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CHROMABRIGHT™

CODE: ES291

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GENERAL DESCRIPTION

The colour of our skin and hair is determined mainly by the amount, distribution and type of melanin. Melanins are pigmented biopolymers that are synthesized by the dendritic melanocytes dispersed at the dermo-epidermal junction. Melanin synthesis takes place in membrane-bound organelles termed melanosomes, which contain specific enzymes controlling the production of the pigments [1]. Then, the mature organelles migrate towards the extremities of the melanocyte dendrites where they are transferred in skin to keratinocytes and in hair bulbs to the hair shaft, where the final distribution patterns of the pigment are determined [2].

The first and rate-limiting step of melanogenesis is mediated by tyrosinase. This enzyme catalyses the hydroxylation of tyrosine into 3,4-dihydroxyphenylalanine (DOPA) and the subsequent oxidation of DOPA into DOPAquinone [2].

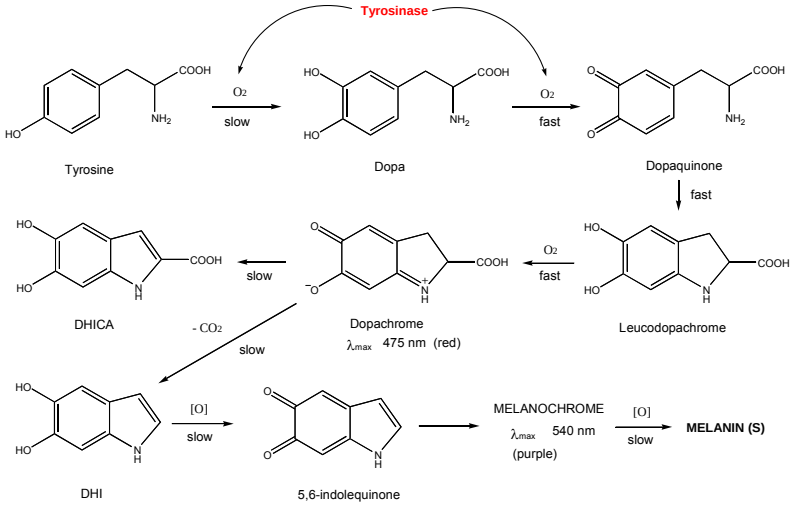


Fig. 1. Raper-Mason pathway for melanin synthesis

The perceived colour of skin and hair is determined by the ratio of eumelanins (brown and black pigments) to pheomelanins (amber and orange pigments) [3]. The resulting pigmentation is finally cleared by degradation of melanin during the ascent of the keratinocytes towards the outer stratum corneum. The remaining melanin pigments are shed with desquamation.

Typical pigmentary changes appear during intrinsic aging and photoaging. Abnormal accumulation of melanin is responsible for hyperpigmentations including melasma, freckles, age spots and senile lentigines, which could be a serious aesthetic problem [4]. Acute or persistent UV exposure can result in the formation of hyperpigmented lesions or regions of skin [3].

In Western countries, skin brighteners are applied for the prevention and treatment of irregular hyperpigmentation, such as age spots [5]. The affected individuals often use depigmenting agents to unify their skin colour. In Asia, lighter skin is a symbol of beauty and femininity. The use of skin brightening agents is widely extended by traditional beliefs.

Tyrosinase inhibition is the most common approach to achieve skin hypopigmentation as this enzyme catalises the rate-limiting step of pigmentation. The *in vitro* mushroom tyrosinase inhibition assay is one basic step to assess the direct effect of a given skin lightener on tyrosinase activity [1]. However, the use of mammalian-derived tyrosinase should be considered much better than mushroom tyrosinase [5]. L-dopa oxidation activities of human tyrosinase and mushroom tyrosinase show very different inhibitory effects depending upon the nature of skin lightening agents. Optimum pH, optimum temperature, IC_{50} values, and kinetic parameters of human tyrosinase are different from those of mushroom tyrosinase. Therefore, it is important to use human tyrosinase for the screening and the evaluation of skin brightening agents [4].

Despite the large number of molecules showing depigmenting properties *in vitro*, only some of them are able to induce an effective hypopigmenting effect measurable in clinical trials. This gap between *in vitro* and *in vivo* studies suggests that a new strategy is needed for discovering new depigmenting agents and validating their efficacy. Unfortunately, statistically significant demonstrations of comparative clinical efficacy and safety of these products are not always met. Many inhibitors in cell-free enzymatic assays are likely to have some toxicity or delivery problems in cell-based assays [1]. Current depigmenting agents found in the cosmetic market show numerous adverse effects such as high irritant and sensitising potential, cytotoxicity and instability in formulations.

There is a perceived need in the market for novel skin brightening agents with increased efficacy and improved safety profiles.

New depigmentation products should include the following desirable features: a) inhibition of mammalian tyrosinase; b) lack of toxicologic or mutagenic potential; c) clinical efficacy; d) formulation stability; and e) novelty and patent protection of the agent and/or formulation [3].

CHROMABRIGHT™ is a new patented molecule designed for skin brightening applications that fulfills all the desirable qualities for a new skin brightener. Its hypopigmenting activity has been proved in *in vitro* and *in vivo* studies.

Melanogenesis inhibition efficacy of CHROMABRIGHT™ has proven *in vitro* to be dose-dependent and noticeably higher than the well-known lightening agents Arbutin, Kojic Acid and Magnesium Ascorbyl Phosphate. In addition, at the same concentration this new molecule has showed a similar depigmenting activity than Hydroquinone but presenting no cytotoxicity at the dose assayed. Low concentrations of CHROMABRIGHT™ have demonstrated a significant skin brightening effect when tested on a panel of human volunteers *in vivo*.

Furthermore, CHROMABRIGHT™ has shown to possess a photoprotective effect on human epidermal keratinocytes. Unlike other depigmenting agents, which can cause skin irritation when exposed to the sun, CHROMABRIGHT™ helps to prevent the skin damage caused by UV radiation.

CHROMABRIGHT™ has shown no cytotoxic effects on melanocytes, keratinocytes or fibroblasts, neither mutagenicity nor any irritation or sensitisation reaction. It is a completely safe skin brightening active for cosmetic applications.

Other important features of CHROMABRIGHT™ are its high stability in formulations, high solubility in oils, and detectability at very low concentrations.

PROPERTIES AND APPLICATIONS

- Inhibits mushroom and endogenous human tyrosinase activity.
- Exhibits a significant dose-dependent depigmenting effect on human melanocytes, which is noticeably higher than Arbutin, Kojic Acid and Magnesium Ascorbyl Phosphate.
- At the same concentration, CHROMABRIGHT™ is not cytotoxic while Hydroquinone clearly presents cytotoxicity, having both a similar depigmenting activity.
- Has a significant photoprotective effect on human epidermal keratinocytes cell cultures, therefore preventing UV-induced skin damage.
- Induces a significant brightening effect on the skin *in vivo*.
- Reliable stability in final formulations.
- CHROMABRIGHT™ has a completely safe profile for cosmetic applications.

CHROMABRIGHT™ can be incorporated in cosmetic formulations such as emulsions, oily sera, and in general in any formulation containing oil or silicon phases.

TECHNICAL INFORMATION

PRODUCT SPECIFICATIONS

Appearance:	Powder
Colour:	White – Off white
Melting point:	42 – 44 ºC
UV max. (in acetonitrile):	284 – 290 nm
Molecular weight:	446.66

PROCESSING AND DOSAGE

CHROMABRIGHT™ can be easily incorporated into cosmetic formulations dissolved in the appropriate solvent (see solubility table). It must be added at the oily phase.

SOLVENT INCI NAME	SOLUBILITY AT ROOM TEMPERATURE
Ethylhexyl Cocoate	26.89%
C-12-15 Alkyl Benzoate	34.61%
Soybean (Glycine Soja) Oil	19.80%
Caprylic/Capric Trigliceride	24.35%
Ethylhexyl Methoxycinnamate	35.88%
Mineral (Paraffinum Liquidum) Oil	13.52%
Isohexadecane	15.66%
Peg-7 Glyceryl Cocoate	9.27%
Cyclopentasiloxane	1.27%
Ethanol	6.39%

A dosage of 0.1 – 0.5% of CHROMABRIGHT™ is recommended to obtain a significant brightening effect.

CHROMABRIGHT™ is also available dissolved in an oil (CHROMABRIGHT™ MFF, code ES292). A 5% dosage of the oily version is recommended in final cosmetic products. See table below to check CHROMABRIGHT™ MFF compatibility with solvents:

SOLVENT INCI NAME	SOLUBILITY AT ROOM TEMPERATURE
Ethylhexyl Methoxycinnamate	9.1%
Mineral Oil (Paraffinum Liquidum)	33.3%
Isohexadecane	Compatible[a]
Sweet Almond (Prunus Amygdalus Dulcis) Oil	4.8%
Soybean (Glycine Soja) Oil	4.8%
Sunflower (Helianthus Annuus) Seed Oil	4.8%
Isopropyl Myristate	Compatible[a]
Isopropyl Palmitate	67.9%
C12-15 Alkyl Benzoate	23.1%
Caprylic/Capric Triglyceride	16.7%
Dimethicone	Compatible[a]
Phenyl Trimethicone	Compatible[a]
Dicaprylyl Ether	Compatible[a]
Cetearyl Isononanoate	33.3%
Squalane	23.1%

[a] The active is miscible in all proportions

STORAGE AND SHELF LIFE

Keep in a clean, cool and dark place. Care should be taken not to exceed 40 ºC during the storage and transport of the CHROMABRIGHT™ powder. If product is stored as recommended it will remain stable for at least 30 months.

STABILITY

The stability of CHROMABRIGHT™ was tested in an O/W emulsion. The emulsion was stored at 40 ºC and at room temperature and analysed at 0, 1, 2, 3, 4 and 6 months by HPLC. CHROMABRIGHT™ was also formulated at different pHs (3.8, 5.0 and 8.4) in order to study the influence of the pH of the final product in the stability of CHROMABRIGHT™. Two different samples were stored at room temperature and at 60ºC during 10 and 30 days. After this time, CHROMABRIGHT™ was analysed by HPLC.

Composition of the O/W emulsion:

Ingredient	**%**
Water	qsp 100
Mineral oil (Paraffinum Liquidum)	10
Polyacrylamide, C13-14 Isoparaffin, Laureth-7	3
CHROMABRIGHT™	0.05

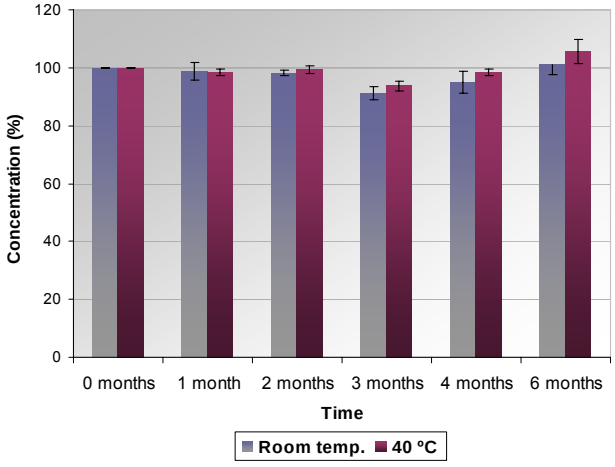


Fig. 2. Stability of CHROMABRIGHT™ in an O/W emulsion after 6 months

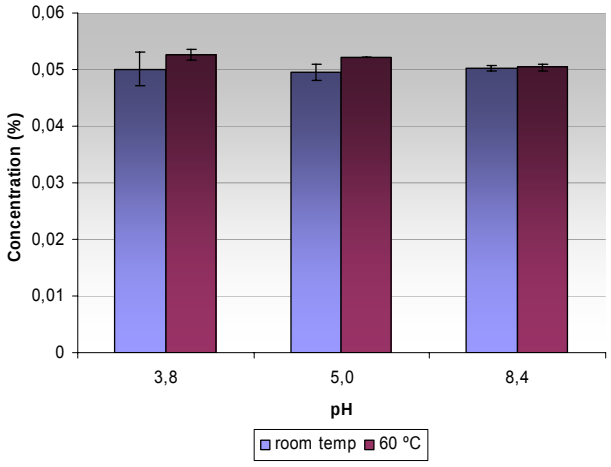


Fig. 3: Concentration of Chromabright™ in an O/W emulsion at different pHs after 30 days

The concentration of CHROMABRIGHT™ experienced no significant variations during the stability study and proved to remain stable in an O/W emulsion after 6 months. CHROMABRIGHT™ also proved to be stable in emulsions at different pH.

A complete stability report is available upon request.

SAFETY

The toxicological profile of CHROMABRIGHT™ for cosmetic purposes was assessed *in vitro* and *in vivo*. A full toxicological report and a summary of all the safety tests performed are available on request.

In vitro tests

Ocular Irritation (HET-CAM test)
The product is potentially not irritating for the eyes.

NRU Phototoxicity test
The results showed no signs of phototoxicity.

Cytotoxicity test on human epidermal keratinocytes
The results showed no signs of cytotoxicity at the concentrations assayed.

Cytotoxicity test on 3T3 fibroblasts
The results showed no signs of cytotoxicity at the concentrations assayed.

Bacterial reverse mutation test (Ames test)
The product produced no mutagenic activity in any of the five bacterial strains used.

Cytotoxicity on human primary melanocytes
The product showed no significant cytotoxic effects on human primary melanocytes.

In vivo tests

Skin sensitisation and cutaneous compatibility test
A HRIPT (Human Repeated Insult Patch Test) was performed on 100 volunteers aged 18 to 68. CHROMABRIGHT™ neither induced skin irritation nor showed any cutaneous sensitisation.

EFFICACY

In vitro

Tyrosinase inhibition

The *in vitro* mushroom tyrosinase inhibition assay is one basic step to assess the direct effect of a given skin brightener on tyrosinase activity. This assay is more relevant when performed using purified mammalian tyrosinase or recombinant enzymes, because of the different substrate and cofactor requirements, as well as sensitivity to inhibitors.

Digestion of L-Dopa (tyrosinase substrate) in the presence of test items and measure of absorbance variations at 475 nm (wavelength of melanin absorption) is a validated assay for measuring the potential inhibitory activity of compounds on the tyrosinase activity.

The results obtained in all experiments were normalised regarding the absorption of a control experiment (without test items). Kojic acid 0.1mM was used as positive control.

- **Inhibition of mushroom tyrosinase**

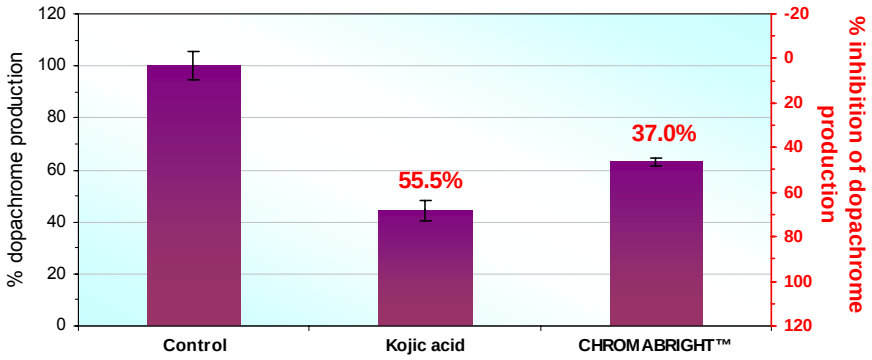


Fig. 4. Inhibition of mushroom tyrosinase activity

CHROMABRIGHT™ was able to inhibit mushroom tyrosinase activity by 37% at the concentration tested (1mM).

- **Inhibition of endogenous human tyrosinase**

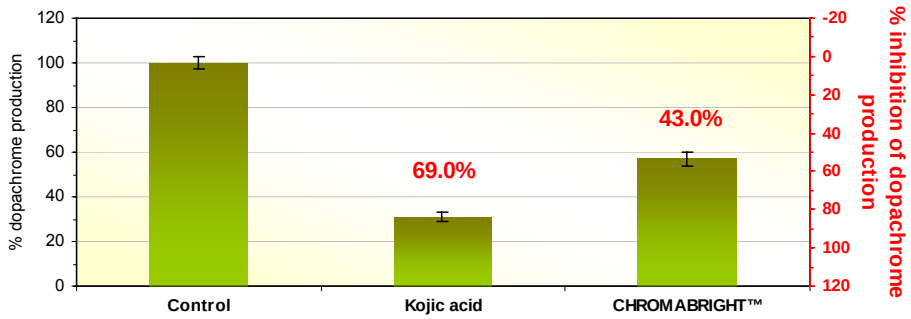


Fig. 5. Inhibition of endogenous human tyrosinase activity

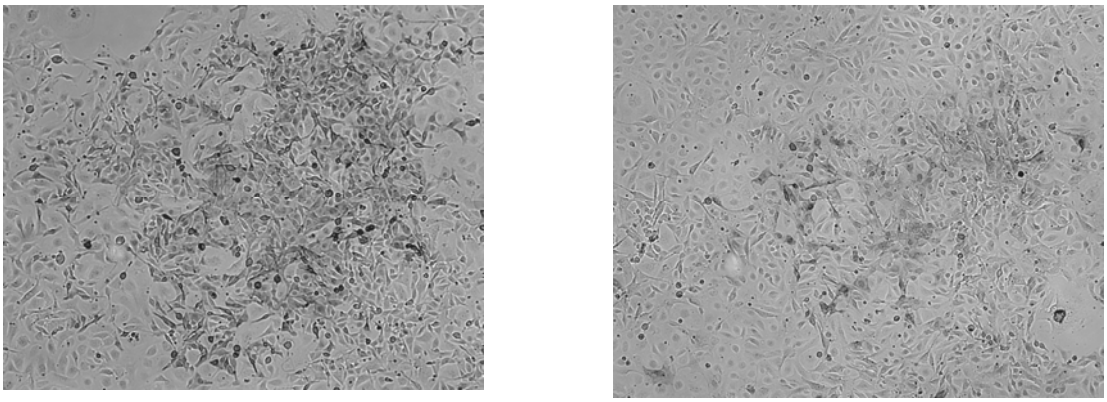
CHROMABRIGHT™ was able to inhibit endogenous human tyrosinase activity by 43% at the concentration tested (1mM).

Depigmenting effect on human melanocyte cultures

Plated human melanocytes were incubated for 5 days or 9 days and fresh medium containing 0.1mM CHROMABRIGHT™ or 0.1mM kojic acid was added daily. The melanin content was visualised directly by image recording through a microscope using a 40x lens. The lightening efficacy was blindly assigned by counting the cells showing melanin staining and the total number of cells.

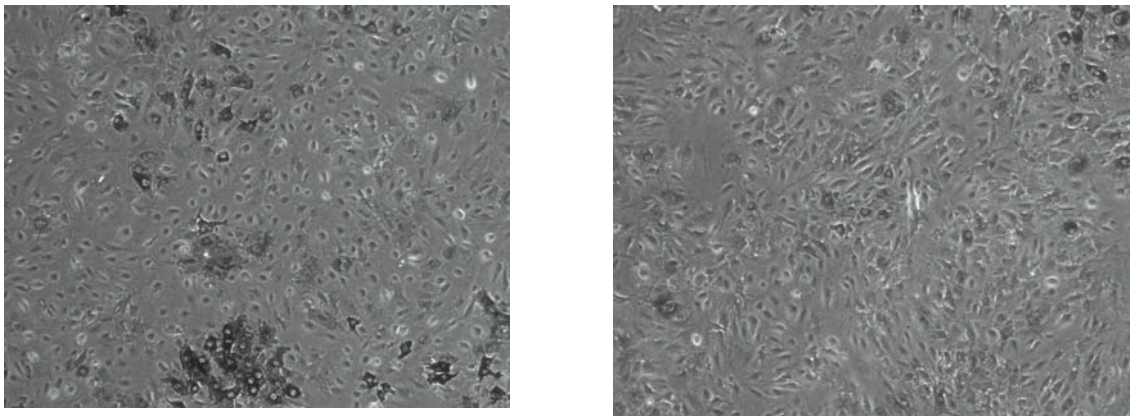
The results obtained in all experiments were normalised regarding the lightening efficacy of a control experiment (without test items).

Kojic acid was used as positive control (n.d.= not determined).



Control 0.1mM CHROMABRIGHT™

Fig. 6. Microscopy images of human melanocytes cultured for 5 days



Control 0.1mM CHROMABRIGHT™

Fig. 7. Microscopy images of human melanocytes cultured for 9 days

As it can be observed in Table 1, the percentage of melanin stained cells decreased after incubation of human melanocyte cultures with CHROMABRIGHT™.

	5 days	9 days
Control	22.09 ± 1.94	24.24 ± 3.75
Kojic acid	15.41 ± 1.81	n.d.
CHROMABRIGHT™	11.96 ± 1.43	9.40 ± 1.54

Table 1. Percentage of melanin stained cells after incubation of human melanocytes.

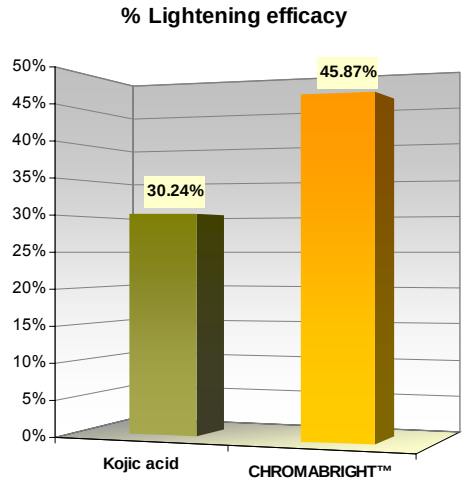


Fig.8 . Lightening efficacy of CHROMABRIGHT™ on human melanocytes cultures after 5 days of incubation

As shown in Fig. 8, CHROMABRIGHT™ showed a better depigmenting effect than Kojic acid after 5 days of incubation. A time-dependent effect is observed after 9 days of incubation, when CHROMABRIGHT™ raised to **61.2% of lightening efficacy**.

Melanogenesis inhibition on human epidermal melanocytes

Primary human melanocytes (HEMn-DP) cell cultures were used to compare the melanogenesis inhibition efficacy of different known depigmenting products (Hydroquinone, Arbutin, Magnesium Ascorbyl Phosphate (MAP) and Kojic Acid) respect to CHROMABRIGHT™.

The concentrations obtained were: 5, 10, 100, 150 and 200 µM for CHROMABRIGHT™ and 10 µM for the other products. Melanocytes were seeded and were allowed to grow until confluence for 2 weeks. After overnight incubation, first treatment with products was done and was repeated on days 3, 6, 8, 10, 13, 15 and 17. A control was performed with medium without treatment.

After 20 days of culture, melanin concentration was determined by measurement of absorbance at 450 nm and values were normalised respect to the number of cells per well. Melanin concentration was determined from a standard curve plotted with synthetic melanin at known concentrations. The percentage of melanin respect to control was calculated as: T/C x 100, where T represents the pg/cell of melanin obtained for treated-cells and C that of the control cells. The evaluation of statistical significance was performed by the one-way analysis of variance (ANOVA).

CHROMABRIGHT™ inhibited melanogenesis at all tested concentrations, showing a dose-dependent depigmenting efficacy.

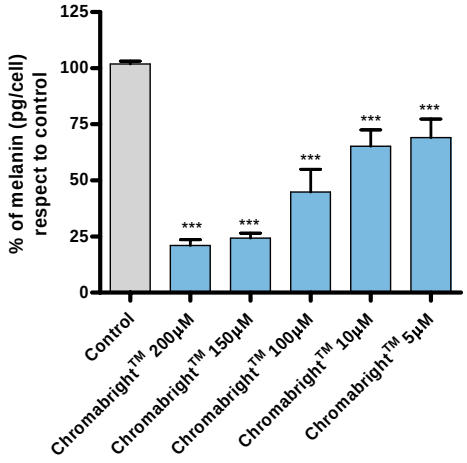


Fig. 9. Percentage of melanin (pg/cell) after treatment with CHROMABRIGHT™ respect to non-treated cells (control) (*** P<0.01)

CHROMABRIGHT™ demonstrated to possess a higher depigmenting activity than MAP, Kojic Acid and Arbutin and a similar level of efficacy than Hydroquinone at the same concentration (10 µM).

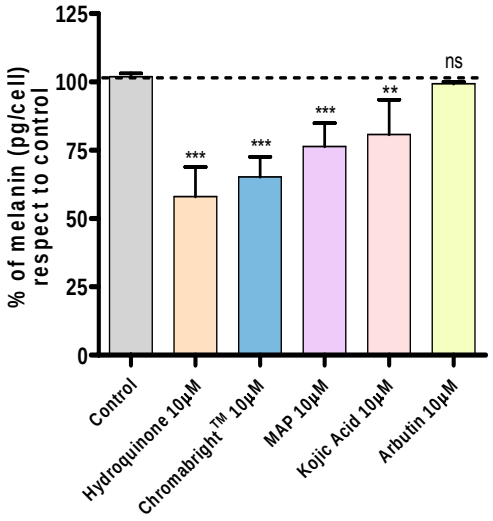
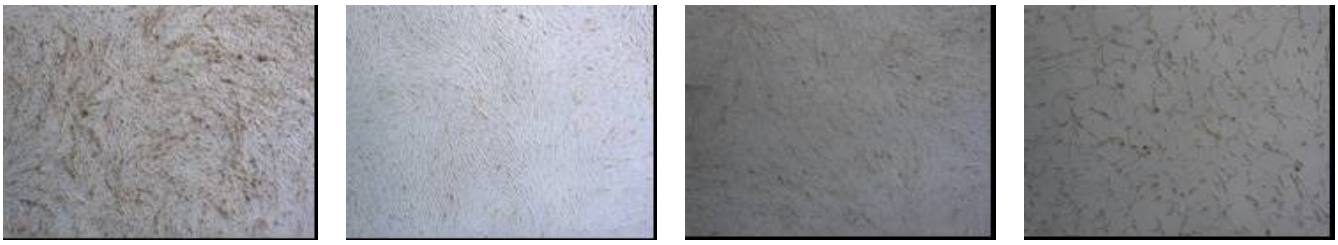


Fig. 10. Percentage of melanin (pg/cell) after treatment with Hydroquinone, CHROMABRIGHT™, MAP, Kojic Acid or Arbutin respect to control (*** P<0.01; ** P<0.05; ns= not significant)

CHROMABRIGHT™ melanogenesis inhibition was also studied by optical microscopy showing that at the same concentration (10µM), CHROMABRIGHT™ presented a similar depigmenting effect than Hydroquinone (Fig. 11). Moreover, Hydroquinone cytotoxic effect was clearly observed. On the contrary, CHROMABRIGHT™ did not present cytotoxicity at the dosages tested.



Control | CHROMABRIGHT™ 200 µM | CHROMABRIGHT™ 10 µM | Hydroquinone 10 µM

Fig 11. Microscopic photographs of human melanocytes treated with CHROMABRIGHT™ or Hydroquinone for 20 days.

CHROMABRIGHT™ melanogenesis inhibition efficacy proved to be dose-dependent and noticeably higher than Arbutin, Kojic Acid and Magnesium Ascorbyl Phosphate. Furthermore, at the same concentration, CHROMABRIGHT™ showed a similar depigmenting activity than Hydroquinone but presenting no cytotoxicity at the dose assayed.

Photoprotection on human epidermal keratinocytes

The *in vitro* HEKa NRU photoprotection test is based on the determination of the protective effect of a chemical when tested in the presence of a cytotoxic dose of simulated solar light.

The photoprotective effect is expressed as an increase of the uptake of the vital dye Neutral Red (NR), when measured 24 hours after treatment of human epidermal keratinocytes with the test chemical and irradiation. NR is a weak cationic dye that readily penetrates cell membranes by non-diffusion, accumulating intracellularly in lysosomes. Alterations of the cell surface of the sensitive lysosomal membrane lead to lysosomal fragility and other changes that gradually become irreversible. Such changes brought by the action of solar light result in a decreased uptake and binding of NR. Cell viability is determined by Neutral Red Uptake, measuring the optical density of the NR extract at 540 nm in a spectrophotometer.

The responses obtained in the presence of 47 µg/mL and 150 µg/mL of CHROMABRIGHT™ were compared to irradiated (CTR+UV) and non-irradiated (CTR-UV) control cells (non-treated cells).

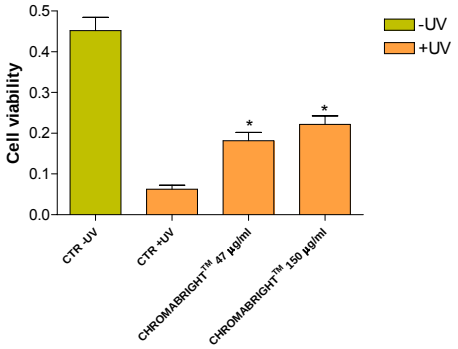


Fig. 12. Cell viability in HEKa cultures (*p<0.01)

Irradiated cells treated with CHROMABRIGHT™ showed an increased viability respect to irradiated non-treated cells. 47µg/mL CHROMABRIGHT™ induced a **190.4% increase** in cell viability respect to irradiated control cells, and 150 µg/mL CHROMABRIGHT™ **increased cell viability by 254.88%**.

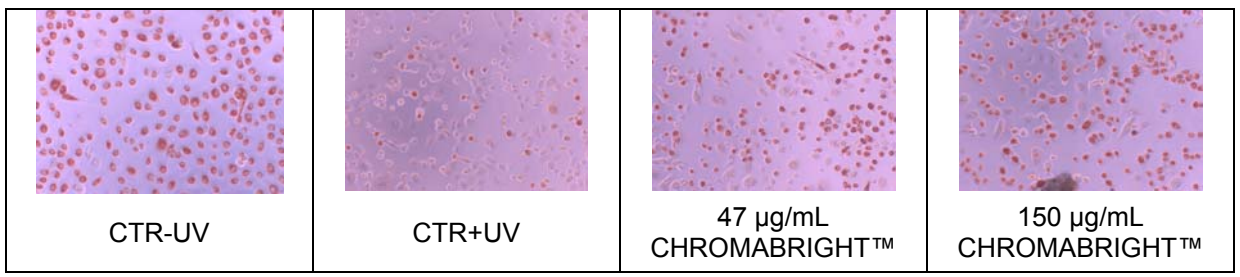


Fig. 13. Neutral Red Uptake in HEKa cultures

CHROMABRIGHT™ proved to have a significant photoprotective effect on human epidermal keratinocytes cell cultures, and can therefore prevent the skin-damaging effects of ultraviolet radiation.

In vivo

Brightening effect

20 healthy Asian female volunteers, aged 18 to 46, from all skin types were selected for this study. The volunteers applied a cream containing 0.1% CHROMABRIGHT™ on one side of the face twice daily for 2 months, and a placebo cream on the other side.

The brightening effect was instrumentally evaluated by means of a Chromameter CR-300. Mean values from 5 successive measurements were calculated. These 5 measurements were rigorously made at the same location at each time.

Measurements were taken before application, after 30 days and after 60 days of treatment.

The following parameters were used to evaluate the *in vivo* effects of CHROMABRIGHT™ on the colour of the skin:

L* (Luminance): represents the relative brightness from total darkness (L*=0) to absolute white (L*=100).
a*: red-green colour axis.
b*: yellow-blue colour axis.

The best description of a brightening effect is given by combining the L* and b* parameters, in the Individual Typologycal Angle ITAº, which is obtained according to the following formula [1]:

$$ITA^{\circ} = \text{Arctg}\,[(L^*-50)/b^*]\cdot(180/\pi)$$

The brighter is the skin, the higher are the L* and ITAº parameters.

After 30 days of treatment, a significant increase ($p<0.01$) in the Luminance L* and in the ITAº was observed for the cream containing CHROMABRIGHT™. The placebo cream presented no significant effects.

After 60 days of treatment, the brightening effect induced by the cream containing CHROMABRIGHT™ increased respect to the results at 30 days, and it was significantly superior ($p<0.001$) to the effect observed for the placebo cream.

At the end of the study, no adverse effects were reported by the volunteers.

The results of the test are summarised in the following table and graphs:

	30 days	60 days
ΔL*	1.1% (p<0.01)	1.7% (p<0.001)
ΔITA°	7.7% (p<0.01)	13.7% (p<0.001)

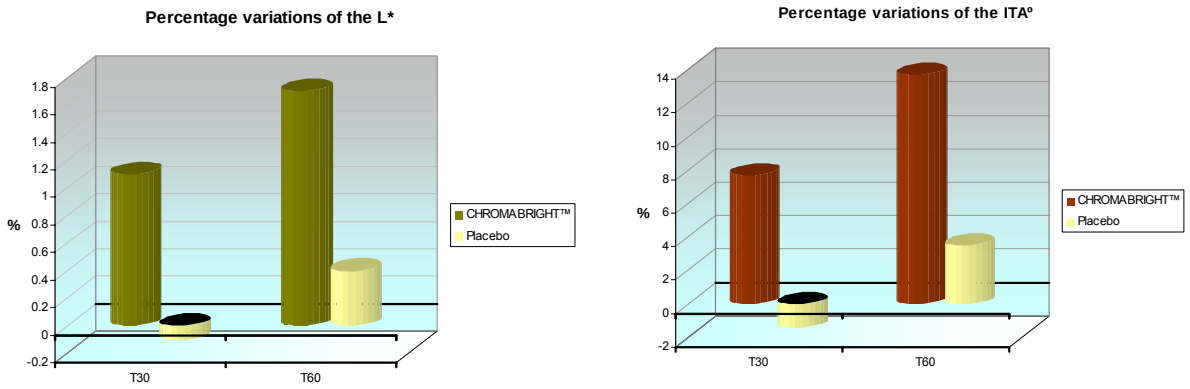


Fig. 14. Variations of the L* and the ITA° parameters after treatment with a cream containing CHROMABRIGHT™

The results of the *in vivo* test prove that CHROMABRIGHT™ is able to induce a significant brightening effect after 30 and 60 days of treatment.

STUDY OF THE EFFICACY AGAINST MELASMA AND LENTIGINES

The aim of the study was to evaluate the efficacy of CHROMABRIGHT™ in a panel of 14 volunteers affected of melasma and/or actinic lentigines. The volunteers applied a cream containing 0.5% CHROMABRIGHT™ on their face and/or hands twice a day for 30 days. The dermatological evaluation was performed at the beginning of the treatment and at 15 and 30 days according to the following score scale:

Score	Clinical evaluation	% of improvement
1	No improvement	0%
2	Slight improvement	10-30%
3	Fairly good improvement	40-70%
4	Good improvement	80-100%

CHROMABRIGHT™ showed to improve melasma by 48% and lentigines by 47% at 30 days of treatment.

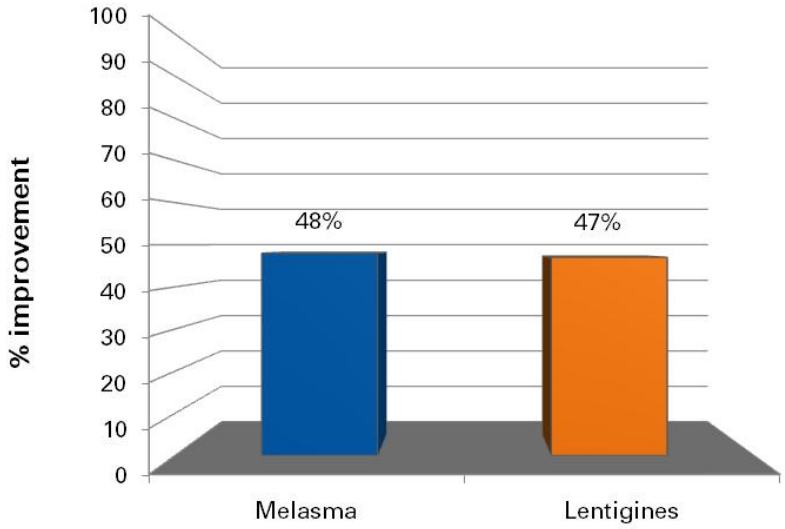
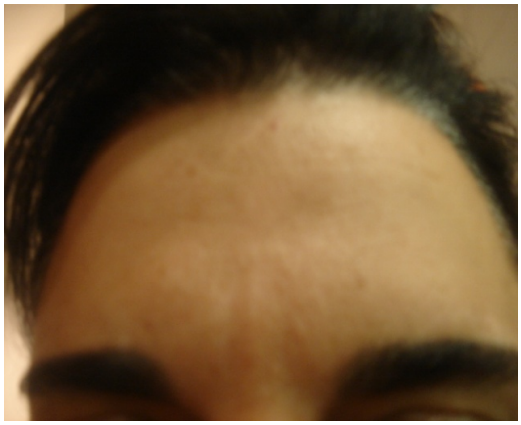


Fig.15. Percentage of improvement of melasma and lentigines at 30 days

0 days



30 days

The treatment was prolonged up to 60 days in 10 of volunteers presenting melasma and lentigines. CHROMABRIGHT™ demonstrated to reduce the appearance of melasma by 72% and lentigines by 67% at 60 days of treatment.

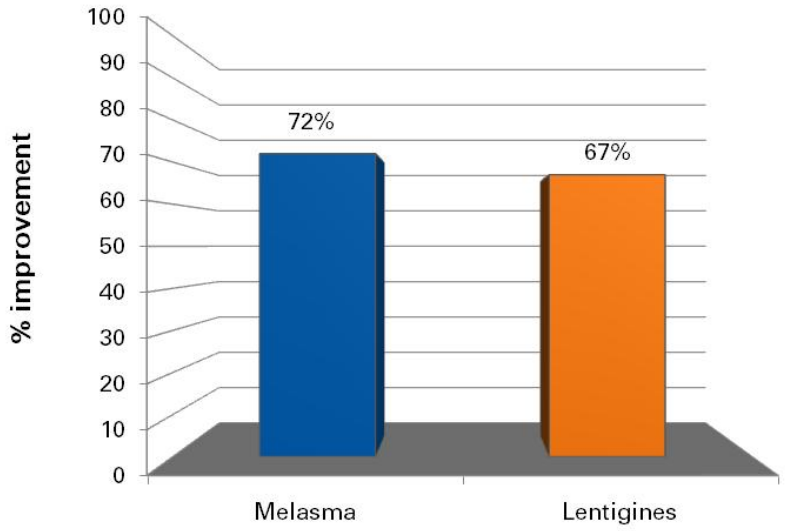


Fig.16. Percentage of improvement of melasma and lentigines at 60 days

0 days

60 days

CHROMABRIGHT™ proved to have high efficacy in reducing the appearance of melasma and actinic lentigines.

GENERAL PRODUCT INFORMATION

Trade name	CHROMABRIGHT™
Product code	ES291

INGREDIENTS

INCI name	CAS No	EINECS No
DIMETHYLMETHOXY CHROMANYL PALMITATE	1105025-85-1	-

Note: Graphs and photographs are available for customer use provided that the final product contains the same concentration of active as the formulations in our tests. Customers must request written permission for use of the graphic material and/or ingredient tradenames to Lipotec. Customers are responsible for compliance with local and international advertising regulations.
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