

IFSCC 2025 full paper (**IFSCC2025-1222**)

## **“Revolutionizing Skin Tone Management: Glucuronyl Glucosyloleanolate, a Natural and AI-Discovered Compound”**

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### **1. Introduction**

Use of cosmetic products claiming skin lightening effect, fading hyperpigmentation spots or medical treatment to solve pigmentation issues, is widespread and a growing habit among the global population.

The market relies mostly on tyrosinase inhibitors such as hydroquinone, kojic acid, azelaic acid, or alpha-arbutin. Copper chelating effect of these molecules can create adverse effects thus leading to either ban or limitation in concentration use by relevant authorities.<sup>1</sup> Abuse of these ingredients outside of the allowed limits and/or without an appropriate supervision could lead to serious and irreversible adverse effects such as permanent exogenous ochronosis.<sup>2</sup>

In light of the limitations associated with existing depigmenting agents, it is imperative to develop new actives that maintain high efficacy while demonstrating superior safety in toxicological assessments.

In this study, we report the successful identification and evaluation of a natural compound, Glucuronyl Glucosyloleanolate as a disruptive depigmenting-brightening active ingredient leveraging a proprietary A.I. enabled discovery platform. The compound exhibits strong efficacy and an excellent safety profile across *in-vitro*, *ex-vivo*, and clinical studies, with an 86% reduction in melanin production *in-vitro*, a 50% decrease in melanin content on human explants, and up to a 24% increase in ITA in clinical trials. These results underscore its potential as a safe and effective solution for skin depigmentation and brightening.

The successful validation of this innovation platform opens opportunities to expand its use to other segments such as melanin boosters and anti-aging, and may lead to the discovery of new active ingredients.

<sup>1</sup> <https://www.canada.ca/en/health-canada/services/consumer-product-safety/cosmetics/cosmetic-ingredient-hotlist-prohibited-restricted-ingredients/changes.html>

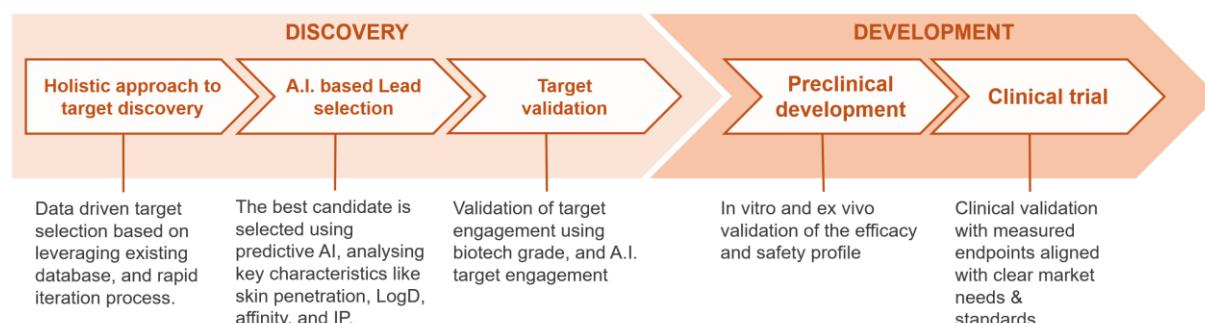
<sup>2</sup> Ajose, F.O.A. et al., “Consequences of skin bleaching in Nigerian men and women”; Int. J. Dermatol. 2005; 44:41-43. doi: 10.1111/j.1365-4632.2005.02812.x.

## 2. Materials and Methods

Natural Glucuronyl Glucosyloleanolate (GGO) was obtained using a proprietary process and quality controlled prior to its physiological characterization (white crystalline powder with a purity of 98.9% determined by HPLC). Chemical structure was confirmed by <sup>1</sup>H NMR and mass spectrometry with comparison to published data.

### a. Lead selection through an A.I. enabled discovery process

Using a proprietary discovery platform (Fig. 1) that integrates public databases with AI-driven analysis, we combine in silico physicochemical predictions (e.g., solubility, lipophilicity, skin penetration) with literature-based insights (mechanism of action, safety, and IP landscape). This platform enables the identification of candidate molecules for skin lightening, whitening, and brightening applications. Virtual hits are subsequently validated through experimental testing to confirm their physiological activity.



**Fig. 1:** AI based technological platform used to identify virtual hits

### b. Cell based assay

Cell based assay were performed at Qima (Gençay, France). Melanocytes (NHEM) were incubated for 9 days with treatments and stimulation (L-tyrosine + α-MSH). An unstimulated control condition was performed in parallel. At the end of incubation, melanin was extracted after cells lysis and quantified by optical density. Results were expressed as µg/ml melanin. All experimental conditions were performed in n=3.

### c. Ex-vivo

Ex-vivo experiments were performed at Genoskin (Toulouse, France). NativeSkin® models (female, 48y, skin type III, BMI 24kg/m<sup>2</sup>) were exposed to 25 mJ/cm<sup>2</sup> UVB and to 2.25 J/cm<sup>2</sup> UVA and systemically treated with/without compound. The systemic treatment was maintained in the medium throughout the culture and UVA/UVB irradiations. Culture medium was renewed after UV exposures. After the treatment, skin biopsies were fixed and processed. Staining quantifications (Hematoxylin - Eosin, Tyrosinase and Fontana Masson) were performed.

### d. Formulation

Formulation was performed at Effervescence Lab (Avignon, France). Both Placebo and Active were identical except Active which contain 0.01% by weight of GGO. The product is an oil-in-water emulsion formulated exclusively with neutral, non-active ingredients. Microbiology and stability studies of both Placebo and Active products were done before the clinical trial.

### e. Patch test

A patch test was performed at Cosmepar (Nantes, France). Pure GGO was applied on the back (scapular zone) of the volunteers (7 women, 5 men ranging from 20 and 70 years old) as a semi occlusive patch (with filter paper) for two days. Erythema and oedema were scored by a qualified physician at the end of the study.

#### f. Skin sensitization

Genomic Allergen Rapid Detection skin (GARD®skin, OECD442E) Dose-Response Assay was performed by Eurofins with DMSO solution of GGO ranging from 500 µM to 15.625µM (serial dilutions). Cells (MUTZ-3) were incubated with the test item for 24 h at 37°C before cell viability study (FACS) and RNA isolation, quality control (Agilent bioanalyser) and hybridization (NanoString).

#### g. Clinical trial

A randomized, single-blind, placebo-controlled clinical study was conducted under the supervision of Gredeco Laboratory (Paris, France). A total of 60 volunteers were enrolled and randomized into two groups of 30: the GGO treatment group (Group A) and the placebo group (Group B). Each group was further stratified by skin phototype and ethnic origin as follows:

- 10 participants of African origin with black skin (phototype V)
- 10 of Asian origin (phototype III or IV)
- 10 of Caucasian or north African origin (phototype III or IV)

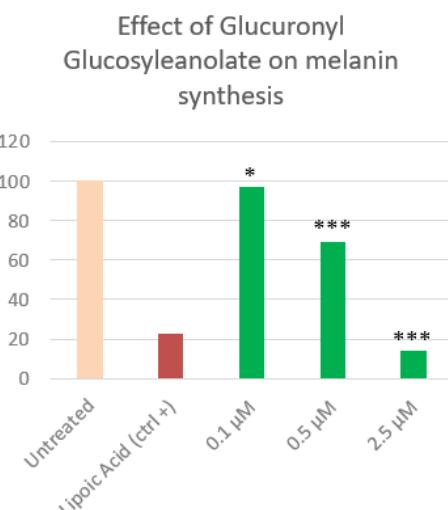
Participants applied their assigned product (GGO or placebo) twice daily—after morning and evening hygiene routines—for 84 days. To prevent UV-related bias, all volunteers also applied SPF50 sunscreen (Avène) during daylight hours.

Clinical assessments were conducted at four time points: Day 0, Day 21, Day 56, and Day 84. The following evaluations were performed:

- Measurement of ITA° (Individual Typology Angle) using a Chromameter CR400 (Konica Minolta)
- 2D facial photography using the VISIA imaging system

### 3. Results

By applying our in-silico discovery platform to the depigmentation and brightening application, we efficiently screened thousands of known compounds to identify 9 lead candidates with promising profiles in terms of predicted efficacy and safety. These shortlisted compounds were advanced to *in-vitro* validation. In particular we picked up Glucuronyl Gluco-syloleanolate, which was then further characterised in an experimental *in-vitro* depigmentation test on melanocytes activated by α-MSH, where a dose-dependent efficacy was demonstrated with significant inhibition of melanin production compared with blank (basal level, no UV exposure), untreated (UV exposed, no compound) but also with a positive reference, lipoic acid (see Figure 2 and Table 1).



*Fig 2: Effect on melanin synthesis in melanocytes stimulated with L tyrosine + alpha-MSH*

**Table 1:** GGO in cell-based depigmentation assay

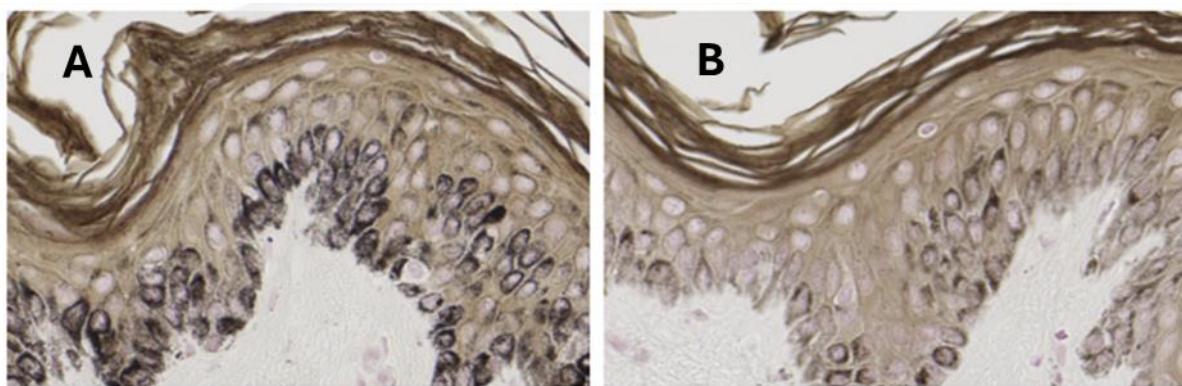
Conditions <sup>a</sup>	Melanin ( $\mu\text{g/ml}$ ) <sup>b</sup>	Melanin (%) <sup>c</sup>	P
Unstimulated - Blank (basal level)	8.1	38	***
Stimulated – untreated (max response)	21.3	100	/
Stimulated – Lipoic acid 5 $\mu\text{M}$	4.8	22	***
Stimulated – <b>GGO</b> 0.1 $\mu\text{M}$	21.7	97	ns
Stimulated – <b>GGO</b> 0.5 $\mu\text{M}$	15.5	69	***
Stimulated – <b>GGO</b> 2.5 $\mu\text{M}$	3.2	14	***

a) stimulation by L-Tyrosine and  $\alpha$ -MSH. b) Melanin content measured by optical density, average of triplicate. c) Melanin content compared to the positive control (Untreated) in %

In this model, the melanin synthesis inhibition achieved up to 86% at 2.5 $\mu\text{M}$  highlighting the strong potency of this compound.

Following the validation of GGO in the cellular model, an efficacy test on isolated organs (human explants) was carried out and demonstrated:

- A significant reduction in tyrosinase expression, with lower levels by up to 2.4 times compared to the positive control, as shown in Figure 4. This result supports the proposed mechanism of action of the compound.
- A reduction in melanin expression identified by assessment of the melanin content using Fontana Masson staining. The obtained results confirmed the efficacy of the compounds in reducing melanin expression, as shown in figure 3, and further reinforce the mechanism of action validation. In this particular ex-vivo model, melanin expression was reduced by 45% versus explant exposed to the same UV regimen.
- An excellent GGO tolerance by the explants demonstrated by evaluation of skin structure and integrity by hematoxylin and eosin staining. This revealed a histologically healthy skin without any signs of impaired viability. The perfect structural integrity allowed us to conclude on the absence of toxicity in human skin ex-vivo. To the best of our knowledge, very few depigmenting compounds demonstrate sufficient safety to avoid inducing adverse effects that compromise the integrity of human tissues in the explant test.



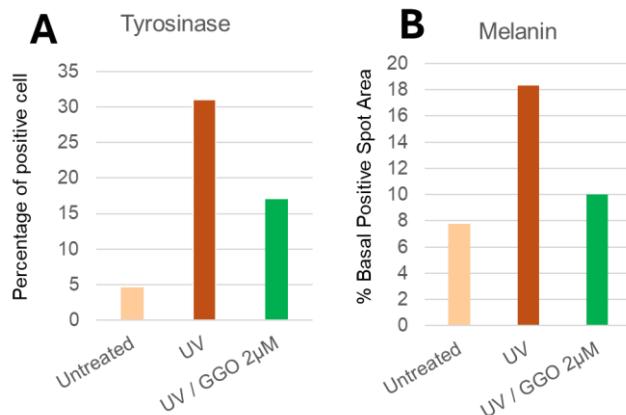
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**Figure 3:** melanin production consecutive to UV exposure on human explants

A: UV irradiation (positive control) – B: UV irradiation + systemic treatment of Glucuronyl Glucosyloleanolate

Interestingly, treatment with GGO significantly reduced the expression of key enzymes involved in melanin biosynthesis, particularly tyrosinase, by up to 58% (Figure 4). This suggests a distinct mechanism of action compared to most existing products, which primarily act through direct tyrosinase inhibition.

These ex vivo findings confirm the compound's potential and support progression to clinical trials the final critical step before registration and industrial-scale production.



**Figure 4:** Tyrosinase (A) & Melanin (B) quantification on human explant followed UV exposure w / wo GGO treatment compared to untreated

To confirm safety prior to clinical testing, a 48-hour patch test was conducted on 12 volunteers using the pure compound. As expected, no skin irritation or adverse effects were observed. The Mean Irritation Index (MII) was 0.00, leading to the conclusion that the compound is non-irritant. In addition, an OECD442E test (GardSkin) demonstrated the absence of any sensitising effect at concentrations of up to 0.5mM, confirming the product's excellent safety.

Consequently, GGO was evaluated in a single blind randomized controlled clinical trial. The trial assess GGO's efficacy for two applications: skin tone brightening and hyperpigmentation spots. The trial includes 60 volunteers divided in two equal groups, GGO at 0.01% and placebo (same formulation but without GGO), applying the product twice daily. All participants apply SPF50 every 4 hours during the day. For the analysis volunteers are divided into three subgroups:

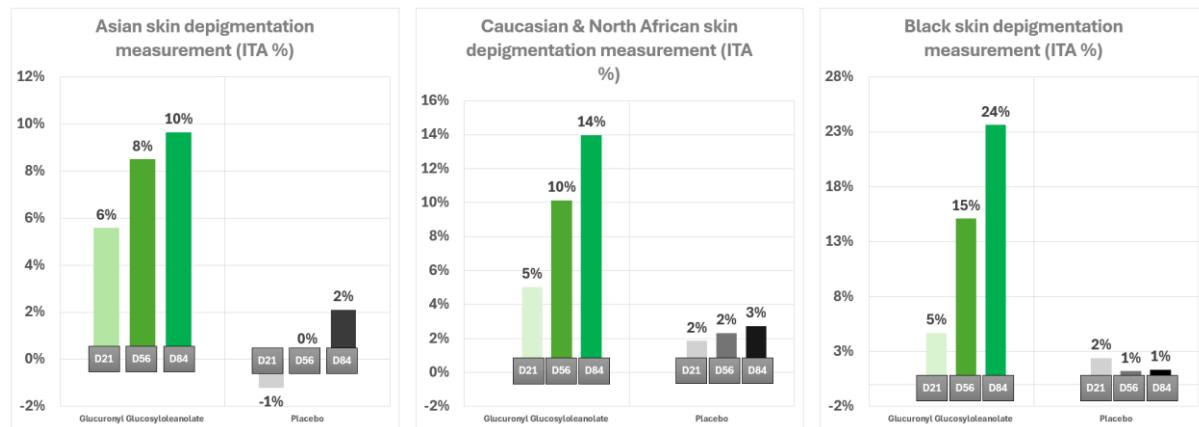
- Subgroup 1: Asian skin of phototypes III and IV
- Subgroup 2: Caucasian and North African skin of phototypes III and IV
- Subgroup 3: Black skin of phototype V

The depigmentation effect of spots was quantified on subgroup 1 and 2. A significant difference in pigmentation was observed as soon as day 21 and kept increasing after each visit reaching up to 17% at day 84.

The skin tone lightening effect was measured on each subgroups. As anticipated, the performance of GGO varied between them. All subgroup saw a significant depigmentation as soon as day 21 that kept increasing similarly to what was observed on the spot depigmentation. After 84 days, the level of depigmentation achieved was:

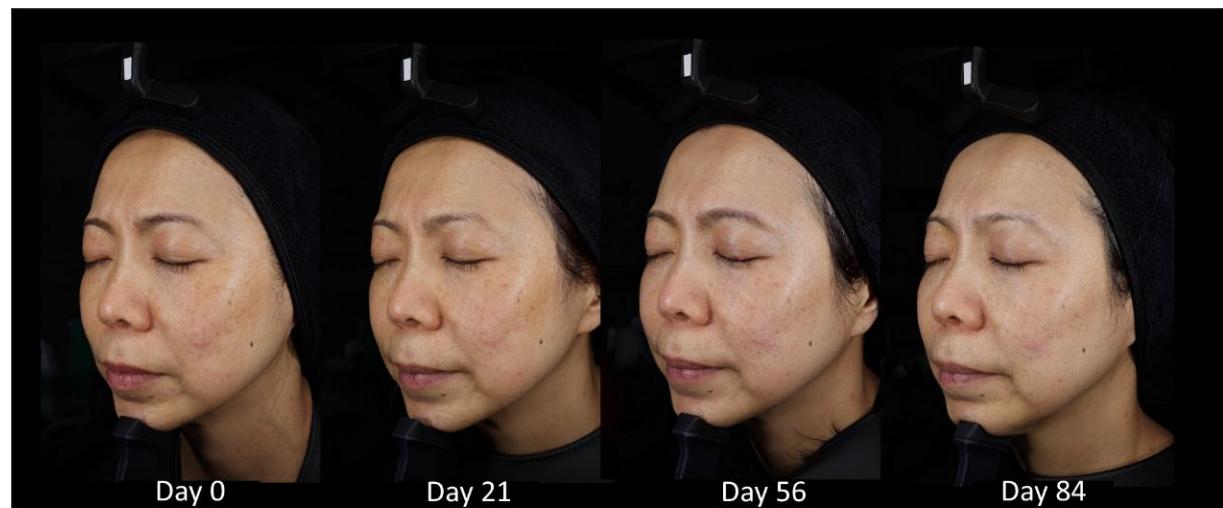
- 10% on Asian skin of phototypes III and IV
- 14% on Caucasian and North African skin of phototypes III and IV
- 24% on Black skin of phototype V

It should be highlighted that the plateau of activity has not yet been reached, so a continuation of the trial would have further enhanced the cutaneous effect.



**Figure 5:** efficacy of global skin tone on volunteers treated with 0.01% of GGO compared to placebo in a 12 weeks clinical study.

Photographs taken during the various visits allow us to follow the evolution of facial pigmentation. An example is given in Figures 6, where the lightening and whitening brightening effects are progressive and clearly visible, while evening out skin tone.



**Figure 6:** volunteer group II facial pigmentation evolution during clinical trial

In addition to global skin tone, a particular attention was made on dark spots where evolution during treatment of few spots were monitored. As illustrated in Figures 7 & 8, pigmentation spots are also evolving favourably. In fact, they depigment more rapidly than the overall skin tone, so much so that they tend to fade and even disappear.

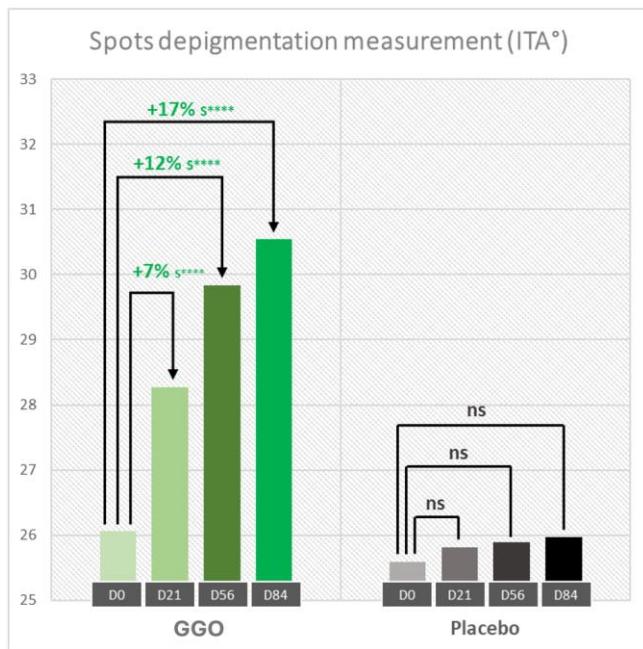


Figure 7: quantification of dark spots pigmentation (ITA measurement)



Figure 8: dark spots at day 0 and day 84

#### 4. Discussion

The discovery and later validation of the depigmenting effect of Glucuronyl Glucosyloleanolate leveraging an *in-silico* approach (Fig 9) demonstrated the validity of this innovation strategy to solve current consumer needs.

The results collected at each steps of the process validated GGO as potential new ingredients for depigmentation of spots and skin lightening with effect respectively achieving an increase in ITA of 17% and up to 24% during the clinical trial. The 2.4x fold reduction in tyrosinase expression quantified during the explants test validated the mechanism of action and offer a viable alternative to tyrosinase inhibitors. At each step of the molecule testing excellent results were observed such as reduction in melanin synthesis of 86% *in-vitro* on human melanocyte or up to 58% *ex-vivo*.

The safety and tolerance profile of GGO was excellent. On human skin explants, tissue structure and integrity were fully preserved which is an important outcome, as some tyrosinase inhibitors can compromise skin integrity in similar test. Based on the results of both the patch test and the skin sensitization assay, GGO is classified as non-irritant and non-sensitizing, confirming its suitability for safe topical use.

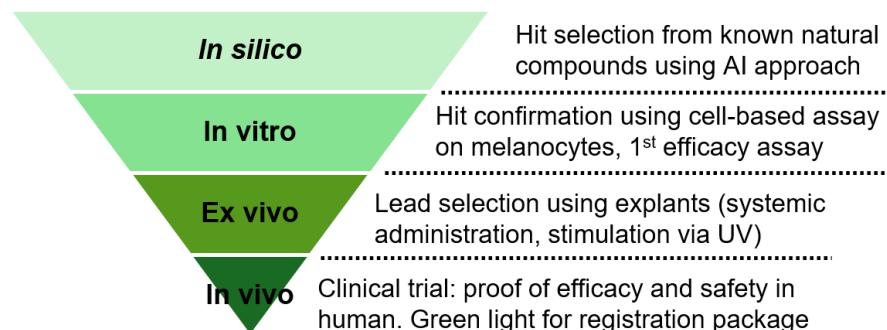
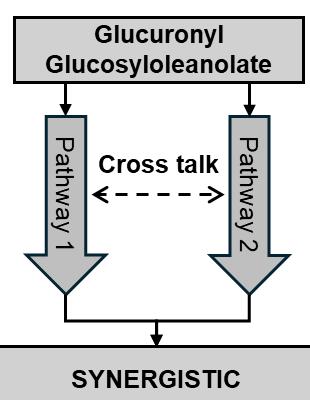


Figure 9: screening tree used to identify and characterize the needle in a haystack

Glucuronyl Glucosyloleanolate belongs to the Saponin family<sup>3, 4</sup> a metabolite derived from oleanolic acid and produced by plants.<sup>5</sup> It is found in leaves and roots, from which it can be extracted, and is associated with numerous beneficial pharmacological properties for humans, such as oncology, diabetes, inflammation, fibrosis, and others.<sup>6</sup>

GGO is known to be an agonist of the Free Fatty acid Receptor 1 (FFAR1, also known as GPR40).<sup>7</sup> FFAR1 is a seven transmembrane G protein-coupled receptor (GPCR). FFAR1 activation by medium-chain fatty acid (MCFA, 6-12 carbon atoms) and long-chain fatty acid (LCFA, more than 12 carbon atoms) endogenous ligands initiates Gαq signaling pathway characterized by phospholipase C-dependent synthesis of diacylglycerol (DAG) and inositol triphosphate (IP3). This second messenger binds to its receptor located within the endoplasmic reticulum and induces the intracellular release of calcium ions. But FFAR1 also couples Gαi proteins, thus leading to a negative modulation of cyclic adenosine monophosphate (cAMP) levels via an increase of phosphodiesterases (PDE).<sup>8</sup> This negative modulation of cAMP will block the natural production mechanism of the enzymes involved in melanin synthesis, Tyrosinase, TRP1 and TRP2, and consequently reduces their expression, as we have observed.

In addition, GGO is known to interfere with NFκB,<sup>9</sup> a mechanism followed by Rhododendrol<sup>10</sup> to limit the transfer of melanosomes to keratinocytes.



Knowing the influence of Calcium, modulated by FFAR1, on tyrosine metabolism, we hypothesise that GGO demonstrates a massive activity on melanin production via multiple and convergent mechanisms. The experimentally demonstrated effect (*in-vitro*, *ex-vivo* and *in-vivo*) of GGO is compatible with a known synergistic effect when different mechanisms targeting the same physiological effect are activated simultaneously leading to an effect which is greater than the sum of individual effect induced by each mechanism individually.<sup>11</sup> Studies are therefore required to demonstrate further this hypothesis.

On the top of the depigmenting effect, a deep analysis of the scientific literature reveals the potential of GGO as an anti-inflammatory,<sup>12</sup> protective,<sup>13</sup> antiaging<sup>14,15</sup> and antiphoto-aging<sup>16</sup>

<sup>3</sup> JP Vincken et al.; Phytochemistry, 68 (2007) ; 275-297. doi:10.1016/j.phytochem.2006.10.008

<sup>4</sup> C. Faustino et al.; Life; 2023, 13 (7), 1514; <https://doi.org/10.3390/life13071514>

<sup>5</sup> T. Dahmer et al; J. Medicinal Food; 15 (12), 2012, 1073-1080. DOI: 10.1089/jmf.2011.0320.

<sup>6</sup> a) Liang et al.; Bio. Org. Chem. Lett. ; 20 (2010) ; 7110-7115. b) Li et al ; Journal of Pharmacy and Pharmacology, 67 (2015), pp. 997–1007. c) Xu et al. Z. Naturforsch. 2021; 76(3–4)c: 103–110. d) Gao et al. Chinese Medicine (2025), 20:36.

<sup>7</sup> Cui et al.; Journal of Ethnopharmacology; 164 (2015) 334–339. doi.org/10.1016/j.jep.2015.02.032

<sup>8</sup> Governa et al.; Bioorg. Med. Chem. Lett. 41 (2021) 127969. doi.org/10.1016/j.bmcl.2021.

<sup>9</sup> Xin Yi et al.; Sci Rep. 2020 Oct 27;10(1):18303. doi: 10.1038/s41598-020-75358-1.

<sup>10</sup> N. Arase et al.; J Dermatol Sci.; 2016;83(2):157-9. doi: 10.1016/j.jdermsci.2016.05.002

<sup>11</sup> Paola Rogliani et al.; Curr Res Pharmacol Drug Discov. 2020 Dec 13;2:100009. doi: 10.1016/j.crphar.2020.100009

<sup>12</sup> Xiao-Juan Wang et al.; Chinese Herbal Medicines, 2021, 13, 64-77, DOI 10.1016/j.chmed.2020.12.003

<sup>13</sup> Hosono-Nishiyama et al.; Planta Med; 2006, 72: 193-198. DOI 10.1055/s-2005-916176

<sup>14</sup> Huan Li et al.; Drug Design, development and Therapy, 2021:15, 4025-4042. DOI: 10.2147/DDDT.S330222

<sup>15</sup> Hao He et al.; Journal of Enzyme Inhibition and Medicinal Chemistry, 2021; 36:1, 1664-1677, DOI: 10.1080/14756366.2021.1956487

<sup>16</sup> Ki Mo Kim et al. ; Applied Biological Chemistry; 2024, 67: 79. DOI 10.1186/s13765-024-00934-2

active ingredient, as shown in table 2, which is compatible with the observations made during the clinical phase (e.g. noticeable reduction in wrinkles) .

**Table 2:** Known properties of Glucuronyl Glucosyloleanolate

Properties	Mechanism	Reference
Anti-aging	Alleviate D-Gal induced aging	Huan Li 2021
Photo-aging	MAPK/AP-1	Ki Mo Kim, 2024
Protective	Fas / FasL	Hosono-Nishiyama 2006
Anti-inflammatory	Decrease ROS	Xia-Juan Wang, 2021
Anti-wrinkles	Hyaluronidase Inhibition	Hao He, 2021

All these activities naturally complement the major activity, skin lightening/brightening effect, first suspected in-silico and further demonstrated (see results) *in-vitro*, *ex-vivo* and *in-vivo*, successively.

The efficacy and the potency of GGO allows to decrease notably the loading and to have a significative effect as low as 0.01% of the final product and therefore compared quite well with the compounds actually incorporated in lightening products (table 3).

**Table 3:** Main depigmenting agents, MoA and loading in final product

Depigmenting agent	MoA	Loading
Hydroquinone	Tyrosinase inhib.	1-5%
Kojic acid	Tyrosinase inhib.	1%
Niaciamide	Melanosome transfer	up to 20%
alpha-arbutine	Tyrosinase inhib.	0.5%
Tranexamic acid	Tyrosinase / Bradykinin Pathway	5%
<b>GGO</b>	Tyr/TRP1/TRP2 expression	0.01%

The effects, as illustrated above (see results), are massive knowing that GGO is the only active ingredient in the used product. We can imagine that the effect will be even more impressive in a commercial product integrating other actives such as exfoliants (fruit acids for example), moisturizer agents and, obviously other skin lightening agents with whom improved synergistic activities can be obtained.

In addition, GGO is a safe compound as proven by complete lack of side effect observed after patch test (48h, pure), skin sensitisation assay and clinical trial (12 weeks, 0.01% wt), and therefore offering a valuable alternative to current commercial product with are often associated with lack of efficacy and/or toxicity/side effects.

This project demonstrated the validity of our proprietary A.I. enabled discovery platform, which could be used to iterate to identify new candidates-ingredients to serve other market segment in needs of effective and/or safer alternatives.

## 5. Conclusion

By leveraging the advanced strategies inspired from the ones used in the biopharma industry, including artificial intelligence based in silico tools, we have identified a compound, Glucuronyl Glucosyloleanolate, which excellent clinical efficacy and safety profile standsouts, thanks to its new mechanism of action, circumvents current limitation of tyrosinase inhibitors.

*In-vitro* and *ex-vivo* studies demonstrated the ingredient potential and mechanism of action. *In-vitro* GGO inhibited melanin production by up to 86% in NHEM models and demonstrated its capacity in to reduce tyrosinase expression *ex-vivo* by up to 58% vs. positive control through a 2.4x fold reduction in tyrosinase expression.

To valide the ingredient potential, a 84 days clinical trial including 60 volunteers, equaly split between tested and placebo groups, was performed. The results demonstrated remarquable efficacy reaching up 17% ITA increase on hyperpigmentation spots and a brightening of the skin tone reaching up to 24% on black skin, 10% on Asian skin, and 14% on caucasian skin.

During the entirety of the development process, safety was assessed with an increased attention during execution of patch, skin sentization tests, and clinical trial. No adverse effect was observed with this non-irritant non-sensitizer ingredient.

These findings indicate that Glucuronyl Glucosyloleanolate holds strong potential to emerge as a leading active ingredient in the skin brightening and depigmenting landscape in the coming years. Leveraging its low effective concentration (0.01%) when formulated in a cream, the patented ingredient is intended for use in both cosmetic and medical applications.

Future investigations will assess the ingredient's potential anti-aging and antioxidative properties to further demontrate its potential and broaden its scope of application.