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Melanin Inhibition Assay

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Tradename: AC LumiVitis

Code: 21032

CAS #: 8013-01-2 & 85594-37-2 (or) 84929-27-1 & 68333-16-4 (or) 1686112-36-6 (or) 9015-54-7

Test Request Form #: 13653

Lot #: 9418748

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Study Director: *Daniel Shill*
Principal Investigator: *Hannah Stade*

Test Performed:
Melanin Inhibition Assay

Introduction
Skin pigmentation is determined by the amount of melanin in the skin. Melanin is produced by dermal melanocytes via melanogenesis. Melanogenesis is initiated in melanosomes (the specialized organelles within melanocytes) by the oxidation of L-tyrosine to L-dopa (L-3,4-dihydroxyphenylalanine) and then to dopaquinone, which is catalyzed by tyrosinase. Dopaquinone is a highly reactive compound which polymerizes to form melanin. Overproduction of dopaquinone and consequently melanin can cause hyperpigmentation leading to melasma, freckles, age-spots, and liver spots. Agents that block this signaling pathway, therefore, exhibit skin lightening effects.

A Melanin Inhibition Assay was conducted to assess the ability of **AC LumiVitis** to inhibit melanin synthesis, thus indicating its skin lightening properties.

Assay Principle
Human epidermal melanocyte cells are exposed to various inhibitors and enhancers of melanin synthesis. The melanin present in the cells is extracted and has an absorbance at 400 nm, which can be quantitated through optical density measurements. The greater the inhibition exhibited by the sample, the lower the optical density value due to reduced melanin present.

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Materials

A. **Cell Line** Normal Human Neo-Natal Epidermal Melanocyte Cells (ATCC; PCS-200-012)
B. **Incubation Conditions** 37°C, 5% CO2, and 95% relative humidity (RH)
C. **Equipment:** Synergy HT Microplate Reader; Forma humidified incubator; ESCO biosafety laminar flow hood; Pipettes
D. **Buffers/Media:** Dermal Cell Basal Medium (ATCC; PCS-200-030); Melanocyte Growth Kit (ATCC, PCS-200-041); Phosphate Buffered Saline (PBS)
E. **Reagents:** α-Melanocyte Stimulating Hormone (α-MSH) (20 µg/mL); Ascorbic Acid 2-Glucoside (AA2G) (5 mM), NaOH (1 N); DMSO
F. **Plates:** 24-Well Tissue Culture Treated Plate; 96-Well Microplate
G. **Software:** Excel Analysis ToolPak (Microsoft)

Methods

Human neo-natal epidermal melanocytes were seeded into 24-well tissue culture plates and allowed to grow to confluency in Complete Media. 0.01%, 0.02%, and 0.05% concentrations of **AC LumiVitis** were diluted in serum-free Complete Media and incubated with melanocytes for 72 hours. α-Melanocyte Stimulating Hormone (α-MSH) (20 µg/mL) and Ascorbic Acid 2-Glucoside (AA2G) (5 mM) were diluted in Complete Media and used as controls. Complete Media was used as the untreated control.

Media was removed from the wells and the monolayers were washed with PBS. A solution of 1 N NaOH + 10% DMSO was added to each well and the plate was incubated for 90 minutes at 80°C with occasional mixing. 100 µL of each solution was added to a 96-well plate in duplicate. Optical density (OD) was read at 400 nm on a Synergy HT Microplate Reader.

Three separate experiments were performed with conditions in duplicate and average values were recorded. Data was analyzed using a one-way ANOVA with statistical significance accepted at $p \leq 0.05$. The percent of melanin inhibition was calculated by the below equation:

$$Percent\ Inhibition\ (\%) = \frac{Optical\ Density_{Sample} - Optical\ Density_{Complete\ Media}}{Optical\ Density_{Complete\ Media}} \times 100$$

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Results

The data obtained met criteria for a valid assay and the controls performed as anticipated. Compared to untreated melanocytes, α-MSH (20 μg/mL) increased melanin production whereas AA2G (5 mM) decreased melanin production. Melanocytes treated with **AC LumiVitis** demonstrated decreases in melanin production.

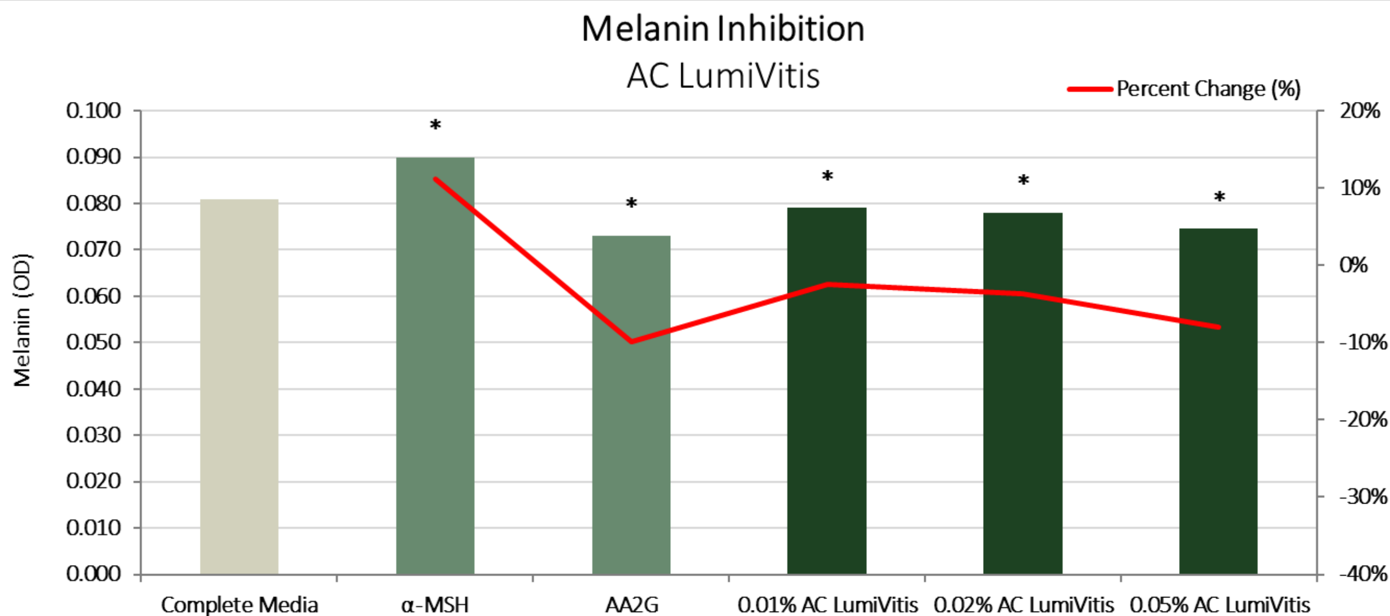

Figure 1. The effect of α-MSH (20 μg/mL), AA2G (5 mM), and **AC LumiVitis** on melanin concentrations in epidermal melanocytes. * indicates significance (p ≤ 0.05) compared to untreated melanocytes.

Table 1. Results from one-way ANOVA Statistical Analysis Compared to Untreated Melanocytes. * indicates significance (p ≤ 0.05) compared to untreated melanocytes.

	α-MSH	AA2G	0.01% **AC LumiVitis**	0.02% **AC LumiVitis**	0.05% **AC LumiVitis**
P-value	0.027*	0.044*	0.035*	0.033*	0.033*

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Discussion

As shown in Figure 1, melanocytes incubated with α-MSH (20 µg/mL), a substance known to stimulate melanin synthesis, exhibited a significant 11% increase in melanin compared to untreated melanocytes (Table 1). Conversely, melanocytes exposed to AA2G (5 mM) elicited a significant 10% reduction in melanin compared to untreated melanocytes (Table 1). These data demonstrate melanogenesis in melanocytes is dynamic and can be manipulated with exogenous compounds.

Similarly, melanocytes treated with **AC LumiVitis** at 0.01%, 0.02%, and 0.05% demonstrated 2%, 4%, and 8% reductions in melanin levels compared to untreated melanocytes, respectively (Figure 1; Table 1). Importantly, the reductions in melanin elicited by **AC LumiVitis** are comparable to the effect of AA2G. Finally, the inhibition activity of **AC LumiVitis** increased as the concentration increased, indicating a dose dependent response. These data demonstrate **AC LumiVitis** reduces melanin synthesis.

Taken together, these results indicate **AC LumiVitis** blunts melanin synthesis when added to personal care applications at recommended use levels. Collectively, **AC LumiVitis** demonstrates skin lightening properties by inhibiting melanin production to counteract the visual appearance of hyperpigmentation.

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