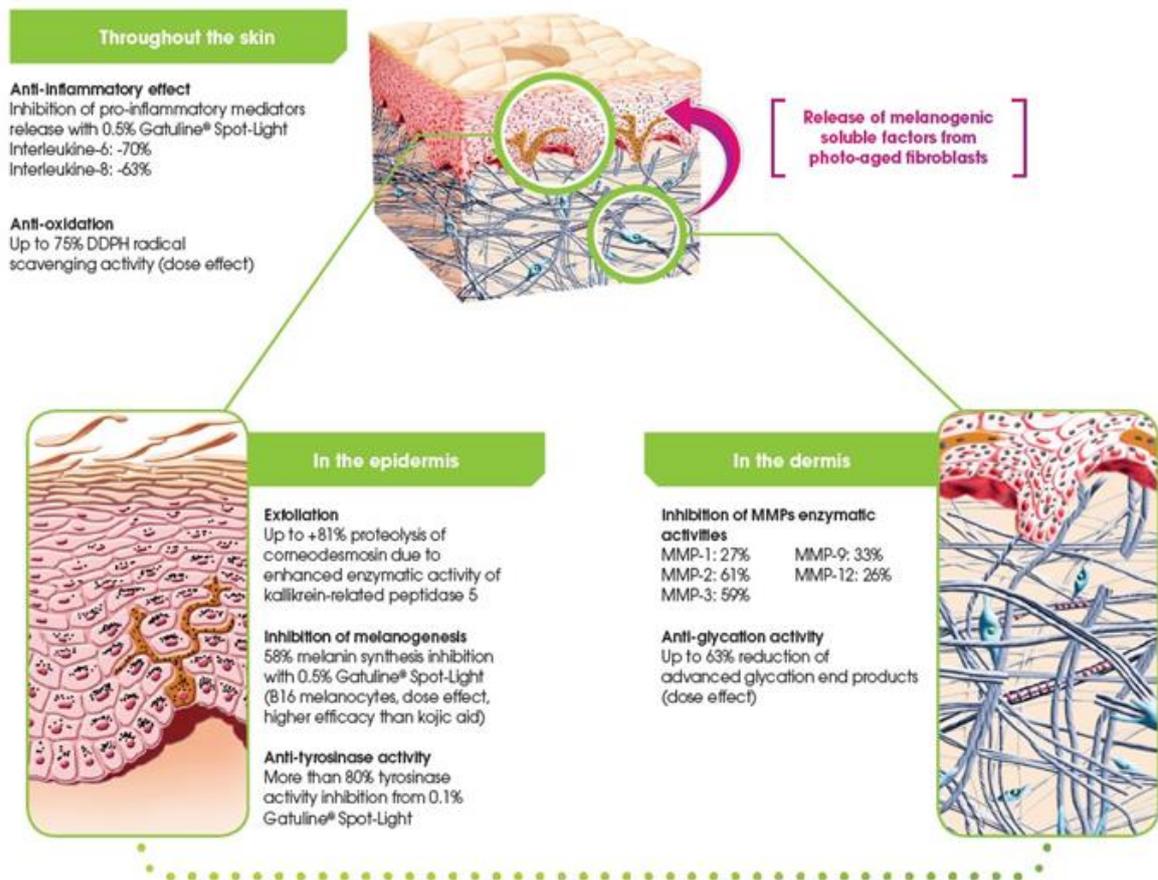
- ✓ **MMP-1: -27%**
- ✓ **MMP-2: -61%**
- ✓ **MMP-3: -59%**
- ✓ **MMP-9: -33%**

✓ **MMP-12: -26%**

## 2. Antiglycation

Model BSA / glucose. Incubation for 7 days with / without Gatuline® Spot-Light and fluorescence measurement with UV spectrophotometer.

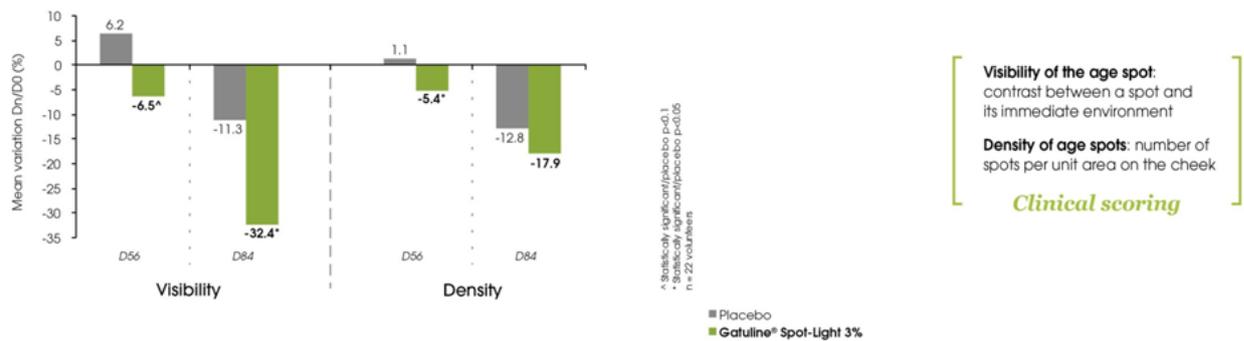
### ■ CONCLUSION:



## ■ IN-VIVO CLINICAL STUDY ON SENESCENT SPOTS:

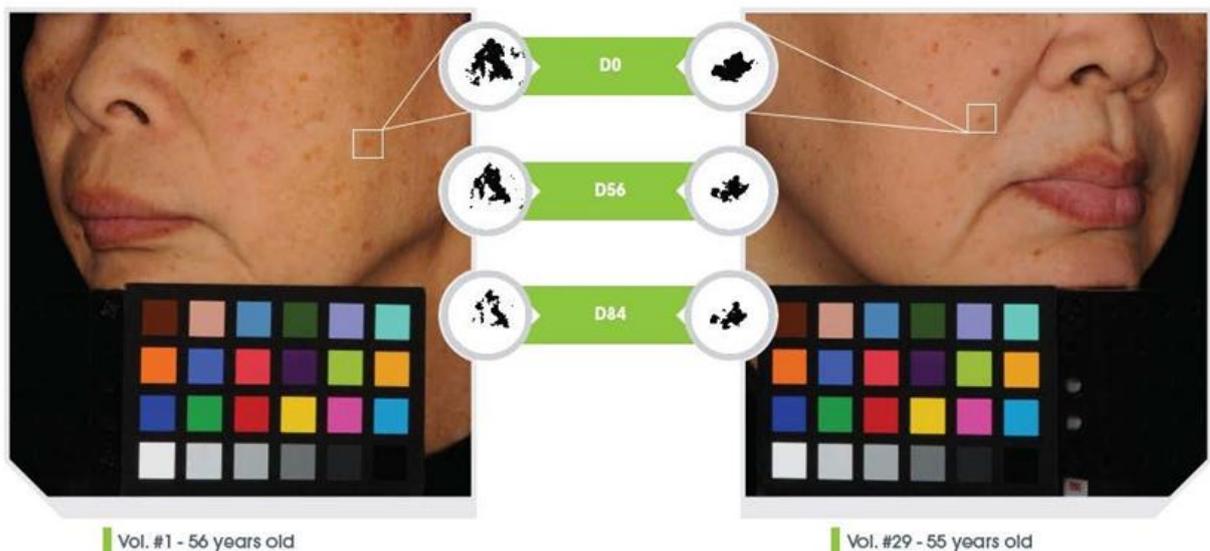
A clinical study carried out in Thailand on 30 Asian volunteers with senescence spots aged 45-63 years. Monocentric and double-blind study. Comparison: active formula (3%) versus placebo.

### Dermatological evaluation: Less blemishes and less visible



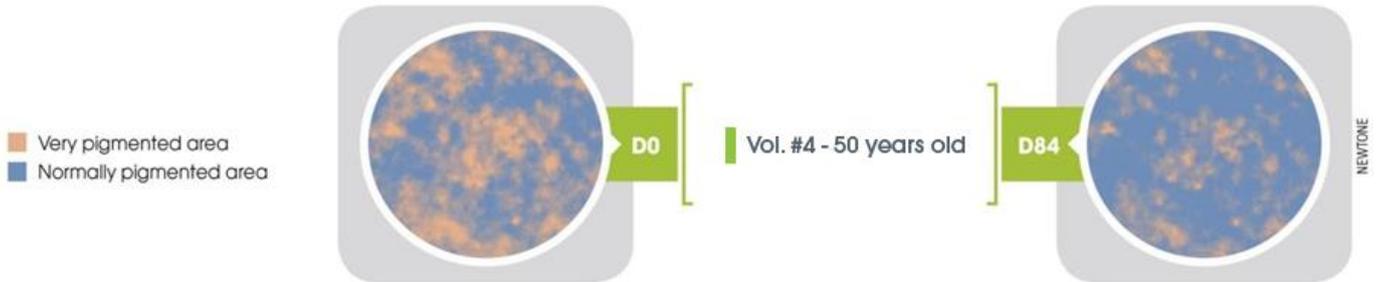
➡ Gatuline® Spot-Light induces a significant reduction of the visibility and density of age spots

### Gatuline® Spot-Light shows a visible correcting action on age spots of different morphologies



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## Visualization at the heart of the age spot



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## CORUM 9515: New generation of stable vitamin C

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**CORUM 9515** is a new generation of stable vitamin C derivative that provides superb whitening effects, serves to promote collagen synthesis and protects DNA damage.



- ✓ **Effective and stable skin lightening agent**
- ✓ **Balance the skin tone**
- ✓ **Reduce dark spot**
- ✓ **Prevent photoaging**
- ✓ **Increase collagen synthesis**
- ✓ **Excellent anti-oxidation properties**
- ✓ **Scavenge radical**
- ✓ **DNA protection**

Vitamin C, or L-ascorbic acid, acts as a cofactor for collagen synthesis. It has a high regenerating ability, by its collagen synthesis stimulating activity.

Vitamin C is essential for the proline hydroxylation, therefore in the development and maintenance of collagen integrity. In addition, vitamin C inhibits the synthesis of Extracellular Matrix Metalloproteinase enzymes of, enzymes which stimulates collagen degradation in the dermis.

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Vitamin C's collagen stimulating properties provides it with wound healing properties, caused by trauma, cuts, burns, or surgery. It is also suitable for the formation of new tissues.

Vitamin C belongs to the group of water soluble vitamins, and like most of them, it is not stored in the body for a long period of time, but in small quantities which are eliminated through urine. For this reason, Vitamin C daily administration is important in order to provide sufficient antioxidant protection.

Its chemical structure is similar to that of glucose (in many mammals and plants, this vitamin is synthesized from glucose and galactose). All compounds which possess the biological activity of ascorbic acid are known as Vitamin C. We should note that the only active form of Vitamin C is L-Ascorbic Acid.

As Vitamin C is a water-soluble substance, it is rapidly eliminated from the organism. Our body tends to protect vital organs, so any vitamin deficiency is felt primarily in the skin (less vital organ), which explains the importance of its topical application.

Pure Vitamin C is very unstable and sensitive to oxidation. Vitamin C contained in **GF LUMINOUS** is stabilized by an ethyl group and its effectiveness has been tested clinically:

## Clinical Efficacy Studies

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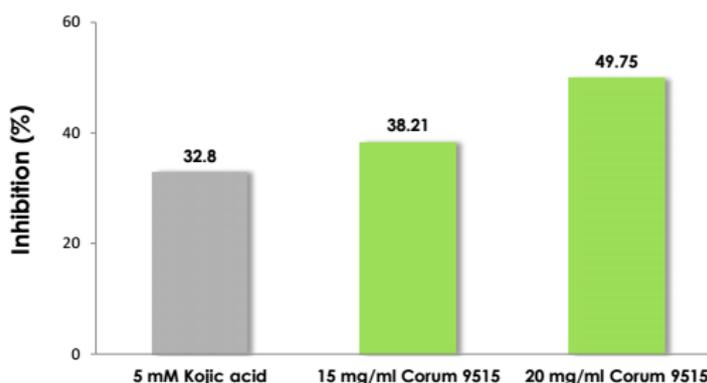
### 1- In-vitro whitening activity study - melanin assessment on human melanocytes:

An in-vitro evaluation of the Ethyl Ascorbic Acid anti-pigment ability was performed (Ethyl Ascorbic Acid is the Vitamin C form contained in VITAMIN C BRIGHTNESS SPRAY).

Theophylline was incubated with melanocytes in order to increase melanin production. Subsequently, Ethyl Ascorbic Acid was added at concentrations of 15 mg / ml and 20 mg / ml respectively, and comparing with kojic acid, a known anti-pigment ingredient.

At both concentrations of Ethyl Ascorbic Acid clear depigmentation effect was observed, obtaining 49.75% whitening effect with 20mg/ml Ethyl Ascorbic Acid:

#### Whitening effect (%)



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## 2- In-vivo whitening efficacy

A study on the in-vivo whitening capacity of Vitamin C (as Ethyl Ascorbic Acid) for 28 days in 20 healthy Asian women aged 25 to 40 years old with skin type III was performed.

A significant improvement in the skin lightening measured by chromatography observed:

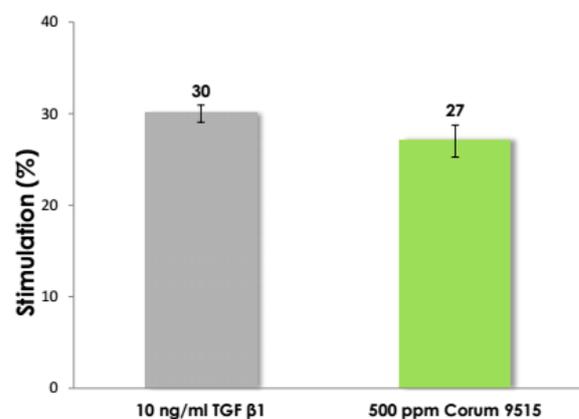


## 3- Stimulation of natural collagen synthesis:

The Stimulation of natural collagen synthesis activity was evaluated on human fibroblast culture.

After 24 hours of culture, the collagen was quantified using a Sircol Quantification Kit. Vitamin C had a similar effect on collagen synthesis that of TGF  $\beta$ 1 (growth factor which stimulates collagen synthesis):

### Stimulation of natural collagen synthesis:

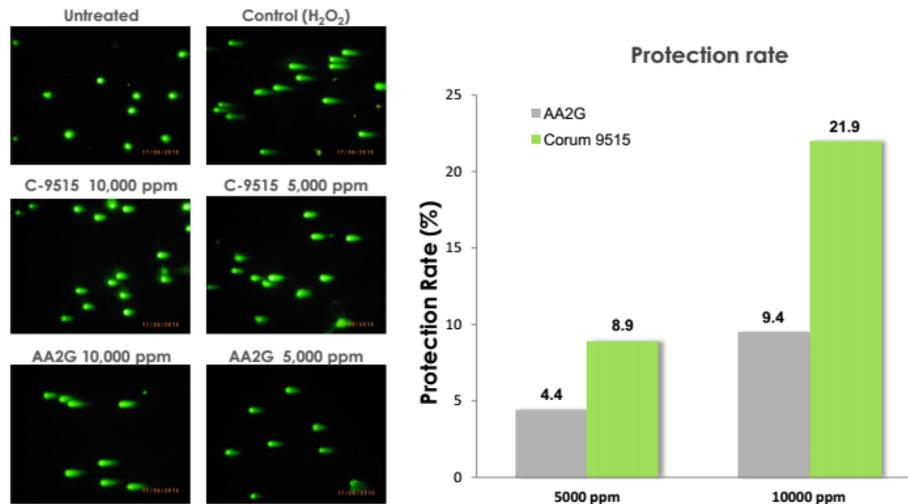


## 4- DNA protection by Comet assay in human fibroblasts:

The comet assay is a technique used to detect DNA damage and cell repair capacity. It is based on the DNA labile alkaline lysis at sites where damage has occurred.

When DNA is in good condition, it has a highly organized association with matrix proteins in the cell nucleus. When damaged, this organization is interrupted. The single DNA strands losing their compact structure and relaxes, expanding out.

Human fibroblasts were treated with Vitamin C (Ethyl Ascorbic Acid) for 24 hours, and then exposed to 100 mM H<sub>2</sub>O<sub>2</sub>. It was shown that Vitamin C at concentrations of 5000 ppm and 10,000 ppm, was able to protect DNA:



## Vitamin C Mechanisms of action:



Vitamin C anti-aging action is exerted through several ways:

### 1. Synthesis and repair of dermal collagen

Deficiency of ascorbic acid (AA) produces significant alterations in connective tissue, since Vitamin C is essential for collagen synthesis.

Vitamin C is essential for the transformation of proline in hydroxyproline and lysine in hydroxylysine (essential constituents of collagen). Consequently Vitamin C offers stability to the extracellular matrix.

The local increase of vitamin C means therefore significantly promote collagen production; therefore improved skin elasticity and greater resistance in wall capillaries are assessed.

### 2. Antioxidant activity

Vitamin C protects cells from free radicals. From all the scientific publications regarding Vitamin C, the most interesting are those related to the photoprotective effect of ascorbic acid when topically applied.

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In mouse and pig it showed that when ascorbic acid is applied before UV radiation exposure, the negative consequences it causes in the skin (erythema, histological changes, "burned cells", wrinkles ...) decreased significantly .

A study published by the Journal of Investigative Dermatology in May 1996, describes how topical application of vitamin C, vitamin E and Selenium protects rats skin cells from damage caused by exposure to UVB rays.

In parallel, the British Journal of Dermatology some years before, evidenced this protective effect of vitamin C, when used topically, on pig skin damaged by ultraviolet radiation.

The Spanish Journal of Physiology published a study showing how direct application of vitamin C protects, and thus prevents the aging in human skin cells in culture subjected to a strong oxidation stimulus with hydrogen peroxide.

One might think that its photoprotective effect was physical, that is to say, topical vitamin C behaves as a sunscreen, and however, its absorption spectrum has nothing to do with the emission of UV radiation. Later it was found that UV radiation produced a significant decrease in the levels of ascorbic acid in the skin.

All this goes to show that the UV light, after exhausting all the vitamin C present in the skin, cause an increase in free radicals, making manifest the neutralizing action of vitamin C.

### **3. Anti-inflammatory action**

Vitamin C inhibits NFκB, which is responsible for the activation of a number of pro-inflammatory cytokines. Therefore, Vitamin C has a potential anti-inflammatory activity and can be used in conditions like acne vulgaris and rosacea. It can promote wound healing and prevent post-inflammatory hyperpigmentation.

### **4. Vitamin C as a whitening agent**

When choosing a whitening agent, it is important to differentiate between substances that are toxic to the melanocyte and substances that interrupt the key steps of melanogenesis. Vitamin C falls into the latter category of depigmenting agents. Vitamin C interacts with copper ions at the tyrosinase-active site and inhibits action of the enzyme tyrosinase, thereby decreasing the melanin formation.